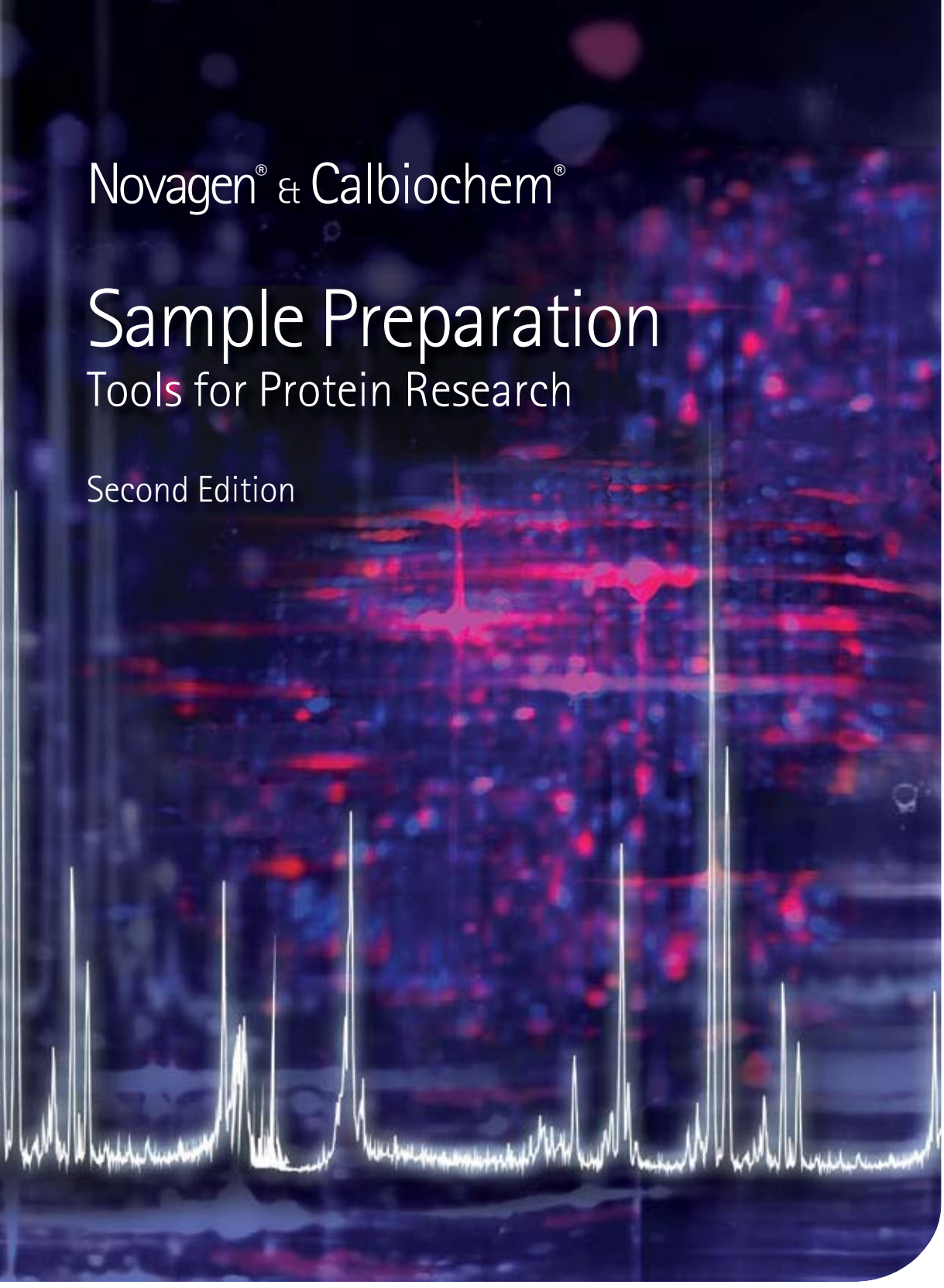

Novagen® & Calbiochem®

Sample Preparation

Tools for Protein Research

Second Edition

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New Products

ProteoExtract® Subcellular Proteome Extraction Kit, Mini, p. 28

ProteoExtract® Subcellular Proteome Extraction Kit, Mini (S-PEK, Mini) is designed for fast and reproducible extraction of subcellular proteomes from adherent and suspension-grown mammalian cells. The S-PEK, Mini takes advantage of the solubility differences of subcellular compartments in the kit's four selected reagents. The optimized stepwise extraction process separates four distinct protein fractions (cytosolic, membrane/organelle, nucleic protein, cytoskeletal). Protein fractions are compatible with downstream applications (1D and 2D gel electrophoresis, immunoblotting, enzyme activity assays, and protein microarrays).

ProteoExtract® Tissue Dissociation Buffer Kit, p. 31

The ProteoExtract Tissue Dissociation Buffer Kit provides a set of optimized reagents for efficient dissociation of fresh tissue samples in combination with a collagenase specific for the tissue type, species origin, and age of animal. Simply mince the tissue, wash with PBS, add collagenase and the provided reagents, and incubate to dissociate. Isolated cells are then ready for subcellular proteome fractionation.

ProteoExtract® Formalin Fixed Tissue Kit, p. 33

The ProteoExtract Formalin Fixed Tissue Kit relies on a proprietary non-detergent formulation to extract full-length, soluble proteins directly from formalin-fixed and paraffin-embedded tissue sections. Extract proteins from tissues of various species, organs, and states (normal or diseased) and use the extracts in downstream applications, such as Western blot and mass spectrometry analysis.

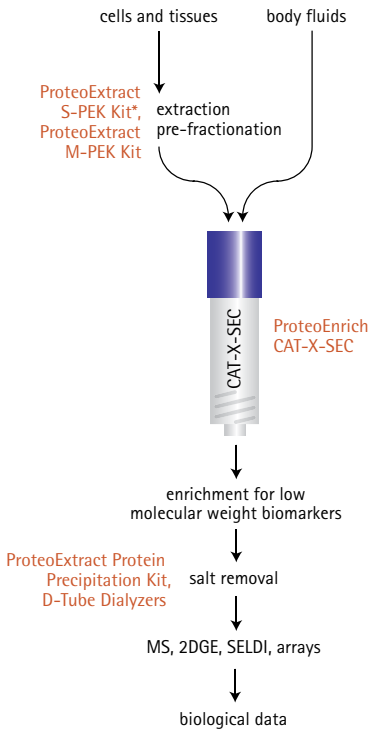
ProteoExtract® Albumin Removal Kit, Maxi, p. 34

This kit contains high-capacity cartridges with a new affinity resin specifically designed for the rapid and efficient removal of >80% of albumin from serum or plasma. Sample capacity for one Albumin Removal cartridge is at least 90-180 µl. These high capacity, reusable cartridges are designed for use either on an LC-instrument or manually (attached to a syringe). Removal of these high abundance proteins allows enhanced detection of low abundance proteins. Each cartridge can be reused up to 10 times.

ProteoExtract® Albumin/IgG Removal Kit, Maxi, p. 36

This kit contains high-capacity cartridges packed with a new affinity resin designed for the rapid and efficient removal of >80% of albumin and IgG from body fluid samples. Sample capacity for one Albumin Removal cartridge is at least 90-180 µl, and for one IgG Removal cartridge is 400 µl. These high-capacity, reusable cartridges are specifically designed for albumin removal (cartridge 1 containing albumin-affinity resin) and IgG removal (cartridge 2 containing immobilized protein A), used either on an LC-instrument or manually (attached to a syringe). Removal of these high abundance proteins allows enhanced detection of low abundance proteins.

New Products *continued*



Processing samples for acquision of biological data

2DGE: two-dimensional gel eletrophoresis; M-PEK: Native Membrane Protein Extraction Kit; MS: mass spectrometry; SEC: size exclusion chromatography; SELDI: surface enhanced laser desorption ionization; S-PEK; Subcellular Proteome Extraction Kit

* Before the cytoskeletal fraction from an S-PEK extraction is applied to a CAT-X SEC cartridge, perform a salt removal step (ProteoExtract® Protein Precipitation Kit or D-Tube™ Dialyzers).

ProteoEnrich™ CAT-X Kit, p. 40

The ProteoEnrich™ CAT-X Kit provides a convenient and reproducible method to fractionate the proteome of biological samples under nondenaturing conditions based on binding to the strong cation exchange resin, Fractogel® EMD SO_3^-. Under slightly acidic conditions, most of the proteome binds to the matrix and partial proteomes can be eluted with a salt gradient. The kit contains two CAT-X cartridges with Luer Lock Adaptors and optimized buffers. Cartridges can be used with a syringe or with liquid chromatography devices and can be reused at least 10 times.

ProteoEnrich™ CAT-X SEC Kit, p. 41

The ProteoEnrich CAT-X SEC Kit provides a highly specific method to enrich the proteome from biological samples, such as body fluids and crude tissue extracts, for low molecular weight proteins. The method uses a unique resin that allows proteins and polypeptides with a globular size of less than 20 kDa to penetrate the resin pores and bind to the inner surface of the pores based on their net charge. Larger proteins flow through the resin-filled cartridge. Proteins can be eluted with a salt gradient or in a single step. The kit contains two CAT-X SEC cartridges with Luer Lock Adaptors and optimized buffers. Cartridges can be used with a syringe or with liquid chromatography devices and can be reused at least 10 times.

ProteoExtract® Phosphoproteome Profiler Kit, p. 44

A convenient and highly reproducible method to enrich for phosphopeptides that result from the activation of signal transduction pathways controlled by protein phosphorylation. After trypsin digestion, phosphopeptides are isolated by affinity chromatography. Each kit contains all reagents and components needed for protein precipitation, trypsin digestion, and enrichment of phosphopeptides.

ProteoExtract® Tryptic Cleavage Modification Kit, p. 46

The ProteoExtract Tryptic Cleavage Modification Kit is an innovative kit based on specific chemical modifications that determine the trypsin cleavage site for in-gel digestions. In combination with the ProteoExtract All-in-One Trypsin Digestion Kit (Cat. No. 650212), the resulting sample contains either trypsin, Arg C-like, or Lys C-like digested peptide fragments, which leads to increased sequence coverage and improved MS profiling to facilitate protein identification.

Protease Inhibitor Cocktails, p. 57–58

Several new animal-free cocktail sets substitute a recombinant version of Aprotinin (instead of Aprotinin prepared from bovine lung): Protease Inhibitor Cocktail Set I, Animal-Free; Protease Inhibitor Cocktail Set III, Animal-Free; and Protease Inhibitor Cocktail Set V, Animal-Free.

Phosphatase Inhibitor Cocktails, p. 60

Phosphatase Inhibitor Cocktails Set III contains four phosphatase inhibitors for broad-spectrum inhibition of both serine/threonine and protein tyrosine phosphatases. Set IV contains three phosphatase inhibitors for inhibition of both serine/threonine and alkaline phosphatases.

iFOLD™ Protein Refolding System 1, p. 67

The system is designed for optimum refolding condition screening of proteins purified as inclusion bodies. The kit contains inclusion body purification and denaturation reagents, and a pre-dispensed 96-well matrix of refolding buffers for the systematic evaluation of 92 buffers representing different combinations of pH, salt, cyclodextrin, redox agent, and refolding additives. Each kit is sufficient for screening up to 96 refolding conditions (92 experimental and 4 control wells) for a single protein.

D-Tube96™ Dialyzers, p. 69

The D-Tube96™ Dialyzer, 6–8 kDa and D-Tube96 Dialyzer, 12–14 kDa offer the same great features as our D-Tube™ Dialyzers Mini in a convenient, 96-tube format suitable for high throughput applications. Select the most appropriate cut-off value and prepare up to 96 × 250 µl samples for buffer exchange or removal of salt, detergents, or urea. If fewer than 96 tubes are required, excess tubes can be removed from the modular device and saved for use at a later time. The D-Tube96 Dialyzers are suited for protein, oligonucleotide, DNA, and RNA dialysis.

ProteoExtract® Detergent Set, p. 73

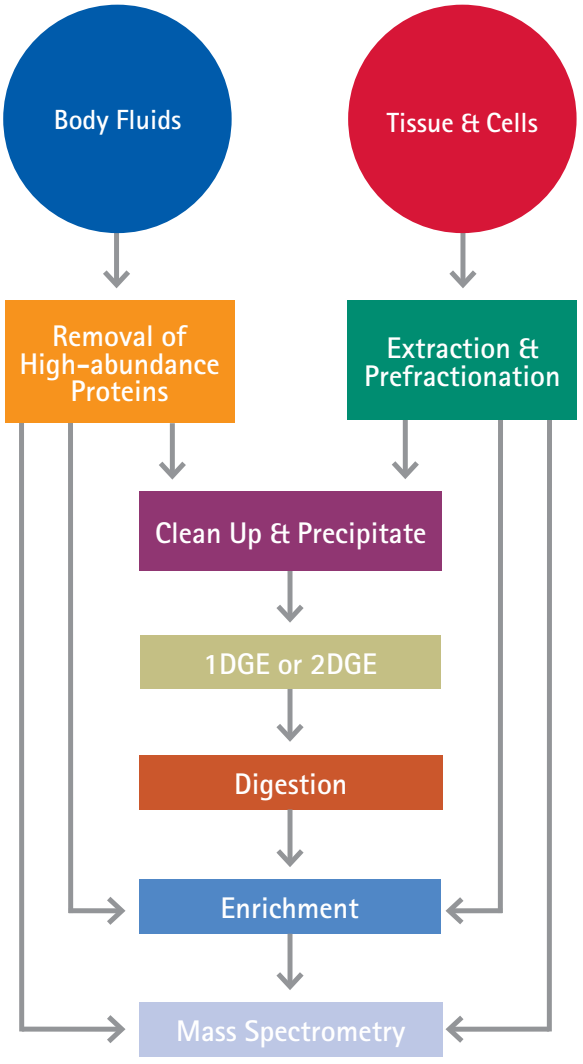
The set, containing nonionic and zwitterionic detergents for membrane protein solubilization, includes 10 g of TRITON® X-100 and 1 g each of ASB-14, ASB-14-4, ASB-16, ASB-C8Ø, CHAPS, *n*-Dodecyl-β-D-maltoside, and ZWITTERGENT® 3-10.

Sample Preparation Overview

Successful proteome analysis depends on standardized, reliable, and convenient sample preparation. EMD Biosciences, through its Calbiochem® and Novagen® brands, offers a comprehensive selection of sample preparation tools for three main areas of protein research: expression proteomics, functional proteomics, and structural proteomics. Most often, expression proteomics includes the comparative analysis of proteins expressed in cells under varying conditions, whether in response to a disease, chemical compound, or therapeutic drug. These differentially expressed proteins can serve as potential drug targets or as biological markers for disease detection. The ProteoExtract® and ProteoEnrich™ kits cover the different steps in sample preparation, from protein extraction and abundant protein removal to concentration of protein mixtures, removal of interfering substances, digestion of proteins, selective capturing of phosphorylated peptides, and selective enrichment for specific protein classes. All kits are compatible with each other. Many of our kits are designed to produce samples that can be used directly in applications such as activity assays, protein microarrays, SDS-PAGE, immunoblotting, ELISA, two-dimensional gel electrophoresis (2DGE), mass spectrometry (MS; including MS/MS, LC-MS, MALDI-MS, SELDI-MS, and ESI-MS), and others.

Structural protein analysis requires a considerable amount of purified protein (20–30 mg on average) and, therefore, these analyses often begin with recombinant protein expression. When purifying proteins for functional or structural proteomics studies, the first step is to disrupt the cells or tissue sample and extract the relevant protein fraction. This step is critical because processing methods that require harsh mechanical, chemical, or enzymatic treatments can directly affect target protein integrity and activity, or otherwise expose proteins to degradative conditions. Our families of protein extraction reagents (BugBuster®, CytoBuster™, NucBuster™, YeastBuster™, PopCulture®, and Insect PopCulture products) are innovative combinations of detergents and other ingredients that enable gentle, efficient, nonmechanical extraction of soluble proteins from bacteria, yeast, plant, mammalian, and insect cells. The reagents are compatible with high-throughput recombinant protein purification and solubility screening.

Example of Protein Sample Preparation Workflow



For more information or to place an order, contact your local office (see back cover).

ProteoExtract® & ProteoEnrich™ Kits for Sample Preparation

One of the major challenges in functional proteomics is the separation of complex protein mixtures to allow detection of low-abundance proteins and enrich for functional protein groups to facilitate downstream analysis. To help meet this challenge, we offer the ProteoEnrich™ and ProteoExtract® product lines. ProteoExtract Kits are unique tools for differential display proteomics studies that allow analysis of complete or partial proteomes from mammalian cells, and from mammalian cells in normal versus diseased or disease-induced states. The Complete Proteome Extraction Kit (C-PEK) is designed for total protein extraction, while the Partial Proteome Extraction Kit (P-PEK) facilitates sequential extraction of protein fractions from mammalian cells according to differences in protein solubility. Both types of kits produce proteome samples ready for 2D gel electrophoresis and mass spectrometry. The Subcellular Proteome Extraction Kit (S-PEK) is designed for sequential extraction of protein fractions from mammalian cells according to their subcellular localization. The S-PEK produces proteins that may be used in activity assays, microarrays, and 1D and 2D gel electrophoresis. ProteoExtract Native Membrane Protein Extraction Kit (M-PEK) is designed for the isolation of non-denatured integral membrane or membrane-associated proteins from mammalian cells and tissues.

ProteoExtract® Abundant Protein Removal Kits

These kits facilitate highly specific depletion of either albumin or albumin/IgG from serum, plasma, or cerebro-spinal fluid. Selective removal of high-abundance proteins improves the detection of low-abundance proteins of interest. The ProteoExtract Albumin Removal Kits are based on an affinity resin, which is highly specific for albumin. The ProteoExtract Albumin/IgG Removal Kits use a combination of the albumin-specific resin and a unique immobilized protein A polymeric resin to remove IgG.

ProteoExtract® Detergent Set

The ProteoExtract Detergent Set contains two non-ionic detergents and six zwitterionic detergents that can be used alone or in combination to solubilize membrane proteins.

ProteoEnrich™ Kits for Group Separations

ProteoEnrich™ CAT-X Kit

The ProteoEnrich CAT-X Kit provides a convenient and reproducible method to fractionate the proteome of biological samples, under non-denaturing conditions, based on binding to the strong cation exchange resin, Fractogel® EMD SO_3^-. Under slightly acidic conditions, most of the proteome binds to the matrix and partial proteomes can be eluted with a salt gradient.

ProteoEnrich™ CAT-X SEC Kit

The ProteoEnrich CAT-X SEC Kit provides a highly specific method to enrich the proteome from biological samples, such as body fluids and crude tissue extracts, for low molecular weight proteins. The method uses a unique resin that allows proteins and polypeptides with a globular size of less than 20 kDa to penetrate the resin pores and bind to the inner surface of the pores based on their net charge. Larger proteins flow through the resin-filled cartridge. Proteins can be eluted with a salt gradient or in a single step.

ProteoEnrich™ ATP-Binders™ Kit

This kit allows group separation of protein kinases and other ATP-binding proteins, yielding cell extracts enriched in active protein kinases. Based on an affinity resin that contains immobilized ATP, the method is compatible with 2D gel electrophoresis, SDS-PAGE/tandem mass spectrometry, Western blot analysis, and activity assays.

Sample Preparation Overview *continued*

ProteoExtract® Kits for Peptide Analysis

ProteoExtract® All-in-One Trypsin Digestion Kit

The ProteoExtract® All-in-One Trypsin Digestion Kit contains a set of optimized reagents for tryptic digestion of various protein samples from polyacrylamide gels, solutions, or cell and tissue extracts to improve LC-MS peak patterns and MS analysis.

ProteoExtract® Tryptic Cleavage Modification Kit

The ProteoExtract Tryptic Cleavage Modification Kit is an innovative kit utilizing specific chemical modifications to control the trypsin cleavage site for in-gel digestions. Used in combination with the ProteoExtract All-in-One Trypsin Digestion Kit, the resulting sample contains either trypsin, Arg C-like, or Lys C-like digested peptide fragments, leading to increased sequence coverage and improved MS profiling.

ProteoExtract® Protein Precipitation Kit

The ProteoExtract Protein Precipitation Kit offers a fast, efficient one-step process for concentrating proteins and removing impurities from a variety of sources.

ProteoExtract® Phosphopeptide Capture Kit

The ProteoExtract Phosphopeptide Capture Kit is a dedicated tool for the analysis of the phosphoproteome. The method relies on the specific interaction of phosphate groups with immobilized zirconium ions on the surface of magnetic particles to capture highly purified phosphopeptides for identification by LC-MS or MALDI-MS.

ProteoExtract® Phosphoproteome Profiler Kit

The ProteoExtract Phosphoproteome Profiler Kit provides a method to determine the protein phosphorylation patterns that result from external and internal signaling. Affinity purification with the MagPrep® PhosphoBind particles included in this kit allows selective enrichment for phosphorylated peptides. Each kit contains all reagents and components needed for protein precipitation, trypsin digestion, and enrichment for phosphopeptides.

Conditions suitable for MALDI/MS

Component or condition	Maximum*
pH	< 4
sodium hydroxide (NaOH)	50 mM
urea	1–2 M
guanidine HCl	1–2 M
dithiothreitol (DTT)	0.5 M
NaCl, KCl	50–100 mM
ammonium carbonate	0.1–0.5 M
Tris	50 mM
HEPES, MOPS	50–100 mM
phosphate buffer	10–20 mM
glycerol	1%
detergents, non-ionic	0.1%
sodium dodecyl sulfate (SDS)	0.01%
octyl-β-D-glucopyranoside	0.1%
octyl glucoside acid	50 mM
dimethylsulfoxide (DMSO)	(avoid)
sodium azide	1 mM
pyridine	0.05%
Coomassie blue stain	0.01%

* These maximum values are those that are primarily compatible with MALDI-MS. Because of online coupling, ESI-MS maxima are significantly lower.

BugBuster® Protein Extraction Reagents

Fast, Efficient, Gentle Protein Extraction from Bacteria

BugBuster® Protein Extraction Reagents are innovative combinations of detergents and other ingredients that enable gentle, efficient, nonmechanical extraction of soluble proteins from bacterial cells. rLysozyme™ Solution increases the efficiency of bacterial lysis with BugBuster Reagent. Addition of Benzonase® Nuclease degrades contaminating DNA and RNA for the preparation of nonviscous, nucleic acid-free extracts ready for target protein purification. Lysonase™ Bioprocessing Reagent combines the functional activities of rLysozyme and Benzonase Nuclease in an optimized, ready-to-use reagent that significantly increases protein extraction efficiency and facilitates processing of protein extracts. In addition, Lysonase can be used to enhance the effectiveness of non-detergent–based cell lysis procedures.

YeastBuster™ and CytoBuster™ Protein Extraction Reagents for Yeast, Plant, Mammalian, and Insect Cell Lysis

YeastBuster™ and CytoBuster™ Protein Extraction Reagents are formulated for a fast, efficient, and gentle extraction of soluble proteins from yeast, plant, mammalian, and insect cells. The YeastBuster reagent avoids harsh conditions of vigorous mechanical or chemical treatment that often result in degradation of target proteins from yeast and plant cells. The CytoBuster reagent is a proprietary formulation of detergents optimized for efficient extraction of proteins from mammalian and insect cells. Both reagents are compatible with downstream protein purification and functional assays.

NucBuster™ Protein Extraction Kit for Nuclear Extract Preparation

The NucBuster™ Protein Extraction Kit is designed for rapid isolation of the nuclear protein fraction from mammalian cells and avoids tedious traditional nuclear extract preparation methods. Nuclear extracts obtained by the NucBuster method are ideal for transcription factor assays.

Reportasol™ and PhosphoSafe™ Extraction Reagents for Functional Activity Assays

Reportasol™ and PhosphoSafe™ Extraction Reagents are specifically optimized for efficient extraction of reporter enzymes and phosphoproteins, respectively, from mammalian cells. Both formulations allow for maximal retention of enzymatic activity of extracted proteins, and do not require shaking or mixing to obtain efficient extraction.

High-throughput Systems: Recombinant Protein Extraction/Purification

PopCulture® Reagent for *E. coli* Lysis in Medium

PopCulture® Reagent is used for extraction of proteins from liquid cultures of *E. coli* without harvesting cells. Addition of 0.1 culture volume of PopCulture Reagent directly to cells in medium, grown at any scale, efficiently extracts proteins while retaining their biological activity. The reagent is used in combination with rLysozyme™ Solution for efficient cell lysis and is compatible with Benzonase® Nuclease and protease inhibitors to reduce viscosity and minimize protein degradation, respectively. This extraction method, combined with the Solubility Screening Plate or with the magnetic- or filtration-based affinity purification provided by the RoboPop™ Kits, enables truly high-throughput protein solubility screening and purification in automated formats.

Insect PopCulture® Reagent for Insect Cell Lysis in Medium

Insect PopCulture Reagent allows centrifugation-free protein extraction from total cultures of insect cells, in suspension or on tissue culture plates. The method increases processing efficiency and target protein yields and is amenable to automated expression screening and affinity purification methods.

Protein Refolding

Overexpression of recombinant proteins in *E.coli* is an efficient method for producing large quantities of target proteins for many biological and new drug development applications. However, proteins often accumulate in the cells as inactive, misfolded, and insoluble aggregates (inclusion bodies). To obtain the target protein in an active, soluble form, the inclusion bodies must be solubilized and the protein refolded.

While the optimal conditions for solubilization and refolding largely depend on the individual protein, some general protocols and reagents have been successfully used for a number of different proteins. The Protein Refolding Kit provides the reagents to perform one general refolding protocol and can serve as a basis for developing an optimized refolding buffer, containing additional reagents such as non-detergent sulfobetaines (NDSBs, see page 68). To determine optimal target protein refolding conditions, the iFOLD™ Protein Refolding System 1 includes all reagents needed to isolate and denature inclusion bodies, and a 96-well plate containing 92 unique refolding buffers. Optimal refolding conditions for target proteins are determined by systematically and simultaneously evaluating the 92 different refolding buffer combinations of pH, salt, cyclodextrin, redox agent, and refolding additives.

Dialysis Devices

D-Tube™ and D-Tube96™ Dialyzers can be used for dialysis or electroelution of proteins or nucleic acids from polyacrylamide or agarose gels. The Electroelution Accessory Kit provides a compatible support tray and precipitation reagents.

Protein Extraction Reagents Application Guide

Product	Starting Material		Applications								Comments
				Analysis							
	Total Culture	Cell Pellet	HT Compatible	1D PAGE	2D PAGE	IEF	MS	Western Blot	Activity Assay	Purification	
E. coli											
BugBuster® Protein Extraction Reagent		✓		✓	✓	✓		✓	✓	✓	Efficient protein extraction from *E. coli* under non-denaturing conditions. Extraction enhanced by the addition of rLysozyme™ Solution and Benzonase® Nuclease. Can be used on cell pellets from any size culture.
BugBuster HT Protein Extraction Reagent		✓	✓	✓	✓	✓		✓	✓	✓	Rapid protein extraction and nucleic acid degradation. Ideal for processing many samples of any volume. Benzonase Nuclease is premixed in the lysis reagent. Extraction enhanced by the addition of rLysozyme Solution.
BugBuster Master Mix		✓	✓	✓	✓	✓		✓	✓	✓	BugBuster Master Mix combines BugBuster Protein Extraction Reagent with Benzonase Nuclease and rLysozyme Solution. Convenient, all-in-one protein extraction reagent efficiently lyses bacteria and digests nucleic acids.
BugBuster (primary amine-free) Extraction Reagent		✓		✓	✓	✓		✓	✓	✓	Ideal as an extraction method for purifying metal-dependent proteins or proteins to be used for immobilization or crosslinking. Extraction enhanced by the addition of rLysozyme Solution and Benzonase Nuclease.
BugBuster 10X Protein Extraction Reagent		✓		✓	✓	✓		✓	✓	✓	A concentrated form of BugBuster Protein Extraction Reagent. Ideal for extraction when a specific buffer is required for protein stability. Extraction enhanced by the addition of rLysozyme Solution and Benzonase Nuclease.
PopCulture® Reagent	✓		✓	✓				✓	✓	✓	Protein extraction from cells directly in the culture medium; no centrifugation required. Designed for small volumes. Extraction enhanced by the addition of rLysozyme Solution and Benzonase Nuclease.
RoboPop™ Purification Kits (magnetic- or filtration-based)	✓		✓	✓			✓	✓	✓	✓	Protein extraction and purification in 96-well format. Ideal for robotic or manual processing; GST•Tag™- or His•Tag®-based purification by magnetic or filtration methods. rLysozyme Solution and Benzonase Nuclease are included.
Yeast											
YeastBuster™ Protein Extraction Reagent		✓		✓				✓	✓	✓	Efficient protein extraction from yeast under non-denaturing conditions from any volume of culture. Add 0.5 M THP Solution (included) and Benzonase Nuclease for enhanced efficiency.
Insect											
CytoBuster™ Protein Extraction Reagent		✓+		✓	✓**	✓**		✓	✓	✓	Gentle lysis of mammalian cells with retention of protein activity for assays and purification. Can use with monolayers or pellets derived from suspension cultures.
Reportasol™ Extraction Buffer		✓+	✓	✓	✓**	✓**		✓	✓R		Optimized for maximal activity of reporter enzymes (β-gal, firefly, and *Renilla* luciferases). Passive lysis of monolayers.
Insect PopCulture Reagent	✓		✓	✓				✓	✓	✓	Lysis of insect cells directly in serum-free medium. Ideal for expression screening of many small samples. Compatible with affinity purification.

Key:
1D PAGE = One-dimensional Polyacrylamide Gel Electrophoresis
2D PAGE = Two-dimensional Polyacrylamide Gel Electrophoresis
IEF = Isoelectric Focusing
MS = Mass Spectrometry
+ = Cell pellet or adherent cells
G = Gel Shift
R = Reporter Assay
* = SDS must be removed before IEF
** = Salt must be removed before IEF

For more information or to place an order, contact your local office (see back cover).

Product		Starting Material: Total Culture	Starting Material: Cell Pellet	Applications: HT Compatible	Applications: Analysis: 1D PAGE	Applications: Analysis: 2D PAGE	Applications: Analysis: IEF	Applications: Analysis: MS	Applications: Analysis: Western Blot	Applications: Analysis: Activity Assay	Applications: Purification	Comments
Mammalian												
CytoBuster™ Protein Extraction Reagent			✓+		✓	✓**	✓**		✓	✓	✓	Gentle lysis of mammalian cells with retention of protein activity for assays and purification. Can use with monolayers or pellets derived from suspension cultures.
Reportasol™ Extraction Buffer			✓+	✓	✓	✓**	✓**		✓	✓R		Optimized for maximal activity of reporter enzymes (β-gal, firefly, and *Renilla* luciferases). Passive lysis of adherent cells.
PhosphoSafe™ Extraction Reagent			✓	✓	✓	✓**	✓**	✓	✓	✓	✓	Ideal for extraction of phosphorylated proteins.
NucBuster™ Protein Extraction Kit			✓		✓G	✓**	✓**		✓	✓	✓	Rapid isolation of nuclear protein fraction from mammalian cells. Ideal for electrophoretic mobility shift assays.
ProteoExtract® Mammalian Complete Proteome Extraction Kit			✓		✓	✓*	✓*	✓*	✓			Total proteome extracted into one fraction.
ProteoExtract Mammalian Partial Proteome Extraction Kit			✓		✓	✓*	✓*	✓*	✓			Produces four protein fractions based on solubility
ProteoExtract Subcellular Proteome Extraction Kit			✓		✓	✓(*)	✓(*)	✓(*)	✓	✓		Produces four native protein fractions based on subcellular localization.
ProteoExtract Native Membrane Proteome Extraction Kit			✓		✓	✓	✓	✓	✓	✓		Produces two native protein fractions, membrane and non-membrane.
Lysis and Extraction Enhancement												
Gram-negative bacteria (*E. coli*)	rLysozyme™ Solution	✓	✓	✓	✓				✓	✓	✓	Cleaves bond in peptidoglycan layer of *E. coli* cell wall. Use alone or combined with BugBuster® or PopCulture® reagents for improved protein extraction. Use with Benzonase Nuclease to reduce sample viscosity and degrade nucleic acids.
	Lysonase™ Bioprocessing Reagent	✓	✓	✓	✓				✓	✓	✓	Convenient mixture of rLysozyme and Benzonase Nuclease minimizes pipetting steps
Gram-positive bacteria	Chicken Egg White Lysozyme Solution	✓	✓	✓	✓				✓	✓	✓	Cleaves bond in peptidoglycan layer of bacterial cell wall.
All cells	Benzonase® Nuclease	✓	✓	✓	✓				✓	✓	✓	Degrades all types of nucleic acids for more efficient protein extraction, faster chromotagraphy, and reduced interference in assays.

Key:
1D PAGE = One-dimensional Polyacrylamide Gel Electrophoresis
2D PAGE = Two-dimensional Polyacrylamide Gel Electrophoresis
IEF = Isoelectric Focusing
MS = Mass Spectrometry
\+ = Cell pellet or adherent cells
G = Gel Shift
R = Reporter Assay
* = SDS must be removed before IEF or MS
(*) = SDS must be removed before IEF or MS; only applicable for cytoskeletal fraction (F4)
** = Salt must be removed before IEF

BugBuster® Protein Extraction Reagents

Simple extraction of soluble protein from *E. coli* without sonication

BugBuster® Protein Extraction Reagent

BugBuster® Protein Extraction Reagent is formulated to gently disrupt the cell wall of *E. coli* to liberate soluble proteins. It provides a simple, rapid, low-cost alternative to mechanical methods, such as French Press or sonication, for releasing expressed target protein in preparation for purification or other applications. The proprietary formulation utilizes a detergent mix that is capable of cell wall perforation without denaturing soluble protein.

In practice, cells are harvested by centrifugation and suspended in BugBuster. The addition of rLysozyme™ Solution, which hydrolyzes N-acetylmuramide linkages in the cell wall, enhances the extraction efficiency, especially for larger proteins. Following a brief incubation, insoluble cell debris is removed by centrifugation. At this point, Benzonase® Nuclease can be added to reduce the viscosity of the extract caused by the release of chromosomal DNA. The clarified extract is ready to use and fully compatible with affinity supports offered by Novagen, including GST•Bind™, GST•Mag™, His•Bind®, His•Mag™, S•Tag™, Strep•Tactin®, and T7•Tag® Resins, or several other chromatography matrices. Following binding to affinity resin, excess BugBuster Reagent is easily removed by washing the column with the appropriate buffer. BugBuster Reagent is also useful for the preparation of high-purity inclusion bodies in instances where expressed proteins are insoluble. The reagent is available in a variety of configurations, BugBuster Reagents and BugBuster plus Benzonase® Nuclease.

The standard BugBuster reagent is supplied as a Tris-buffered "1X" ready-to-use liquid that is stable at room temperature. The 500-ml size is also available with 10 KU Benzonase Nuclease (provided in a separate vial) for the preparation of low viscosity extracts by removal of nucleic acids from protein preparations. BugBuster and Benzonase are compatible with common protease inhibitors.

BugBuster® Master Mix

BugBuster Master Mix combines BugBuster Protein Extraction Reagent with Benzonase Nuclease and rLysozyme Solution in one convenient reagent. BugBuster Master Mix allows maximum recovery of active, soluble protein from both Gram-negative and Gram-positive bacteria. With the Master Mix, there is no need for dilution or additional steps. The two available package sizes provide sufficient reagents for protein extraction from 20 g and 100 g cell paste.

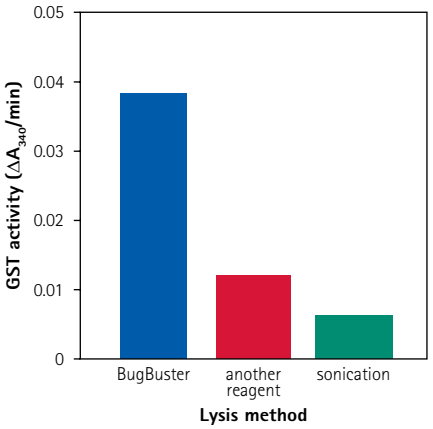
Product	Size	Cat. No.	Price
BugBuster® Protein Extraction Reagent	100 ml 500 ml	70584-3 70584-4	
BugBuster® plus Benzonase® Nuclease	1 kit	70750-3	
BugBuster® Master Mix	100 ml 500 ml	71456-3 71456-4	
Benzonase® Nuclease, Purity >90%	2.5 KU 10 KU	70746-4 70746-3	

Components

Cat. No. 70750

- 500 ml — BugBuster Protein Extraction Reagent
- 10 KU — Benzonase Nuclease, Purity >90%

Note: 1 KU = 1000 units



Comparison of *E. coli* lysis methods

50-ml samples of an induced 500-ml culture of BL21(DE3) containing pET-41a(+) encoding GST were harvested by centrifugation and resuspended in 2 ml 1X PBS, another commercially available protein extraction reagent, or BugBuster Reagent. The sample in PBS was sonicated with 10 pulses at 50% duty for 30 s total. Samples in lysis reagent were treated according to their respective protocols. Extracts were clarified by centrifugation and assayed for GST enzymatic activity using the GST•Tag™ Assay Kit (Cat. No. 70532-3).

BugBuster® Protein Extraction Reagents

Additional configurations increase convenience and versatility

BugBuster® HT Protein Extraction Reagent

BugBuster® HT combines BugBuster Protein Extraction Reagent and Benzonase® Nuclease in one convenient reagent. BugBuster HT eliminates common bioprocessing problems resulting from traditional cell lysis procedures. Soluble proteins are gently extracted from *E. coli* without exposure to heat or oxidative damage and viscosity is eliminated by nucleic acid digestion in a single step. The resulting protein extract can easily be fractionated by conventional purification techniques. BugBuster HT is ideally suited for application in high-throughput protein purifications. Compatible with rLysozyme™ Solution.

BugBuster® 10X Protein Extraction Reagent

BugBuster 10X is a concentrated formulation of the proprietary detergents employed in BugBuster Reagent without the addition of buffer components. BugBuster 10X provides a flexible, concentrated alternative to the ready-to-use standard BugBuster Reagent, allowing user-defined dilution and addition of buffer components. BugBuster 10X has all of the bioprocessing benefits of standard BugBuster Reagent plus the freedom to control pH, reagent concentration, and buffer additives necessary for maximum extraction and activity of target proteins.

BugBuster® (primary amine-free) Protein Extraction Reagent

BugBuster (primary amine-free) is a special formulation of BugBuster Reagent designed for applications where primary amines would interfere if present in the protein extract, such as protein immobilization or cross-linking. The PIPPS buffer used in BugBuster (primary amine-free) has a similar buffering capacity and pH range as the original Tris-buffered BugBuster Reagent, but will not complex metal ions, making it also ideally suited for extraction of metal-dependent proteins.

BugBuster® Plus Lysonase™ Kit

The BugBuster Plus Lysonase™ Kit combines the activities of both reagents to significantly increase protein extraction efficiency and facilitate downstream processing of protein extracts, enabling maximum recovery of active soluble protein from Gram-negative and Gram-positive bacteria. Lysonase Bioprocessing Reagent is an optimized, ready-to-use blend of rLysozyme Solution and Benzonase Nuclease. Use 5 ml BugBuster Reagent and 10 µl Lysonase Reagent per gram of cell paste. Two kit sizes provide sufficient reagents for protein extraction from either 20 or 100 g cell paste.

Product	Size	Cat. No.	Price
BugBuster® HT Protein Extraction Reagent	100 ml	70922-3	
	500 ml	70922-4	
	2 × 500 ml	70922-5	
BugBuster® 10X Protein Extraction Reagent	10 ml	70921-3	
	50 ml	70921-4	
	100 ml	70921-5	
BugBuster® (primary amine-free) Extraction Reagent	100 ml	70923-3	
	500 ml	70923-4	
Lysonase™ Bioprocessing Reagent	0.2 ml	71230-3	
	1 ml	71230-4	
	5 ml	71230-5	
BugBuster® Plus Lysonase™ Kit	1 kit (100 ml)	71370-3	
	1 kit (500 ml)	71370-4	

Components	
Cat. No. 71370	
• 100 ml *or* 500 ml	BugBuster Protein Extraction Reagent
• 200 µl *or* 1 ml	Lysonase Bioprocessing Reagent

Lysonase™ Bioprocessing Reagent

Convenient blend of rLysozyme™ Solution and Benzonase® Nuclease

Lysonase™ Bioprocessing Reagent is an optimized, ready-to-use blend of rLysozyme™ Solution and Benzonase® Nuclease. rLysozyme Solution contains a highly purified and stabilized recombinant lysozyme with specific activity 250 times greater than that of chicken egg white lysozyme. Benzonase Nuclease is a genetically engineered nonspecific endonuclease that degrades all forms of DNA and RNA (single-stranded, double-stranded, circular, linear), reducing extract viscosity, and increasing protein yield. The combined activities of rLysozyme and Benzonase Nuclease significantly increase protein extraction efficiency and facilitate downstream processing of protein extracts.

For efficient protein extraction from bacterial pellets with BugBuster® Protein Extraction Reagent, use 10 µl Lysonase Reagent per 1 g cell paste. For efficient protein extraction from bacterial liquid cultures with PopCulture® Reagent, add 2 µl Lysonase Reagent per 1 ml culture. In addition, Lysonase Reagent can be used to enhance the effectiveness of non-detergent based cell lysis procedures. Store at –20°C.

Product	Size	Cat. No.	Price
Lysonase™ Bioprocessing Reagent	0.2 ml	71230-3	
	1 ml	71230-4	
	5 ml	71230-5	

rLysozyme™ Solution

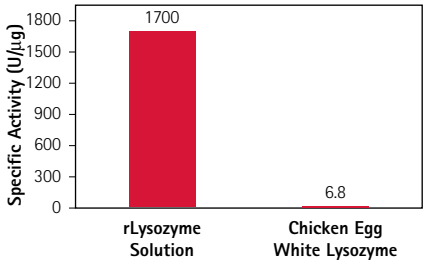
Stabilized recombinant lysozyme

rLysozyme™ Solution contains a highly purified and stabilized recombinant lysozyme that can be used for lysis of Gram-negative bacteria, such as *E. coli*. The enzyme catalyzes the hydrolysis of *N*-acetylmuramide linkages in bacterial cell walls. The specific activity of rLysozyme (1700 KU/mg) for *E. coli* lysis is 250 times greater than that of chicken egg white lysozyme. rLysozyme is optimally active at physiological pH. Very small amounts of rLysozyme Solution (3–5 KU/g cell paste) enhance the efficiency of protein extraction with BugBuster, BugBuster HT, and PopCulture reagents. In the absence of protein extraction reagents, direct lysis of *E. coli* can be achieved by treatment of 1 g cell paste with 45–60 KU rLysozyme Solution. The product is supplied as a ready-to-use solution at a concentration of 30 KU/µl in 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM DTT, and 0.1% TRITON® X-100. rLysozyme Solution is stable at –20°C.

Unit definition: one unit of rLysozyme is defined as the amount of enzyme necessary to cause a decrease of 0.025 unit per minute at 25°C in a 1-ml suspension (1 mg/ml) of Tuner™(DE3) cells in 0.5X BugBuster Reagent diluted with 50 mM Tris-HCl, pH 7.5.

Product	Size	Cat. No.	Price
rLysozyme™ Solution	300 KU	71110-3	
	1200 KU	71110-4	
	6000 KU	71110-5	

Note: 1 KU = 1000 units



Comparison of rLysozyme and chicken egg white lysozyme activities

Activities were measured in a standard activity assay.

rLysozyme™ Solution, Veggie™ Grade

Certified animal-free recombinant lysozyme

rLysozyme™ Solution, Veggie™ Grade is a special grade of rLysozyme Solution prepared using certified animal-free or disease-free reagents. All of the steps in the preparation of the recombinant enzyme are carried out using reagents of nonanimal origin, with the exception of the IPTG used to induce protein expression. IPTG is chemically synthesized by a stringent process from D-galactose isolated from lactose, a milk sugar. The lactose is derived from certified disease-free cows. rLysozyme Solution, Veggie Grade has the same stability and specific activity as the rLysozyme Solution and requires no change to the protocol. With its high specific activity (1700 KU/mg, Note 1 KU=1000 units), only a small amount of rLysozyme Solution, Veggie Grade is required to break the cell wall of *E. coli*. Use the enzyme at 3–5 KU/g cells to enhance protein recovery from cells treated with BugBuster® Reagent or BugBuster HT protein extraction reagents or with PopCulture® Reagent. Alternately, treat thawed cell pellets with only the enzyme using 45–60 KU/g cells. Optimal protein purification is achieved by treating the cell lysate with Benzonase® Nuclease, which efficiently reduces extract viscosity by degrading the nucleic acids liberated as cells lyse. rLysozyme Solution, Veggie Grade is supplied as a ready-to-use solution at a concentration of 30 KU/µl in 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM DTT, and 0.1% TRITON® X-100. The solution should be stored at –20°C.

Product	Size	Cat. No.	Price
rLysozyme™ Solution, Veggie™ Grade	1200 KU	71297-4	
	6000 KU	71297-5	

Note: 1 KU = 1000 units

Chicken Egg White Lysozyme Solution

A ready-to-use, stabilized lysozyme solution

Chicken Egg White Lysozyme Solution is a ready-to-use, stabilized solution of lysozyme. The enzyme catalyses the hydrolysis of *N*-acetylmuramide linkages in bacterial cell walls. The solution can be used for the purification of both DNA and protein. We recommend Chicken Egg White Lysozyme Solution for Gram-positive bacterial lysis. The solution is supplied as 10 mg/ml lysozyme in 25 mM sodium acetate with 50% glycerol, pH 4.5 (200 KU/ml, Note: 1 KU = 1000 units). The product is stable at –20°C.

Unit definition: One unit of Chicken Egg White Lysozyme is defined as the amount of enzyme that causes a decrease of 0.001 A_{450} unit per minute, 25°C, pH 6.2, using a suspension of *Micrococcus lysodeikticus* as the substrate.

Product	Size	Cat. No.	Price
Chicken Egg White Lysozyme Solution, 10 mg/ml	10 × 1 ml	71412-3	

YeastBuster™ Protein Extraction Reagent

Efficient extraction of protein from yeast and plants without mechanical disruption and enzymatic lysis

YeastBuster™ Protein Extraction Reagent is formulated for fast, efficient, and gentle extraction of active proteins from yeast cells. The reagent avoids the harsh conditions of vigorous mechanical or chemical treatment that often result in heat degradation of target proteins. The proprietary formulation utilizes a mix of mild detergents, protein stabilization buffer, and tris(hydroxypropyl)phosphine (THP) reducing agent (THP concentrate provided in a separate tube). This powerful combination eliminates the inconsistencies associated with tedious mechanical disruption of yeast cells with glass bead abrasives, ultrasonication, and pressure disruption, or enzymatic digestion with β-1,3-glucanase lytic enzymes. The reagent has been tested with *Saccharomyces cerevisiae*, *Pichia pastoris*, *P. stipidis*, and *Schizosaccharomyces pombe* strains and with plant cells.

In practice, cells are harvested by centrifugation and suspended in YeastBuster. Following a brief incubation, insoluble cell debris is removed by centrifugation, and the clarified extract is ready to use. In addition to greater total protein yields in crude extracts and recovery of enzymatically active protein, the extracts are fully compatible with GST•Bind™ and Ni-NTA His•Bind® immobilized metal affinity chromatography (IMAC) purification methods. The reagent is available in 100- and 500-ml sizes.

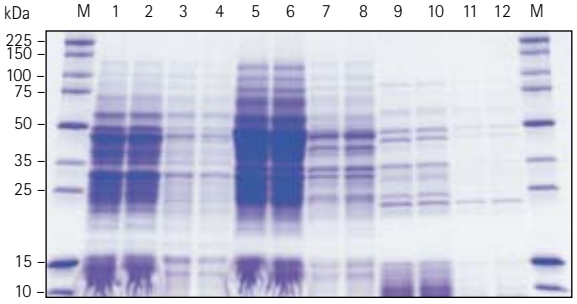
Products	Size	Cat. No.	Price
YeastBuster™ Protein Extraction Reagent	100 ml	71186-3	
	500 ml	71186-4	
0.5 M THP Solution	1 ml	71194-3	
	5 × 1 ml	71194-4	

Components	
Cat. No. 71186	
• 100 ml or 500 ml	YeastBuster™ Protein Extraction Reagent
• 1 ml or 5 ml	100X THP Solution

Related application note on page 81

Features

- Gentle, rapid, efficient extraction of proteins from yeast and plant cells
- Eliminates the inconsistencies associated with abrasive grinding, ultrasonication, and pressure disruption of yeast cells
- Higher yield of total and enzymatically active proteins
- Fully compatible with Ni-NTA His•Bind and GST•Bind affinity purification methods



Protein yield (mg)

	S. cerevisiae	*P. stipidis*	*S. pombe*
YeastBuster	2.7	3.3	0.9
Glass Bead Lysis Buffer	0.8	1.0	0.2

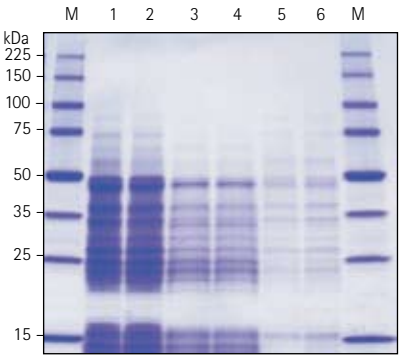
Lane	Sample
M	Perfect Protein™ Markers, 10–225 kDa
1	*S. cerevisiae* YB
2	*S. cerevisiae* YB
3	*S. cerevisiae* GB
4	*S. cerevisiae* GB
5	*P. stipidis* YB
6	*P. stipidis* YB
7	*P. stipidis* GB
8	*P. stipidis* GB
9	*S. pombe* YB
10	*S. pombe* YB
11	*S. pombe* GB
12	*S. pombe* GB

YB = YeastBuster
GB = Glass bead lysis buffer

SDS-PAGE analysis and Coomassie blue staining of extracted soluble proteins from *S. cerevisiae, P. stipidis, and S. pombe*

Duplicate samples of pelleted yeast cells (100 mg wet weight) in 1.5-ml microcentrifuge tubes were resuspended in 500 µl YeastBuster Reagent plus 5 µl 100X THP Solution, or 500 µl glass bead lysis buffer containing 50 mM Tris-HCl, 250 mM LiCl, 100 mM $(NH)_2SO_4$, 1 mM DTT, and 2% glycerol, plus 100 µl acid-washed glass beads (100–150 µm diameter). All samples contained protease inhibitors: 1 mM EDTA, 0.5 µM AEBSF, and 15 µg/ml benzamidine. After resuspension, the pellets were processed according to the standard YeastBuster and glass bead protocols. All samples were centrifuged at 16,000 × g for 5 min prior to SDS-PAGE analysis. Equal-volume samples (7.5 µl) of the extracts were loaded on the gel. Protein yields based on Non-Interfering Protein Assay™ are also shown.

A. SDS-PAGE



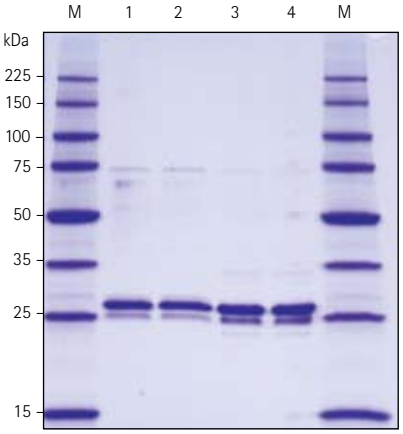
Lane	Sample
M	Perfect Protein™ Markers, 10–225 kDa
1	5 µl YeastBuster™ extract
2	5 µl YeastBuster extract
3	5 µl competitor reagent extract
4	5 µl competitor reagent extract
5	5 µl glass bead extract
6	5 µl glass bead extract

B. Protein and reporter assays

	YeastBuster	Competitor	Glass Beads
Protein (mg/ml)	6.1	3.2	0.65
GST (Δ A_{340}/min)	0.071	0.023	0.007
β-gal (Δ A_{570}/min)	0.113	0.003	0.187

Performance comparison of YeastBuster Protein Extraction Reagent, another commercial reagent, and the glass bead method

Panel A. SDS-PAGE analysis (4–20% gradient gel) and Coomassie blue staining of extracted proteins. *S. cerevisiae* cells containing a recombinant plasmid expressing a 35.6-kDa GST•Tag™/His•Tag® fusion protein were incubated at 30°C, induced for expression, and harvested at OD_{600} of 1.2. Cells were collected by centrifugation at 3000 × g and resuspended in ice cold sterile water. Equal volumes of cells were dispensed into microcentrifuge tubes and pelleted at 3000 × g. Cell pellets (~65 mg wet weight) were resuspended in 330 µl of the respective extraction reagents supplemented with 0.5 mM AEBSF and 15 µg/ml benzamidine. The YeastBuster Reagent also included 0.01 volume 100X THP Solution as directed in the protocol. Glass bead extraction was performed according to the standard protocol. All samples were centrifuged at 16,000 × g for 5 min prior to SDS-PAGE analysis.
Panel B. Analysis of total protein and reporter activities. Total protein extracted by the three methods was determined using the Non-Interfering Protein Assay™ Kit. GST activity was determined using the GST•Tag Assay Kit. β-gal activity was determined using the host expressing *lacZ*. Cells were grown and processed as described for panel A. Samples of the extracts were assayed using the Novagen BetaRed™ β-Gal Assay Kit. Data reflect the average of duplicate assays.



Lane	Sample
M	Perfect Protein Markers, 10–225 kDa
1	2 µg GST•Bind™ eluate
2	2 µg GST•Bind eluate
3	2 µg Ni-NTA His•Bind™ eluate
4	2 µg Ni-NTA His•Bind eluate

SDS-PAGE analysis of GST•Bind and Ni-NTA His•Bind purified samples

S. cerevisiae cells containing a recombinant plasmid expressing a 30.5-kDa GST•Tag/His•Tag fusion protein were incubated and processed as described in Figure 1, panel A. The sample was centrifuged at 16,000 × g for 5 min and 4.5-ml aliquots of the supernatant were purified using GST•Bind or Ni-NTA His•Bind Resins. The protein content of the eluates was determined by BCA and Coomassie blue binding assays. Duplicate samples were analyzed by SDS-PAGE (4–20% gradient) and Coomassie blue staining.

NucBuster™ Protein Extraction Kit

Rapid and convenient extraction of nuclear proteins

The NucBuster™ Protein Extraction Kit provides an alternative to the time-consuming and cumbersome traditional methods for preparing nuclear extracts from mammalian cells. Traditional methods (up to 7 hours), based on a procedure originally described by Dignam et al. (1983), include suspending cells in hypotonic solution, Dounce homogenization, centrifugation, and dialysis. The NucBuster Kit protocol is rapid and allows easy processing of multiple samples. The entire procedure from start to finish yields ready-to-use nuclear extract within 30 minutes. The concentrations of gentle detergents and salt in the final NucBuster extract is directly compatible with electrophoretic mobility shift assays (EMSA).

The NucBuster protocol is based on two proprietary detergent-based solutions: NucBuster Extraction Reagent 1, optimized for cell lysis and removal of cytoplasmic components, and NucBuster Extraction Reagent 2, optimized for extraction of nuclear proteins. In addition, NucBuster extract is free of the viscosity associated with release of genomic DNA, a problem associated with some traditional methods. No Dounce homogenization is required and there is no need for dialysis. The entire procedure is performed in a single microcentrifuge tube and requires only a vortex mixer and microcentrifuge. The kit provides enough reagents for 100 preparations of nuclear extract from 1×10^7 to 5×10^7 cells and the protocol is scalable.

Features

- Nuclear extract preparation obtained within 30 minutes
- No homogenization or dialysis required
- Procedure performed in a single microcentrifuge tube
- Extracts suitable for activity assays, electrophoretic mobility shift assays (EMSA), and NoShift™ and NoShift II Transcription Factor Assays

Reference:

Dignam, J. D., et al. 1983 *Nucleic Acids Res.* **11**, 1475.

Lane	Sample
1	No extract
2	Traditional extract
3	No extract
4	NucBuster extract

NucBuster Protein Extraction Kit versus traditional methodology
The table illustrates the protocol steps and processing times of the NucBuster Protein Extraction Kit and the traditional method. The gel photo shows an electrophoretic mobility shift assay (EMSA) performed with CHO-K1 nuclear extracts prepared using the NucBuster Kit and traditional methods. Extracts were run on a 6% DNA retardation gel, dried on DEAE paper, and exposed to film.

Products	Size	Cat. No.	Price
NucBuster™ Protein Extraction Kit	1 kit (100 rxn)	71183-3	
Use this kit as an accessory product with:			
Protease Inhibitor Cocktail Set I	1 vial 10 vials	539131	
ProteoExtract® Subcellular Proteome Extraction Kit (S-PEK)	1 kit	539790	
ProteoExtract® Subcellular Proteome Extraction Kit, Mini (S-PEK Mini)	1 kit	539791	
ProteoExtract® Native Membrane Protein Extraction Kit (M-PEK)	1 kit	444810	
EMSA Accessory Kit	100 rxn	71282-3	
No Shift™ Transcription Factor Assay Kit	96 rxn	71377-3	
NoShift™ Transcription Factor Assay Kit Plus NucBuster™	96 rxn	71378-3	
NoShift™ II EGR Transcription Factor Assay	96 rxn	71678-3	
NoShift™ II NF-1 Transcription Factor Assay	96 rxn	71679-3	
NoShift™ II NF-κB Transcription Factor Assay	96 rxn	71680-3	
NoShift™ II PPAR Transcription Factor Assay	96 rxn	71681-3	

Components

Cat. No. 71183

• 2 × 7.5 ml	NucBuster Extraction Reagent 1
• 7.5 ml	NucBuster Extraction Reagent 2
• 100 µl	100 mM DTT
• 1 set	Protease Inhibitor Cocktail Set I (lyophilized, makes 100 µl)

Cat. No. 71282

• 1 ml	4X EMSA Buffer
• 125 µl	Poly(dI-dC)•Poly(dI-dC) Solution
• 150 µl	Salmon Sperm DNA
• 100 µl	100 mM DTT

Cat. Nos. 71377 and 71378*

• 4 × 1 ml	4X NoShift Bind Buffer
• 150 µl	Salmon Sperm DNA
• 125 µl	Poly(dI:dC):Poly(dI:dC)
• 30 ml	10X NoShift Wash Buffer
• 20 ml	NoShift Antibody Dilution Buffer
• 1 ea	Streptavidin Plate
• 12 ml	TMB Substrate
• 100 µl	100 mM DTT
• 3 ea	Aluminum Plate Sealer

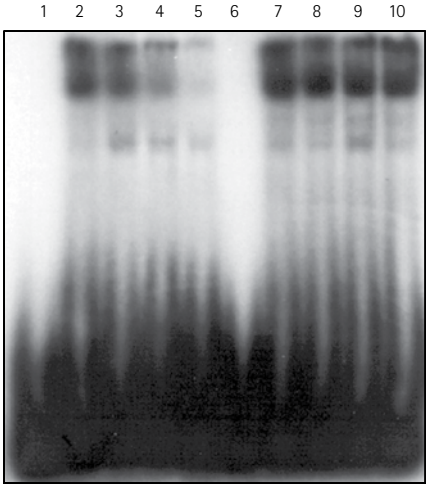
* 71378 also includes 1 kit NucBuster Protein Extraction Reagent

NucBuster Protein Extraction Kit

Procedure	NucBuster Kit	Traditional method**
Cell preparation	10 min	10 min
Cytoplasmic extraction	10 min	25 min
Nuclear extraction	10 min	65 min
Dialysis	–	3 h
Final centrifugation	–	20 min
Total	30 min	5 h

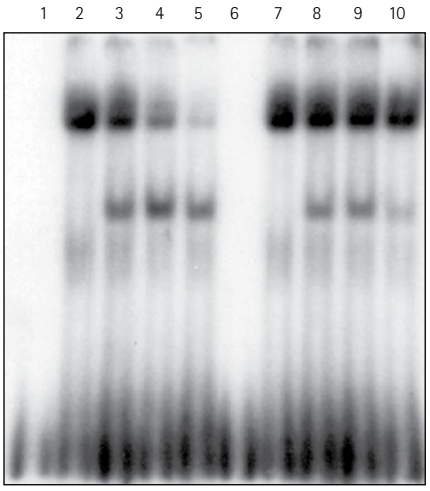
** Traditional method is based on a modification of the procedure of Dignam et al. NP-40 is used in the extraction and the dialysis is shortened to 3 h.

A. Sp1

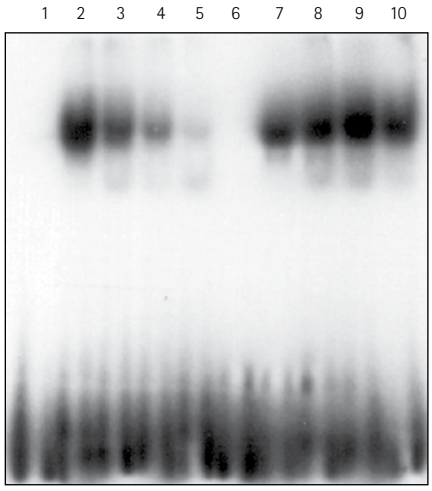


Lane	Sample
1	No extract
2	No competitor
3	10X specific competitor
4	25X specific competitor
5	100X specific competitor
6	No extract
7	No competitor
8	10X nonspecific competitor
9	25X nonspecific competitor
10	100X nonspecific competitor

B. Oct-1



C. CREB



Binding specificity of NucBuster™ extracted nuclear proteins

Nuclear extract from 2 × 10^7 CHO-K1 cells was isolated using the NucBuster Protein Extraction Kit. Nuclear extract samples (2.5 µl) were complexed for 30 min with 0.03 pmol of ^{32}P-labeled ds DNA specifying a Sp1, Oct-1, or CREB binding site and no competitor, or a 10X, 25X, or 100X molar ratio of unlabeled specific or non-specific competitors. The binding reactions were performed as specified in the NucBuster protocol. Samples were analyzed by 6% DNA retardation gel, dried on DEAE paper, and exposed to film.

Reportasol™ Extraction Buffer

Extraction of maximal reporter enzyme activity from mammalian and insect cells

Reportasol™ Extraction Buffer is designed to efficiently extract soluble reporter enzymes from mammalian and insect cells while maintaining maximal activity. The formulation has been optimized for high-throughput assays of firefly luciferase, *Renilla* luciferase, and β-galactosidase. Efficient extraction is achieved in a passive mode (i.e., no shaking or mixing is required) while maintaining extremely high reporter enzyme activity. Reportasol Buffer is also compatible with standard protein assay methods. This buffer is provided in convenient 25-ml bottles (1 bottle for 70909-3, 5 bottles for 70909-4).

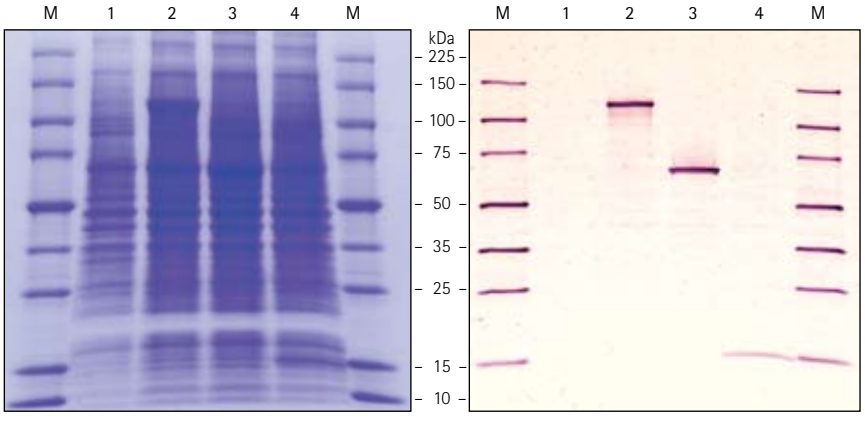
Product	Size	Cat. No.	Price
Reportasol™ Extraction Buffer	25 ml	70909-3	
	125 ml	70909-4	

CytoBuster™ Protein Extraction Reagent

Simple extraction of soluble protein from mammalian and insect cells

The CytoBuster™ Protein Extraction Reagent is a proprietary formulation of detergents optimized for efficient extraction of soluble proteins from mammalian and insect cells. The gentle, non-ionic composition of CytoBuster Reagent enables isolation of functionally active endogenous or expressed proteins without secondary treatment such as sonication or freeze/thaw. CytoBuster Reagent has been specifically formulated for utilization in Western blotting, immunoprecipitation, and kinase/phosphatase assays. The reagent is compatible with protease inhibitors, kinase inhibitors, and phosphatase inhibitors. Store at room temperature. This reagent is provided in convenient 50-ml bottles (1 bottle for 71109-3, 5 bottles for 71009-4).

Product	Size	Cat. No.	Price
CytoBuster™ Protein Extraction Reagent	50 ml	71009-3	
	250 ml	71009-4	



Coomassie blue stained gel — Western blot

Lane	Sample
M	Perfect Protein™ Markers (stained gel), Western Markers (blot)
1	Mock transfection
2	S•Tag β-galactosidase
3	S•Tag firefly luciferase
4	S•Tag 11-kDa fusion protein

Analysis of S•Tag™ Fusion Proteins Extracted with CytoBuster Reagent

COS-1 cells were transfected with a pTriEx™ vector encoding the indicated S•Tag fusion proteins using GeneJuice® Transfection Reagent. After 48 h the cells were treated with CytoBuster Protein Extraction Reagent and equal sample volumes analyzed by Coomassie stained SDS-PAGE (left panel) and Western blot (right panel). The S•Tag fusion proteins were detected on the Western blot using the S-protein AP Conjugate and NBT/BCIP AP substrates. The Perfect Protein Western Markers were detected simultaneously with the S-protein AP Conjugate.

PhosphoSafe™ Extraction Reagent

When phosphorylation state matters

novagen.com/phosphosafe

PhosphoSafe™ Extraction Reagent efficiently extracts cytosolic proteins from mammalian and insect cells while preserving their phosphorylation state. This reagent contains the same formula as CytoBuster™ Protein Extraction Reagent (Trim 2001), but also includes four phosphatase inhibitors: sodium fluoride, sodium vanadate, β-glycerophosphate, and sodium pyrophosphate. PhosphoSafe Reagent is compatible with kinase assays, protein interaction analysis, and other applications. This reagent is provided in convenient 25-ml bottles (1 bottle for 71296-3, 5 bottles for 71296-4).

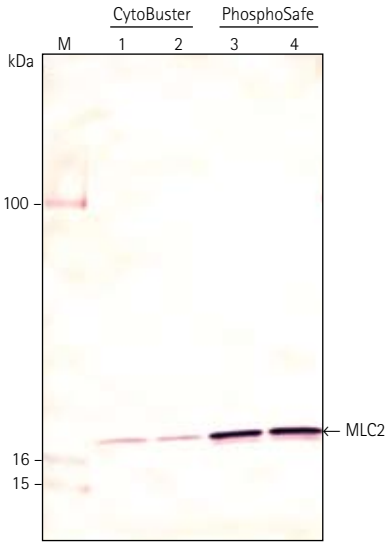
Product	Size	Cat. No.	Price
PhosphoSafe™ Extraction Reagent	25 ml	71296-3	
	125 ml	71296-4	

Features

- Phosphorylation state preserved during extraction
- Compatible with kinase assays and other applications

Reference:

Trim, J. E. and Sawyer, D. L. 2001 *inNovations* **12**, 5.

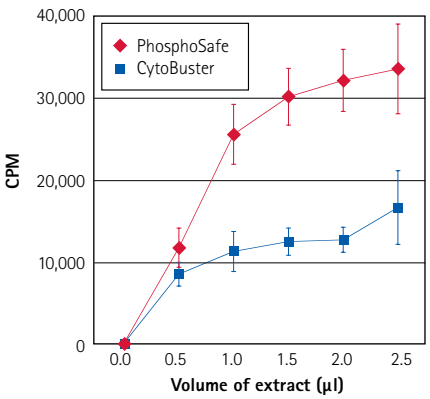


Detection of phosphorylated MLC2

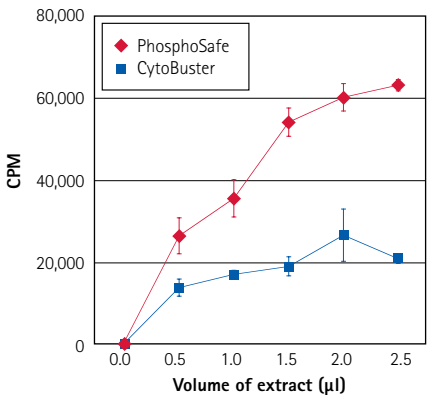
Monolayers of subconfluent L6 myoblasts were extracted with CytoBuster reagent or PhosphoSafe buffer for 10 min at room temperature. Extracts were centrifuged and assayed for protein concentration using the BCA Protein Assay Kit. Duplicates of each extract (10 µg) were analyzed by SDS-PAGE (4–20% gradient gel). Proteins were transferred to a nitrocellulose membrane and protein phosphorylation state was assessed with anti-phospho-MLC2 as the primary antibody. Goat Anti-Rabbit IgG AP Conjugate was added and detected by staining with the AP Detection Reagent Kit. M: Trail Mix™ Western Markers.

Related application note on page 100

A. PKA



B. PKC



Kinase assays for PKA and PKC

Monolayers of subconfluent CHO-K1 cells were extracted with CytoBuster Reagent or PhosphoSafe Buffer for 10 min at room temperature. Biotinylated peptides corresponding to the PKA phosphorylation site of HNF-6 and the pseudosubstrate region of PKC were incubated with increasing amounts of extract (0.6 mg/ml) in the presence of γ-^{32}P ATP. Phosphate transfer mediated by PKA (panel A) and PKC (panel B) was detected by scintillation counting. Protein concentration was determined using the BCA Protein Assay Kit.

ProteoExtract® Complete Proteome Extraction Kit

Fast and easy extraction of total proteins from mammalian cells and tissues

calbiochem.com/proteoextract

The ProteoExtract® Complete Proteome Extraction Kit (C-PEK) is designed for fast and easy extraction of total proteins from mammalian cells and tissues, without the need for sonication or precipitation. C-PEK provides a straightforward two-step isolation of complete proteomes in a single microcentrifuge tube. C-PEK uses optimized extraction reagents to provide improved solubilization of proteins resulting in an increased total number of spots on 2D gels. The procedure uses proprietary Benzonase® Nuclease, a nonspecific nuclease leading to clear, nonviscous protein solutions and improved resolution on 2D gels. Using the C-PEK procedure, protein concentration is not necessary—extracted proteins are ready for immediate use in standard downstream proteomics applications. Each kit contains all the reagents needed for 20 reactions (1×10^8 to 2×10^8 cells per reaction).

Features

- Improved solubilization of total cellular protein
- Ready-to-use proteins for 2D gel electrophoresis —no concentration needed
- Excellent spot resolution facilitated by nucleic acid digestion with Benzonase Nuclease

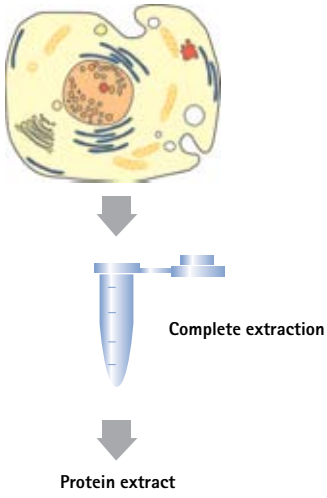
Product	Size	Cat. No.	Price
ProteoExtract® Complete Mammalian Proteome Extraction Kit	1 kit	539779	
Benzonase® Nuclease, Purity >99%	10 KU	70664-3	
Benzonase® Nuclease HC, Purity >99%	25 KU	71206-3	
Benzonase® Nuclease, Purity >90%	2.5 KU 10 KU	70746-4 70746-3	
Benzonase® Nuclease HC, Purity >90%	25 KU	71205-3	

Note: 1 KU = 1000 units

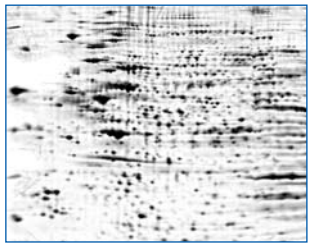
Components

Cat. No. 539779

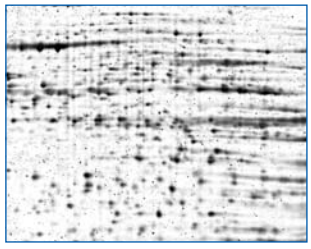
• 2 × 100 ml	Wash Buffer
• 3 ml	Resuspension Buffer
• 25 ml	Extraction Reagent
• 2.27 ml	Reducing Agent
• 1 vial	Benzonase Nuclease
• 1 vial	Glass Beads



Human HepG2-Cells



Dog adrenal gland



Different samples show high resolution in 2D gel electrophoresis

ProteoExtract® Partial Proteome Extraction Kit

Efficient and standardized extraction of cellular proteins into four distinct partial proteomes

calbiochem.com/proteoextract

ProteoExtract® Partial Proteome Extraction Kit (P-PEK) provides efficient and standardized extraction of mammalian cellular proteins into four distinct partial proteomes. Analysis of the partial proteome fractions enriched in subsets of cellular proteins allows detection of low abundance proteins and screening by 2D gel electrophoresis and Western blotting

P-PEK contains uniquely balanced combinations of chaotropes and detergents to solubilize and enrich different subsets of cellular proteins in each of four fractions. The most soluble proteins are released by mechanical disruption in extraction reagent 1, while extraction reagent 2 releases proteins of intermediate solubility. Reagent 3 provides efficient extraction of membrane proteins. SDS buffer solubilizes proteins still insoluble in reagent 3.

Effective removal of nucleic acids with Benzonase® Nuclease eases sample handling and significantly improves resolution on 2D gels. P-PEK protocols generally work without a sonication step, thereby avoiding protein modifications due to temperature effects. P-PEK–extracted proteins are ready for immediate use in 2D gel electrophoresis applications (except fraction 4, which requires dialysis). Each kit contains all the reagents needed for 20 reactions 1×10^8 to 2×10^8 cells per reaction).

Product	Size	Cat. No.	Price
ProteoExtract® Partial Mammalian Proteome Extraction Kit	1 kit	539789	
Benzonase® Nuclease, Purity >99%	10 KU	70664-3	
Benzonase® Nuclease HC, Purity >99%	25 KU	71206-3	
Benzonase® Nuclease, Purity >90%	2.5 KU 10 KU	70746-4 70746-3	
Benzonase® Nuclease HC, Purity >90%	25 KU	71205-3	

Note: 1 KU = 1000 units

Components

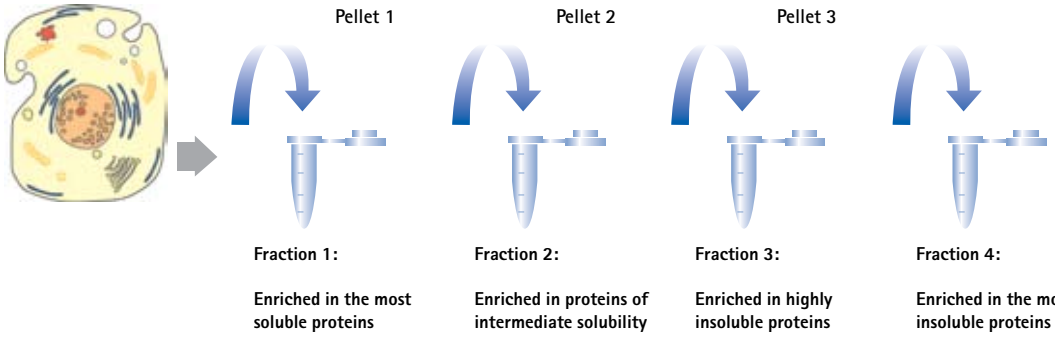
Cat. No. 539789

• 2 × 100 ml	Wash Buffer
• 2 × 110 ml	Extraction Reagent 1
• 40 ml	Extraction Reagent 2
• 15 ml	Extraction Reagent 3 A
• 10 ml	Extraction Reagent 3 B
• 0.04 g	SDS-Buffer A
• 6 ml	SDS-Buffer B
• 0.45 ml	Protease Inhibitor Cocktail
• 45 µl	Benzonase Nuclease
• 19 g	Glass Beads

Features

- Visualize low-abundance proteins from enriched subsets of cellular proteins
- Ready-to-use proteins for 1D and 2D gel electrophoresis
- Excellent spot resolution facilitated by nucleic acid digestion with Benzonase Nuclease
- Includes Protease Inhibitor Cocktail to preserve protein profiles

Related application note on page 87



Sequential partial proteome extraction: the P-PEK method

Samples are sequentially extracted using four buffers with increasing solubilization strengths. The proteome of interest is partitioned into four partial proteomes enriched in proteins of increasing hydrophobicity.

ProteoExtract® Subcellular Proteome Extraction Kits

Fast and reproducible extraction of subcellular proteomes from mammalian cells

calbiochem.com/proteoextract

ProteoExtract® Subcellular Proteome Extraction Kit (S-PEK) is designed for fast and reproducible extraction of subcellular proteomes from mammalian tissue and adherent and suspension-grown cells. S-PEK takes advantage of the different solubilities of certain subcellular compartments in the four selected reagents. In the case of adherent cells, the procedure is performed directly in the tissue culture dish without the need for cell removal. Cells or the parts of the cells remain attached to the plate during sequential extraction of subcellular compartments, until the appropriate extraction reagent is used. Thus, the early destruction of the cellular structure by enzymatic or mechanical detachment of cells from the tissue culture plate and any mixing of different subcellular compartments is prevented. For suspension-grown cells, extraction starts with gentle sedimentation and washing of the cells. For tissues, fragmentation is required before proceeding with the extraction protocol or use the ProteoExtract Tissue Dissociation Buffer Kit and a collagenase of choice. Each kit contains all the reagents needed for 20 reactions (Cat. No. 539790) or 10 reactions (Cat. No. 539791). Each reaction is for 1×10^6 cells.

The stepwise extraction delivers four distinct protein fractions from one sample:

- Cytosolic protein fraction (F1)
- Membrane/organelle protein fraction (F2)
- Nuclear protein fraction (F3)
- Cytoskeletal protein fraction (F4)

Proteins are obtained in the native state (except F4) making the S-PEK suitable for many downstream applications such as 1D and 2D gel electrophoresis, immunoblotting, enzyme activity assays, and protein microarrays.

Product	Size	Cat. No.	Price
ProteoExtract® Subcellular Proteome Extraction Kit	1 kit	539790	
ProteoExtract® Subcellular Proteome Extraction Kit, Mini	1 kit	539791	
Benzonase® Nuclease, Purity >99%	10 KU	70664-3	
Benzonase® Nuclease HC, Purity >99%	25 KU	71206-3	

Note: 1 KU=1000 units

Components

Cat. No. 539790

• 100 ml	Wash Buffer
• 22 ml	Extraction Buffer I
• 22 ml	Extraction Buffer II
• 11 ml	Extraction Buffer III
• 10 ml	Extraction Buffer IV
• 500 µl	Protease Inhibitor Cocktail
• 45 µl	Benzonase Nuclease

Cat. No. 539791

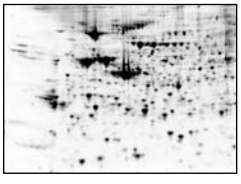
• 50 ml	Wash Buffer
• 12 ml	Extraction Buffer I
• 12 ml	Extraction Buffer II
• 6 ml	Extraction Buffer III
• 5 ml	Extraction Buffer IV
• 250 µl	Protease Inhibitor Cocktail
• 25 µl	Benzonase Nuclease (≥250 U/µl)

Related application notes on pages 83 & 87

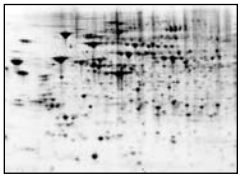
Features

- Stepwise extraction resulting in four distinct subcellular proteomes from one sample
- Highly reproducible
- No ultracentrifugation steps
- Fast—needs just 2 hours, with 45 minutes hands-on time
- Produces proteins suitable for functional studies

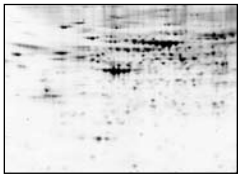
Fraction 1: Cytosol



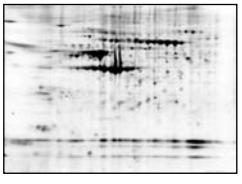
Fraction 2: Membrane/Organelle



Fraction 3: Nucleus

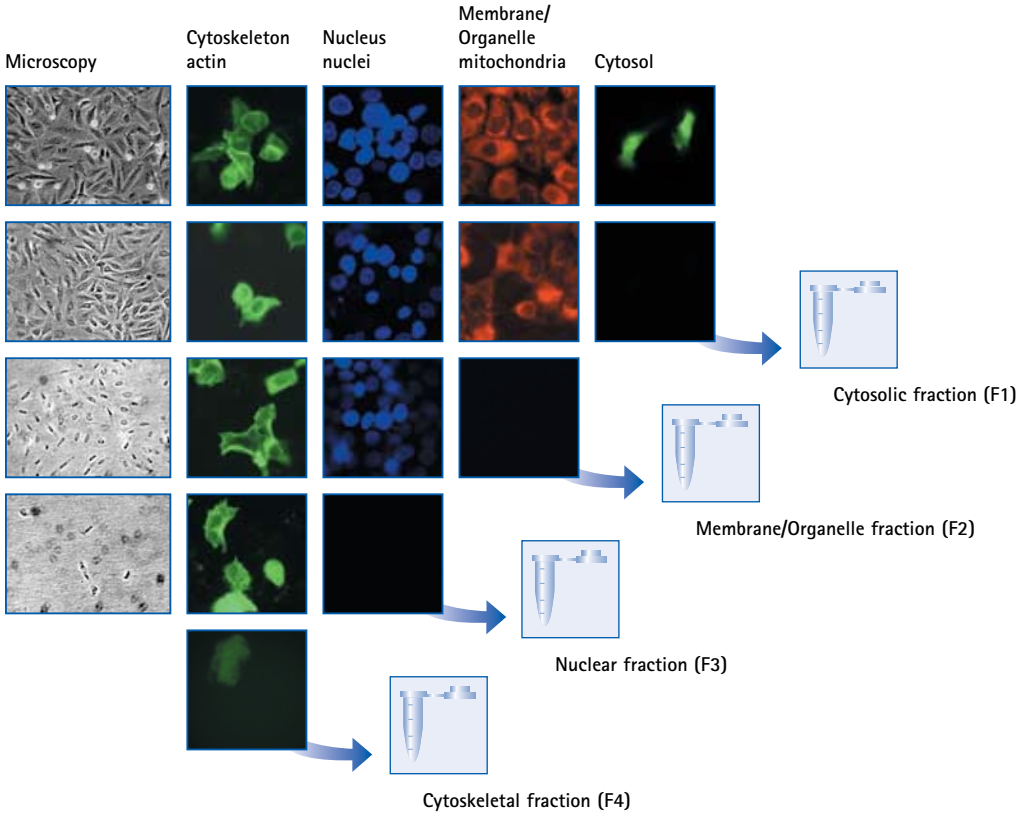


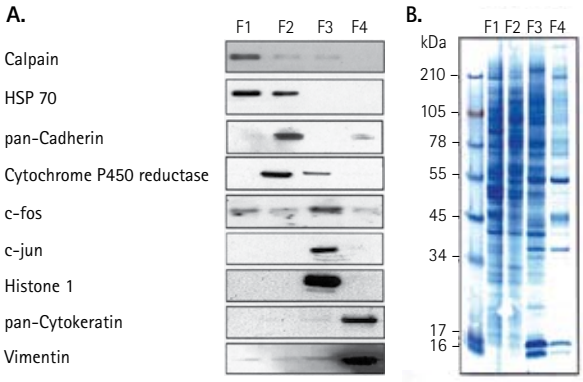
Fraction 4: Cytoskeleton



Protein profiling by 2D gel electrophoresis

The comparison of fractions 1 through 4 by two-dimensional SDS-PAGE indicates a large number of protein spots that are specific for the respective subcellular fraction.





Stepwise extraction with S-PEK demonstrates distinct protein patterns for each isolated subcellular fraction

Panel A: Immunoblots of selected marker proteins demonstrate separation efficiency greater than 80% for subcellular compartments of mammalian tissue culture cells. For detection of c-fos, the protein was immunoprecipitated before electrophoresis.

Panel B: Polyacrylamide gel analysis of subcellular fractions after S-PEK extraction of adherent tissue culture cells demonstrates clearly distinct protein patterns for each fraction. Fractions are as follows: F1, cytosolic fraction; F2, membrane/organelle fraction; F3, soluble nuclear fraction; F4, cytoskeletal/nuclear matrix fraction.

ProteoExtract® Native Membrane Protein Extraction Kit

For the selective extraction of native membrane proteins

calbiochem.com/proteoextract

The ProteoExtract® Native Membrane Protein Extraction Kit (M-PEK) is designed for the isolation of native membrane proteins from mammalian cells and tissue. Rather than separating proteins solely by their intrinsic hydrophobicity, M-PEK extracts proteins from mammalian samples based on an actual association of proteins with cellular membranes. Samples from adherent and suspension tissue culture cells (3×10^6 to 5×10^6 cells) or mammalian tissue (25–50 mg) can be separated as the membrane protein fraction in less than two hours without the need for ultracentrifugation or incubation of samples at elevated temperatures. The extremely mild conditions yield a solution of integral membrane and membrane-associated proteins in their native state. These proteins are suitable for enzyme activity assays, non-denaturing gel electrophoresis, Western blot analysis, ELISAs, assays to examine post-translational modifications of membrane proteins, SELDI-profiling of integral and membrane-associated proteins, and NHS ester labeling of membrane proteins for array detection with dyes or biotin. Each kit contains all the reagents needed for 20 reactions.

Features

- Offers 3- to 5-fold enrichment of native membrane proteins
- Yields proteins in native, functional state
- Allows parallel processing of multiple samples using only 2 steps

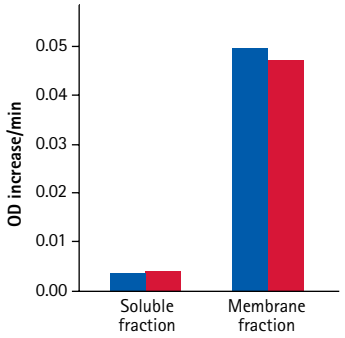
Product	Size	Cat. No.	Price
ProteoExtract® Native Membrane Protein Extraction Kit	1 kit	444810	

Components

Cat. No. 444810

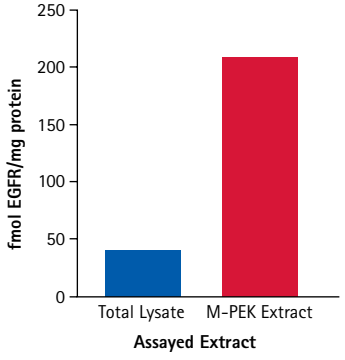
- 100 ml Wash Buffer
- 40 ml Extraction Buffer I
- 20 ml Extraction Buffer II
- 450 µl Protease Inhibitor Cocktail

Related application note on page 83



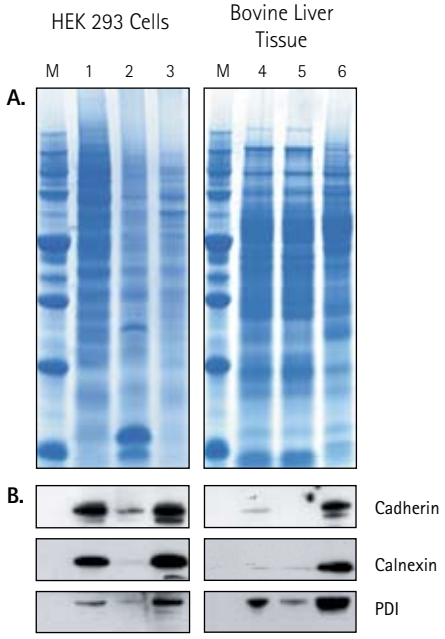
Mild extraction conditions are compatible with enzyme assays

HEK 293 cells were extracted with M-PEK using the two-step procedure to yield a soluble protein and a membrane protein extract. Volume equivalents of the two fractions obtained from two independent experiments were assayed for endogenous alkaline phosphatase activity. The activity profile not only reveals selective separation of this GPI-anchored, membrane-associated enzyme, but also demonstrates that the assayed component is extracted in an active state.



Enrichment for EGF-receptor (EGFR)

HEK 293 cells were extracted with buffered 1% TRITON® X-100 to generate a total lysate or extracted with M-PEK to yield a membrane fraction. The membrane extract obtained with the M-PEK Kit demonstrated a 4.5-fold enrichment of EGFR.



Lane(s)	Sample
M	Markers
1, 4	Total lysates
2, 5	Nonmembrane fraction
3, 6	Membrane fraction

Selective extraction of membrane proteins from cell and tissue samples

Panel A. HEK 293 suspension cells and frozen bovine liver tissue were extracted either with SDS to yield a total lysate or with M-PEK to yield a membrane fraction and remaining "nonmembranous" proteins. Protein equivalents of extracted fractions were separated on SDS-PAGE and visualized by Coomassie blue staining. The membrane protein pattern (lanes 3 and 6) is clearly distinct from the patterns of both total and nonmembraneous fractions (lanes 1, 2, 4, and 5), indicating the selectivity of the M-PEK extraction.

Panel B. Immunoblotting of an equivalent gel using membrane-associated and integral membrane protein markers demonstrates the selectivity of the M-PEK extraction procedure.

NEW ProteoExtract® Tissue Dissociation Buffer Kit

Optimized buffer system for efficient dissociation of fresh tissues with a collagenase of choice

www. calbiochem.com/proteoextract

The ProteoExtract® Tissue Dissociation Buffer Kit provides a set of reagents for efficient dissociation of fresh tissue samples. Simply add the Tissue Dissociation Buffer components with the Protease Inhibitor Cocktail and add a collagenase (not provided) of choice. The choice of collagenase depends on tissue type. After incubating fresh minced tissue with Tissue Dissociation Buffer and collagenase, the dissociated sample is passed through a tissue sieve. Cells isolated using this procedure are then stained with trypan blue to count viable cells. The Tissue Dissociation Kit is compatible with the ProteoExtract Subcellular Proteome Extraction Kit and the ProteoExtract Native Membrane Extraction Kit to facilitate proteome analysis from different cellular fractions. Kit contains sufficient reagents for 10 tissue dissociation reactions (1 g per reaction).

Product	Size	Cat. No.	Price
NEW ProteoExtract® Tissue Dissociation Buffer Kit	1 kit	539720	

Use this kit as an accessory product with:

ProteoExtract® Subcellular Proteome Extraction Kit (S-PEK)	1 kit	539790	
ProteoExtract® Subcellular Proteome Extraction Kit, Mini (S-PEK Mini)	1 kit	539791	
ProteoExtract® Native Membrane Protein Extraction Kit (M-PEK)	1 kit	444810	

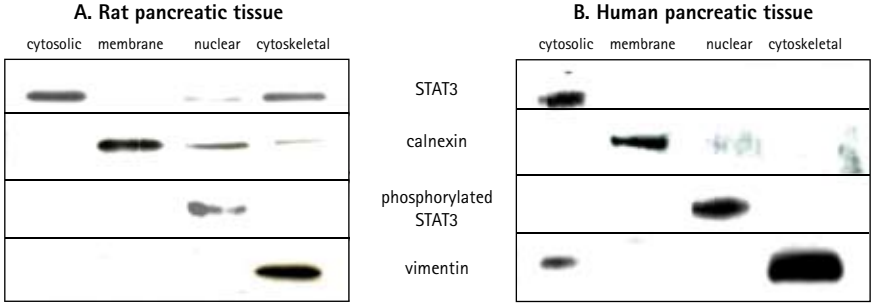
Components

Cat. No. 539720

- 50 ml 10X Tissue Dissociation Buffer
- 1 ml Protease Inhibitor Cocktail
- 100 µl Tissue Dissociation Buffer Additive

Features

- Optimized buffer system for efficient dissociation of fresh tissues
- Provides reagents compatible with a collagenase of choice
- Improved isolation of viable cells from fresh tissues
- Compatible with ProteoExtract Subcellular Proteome Extraction Kits and Native Membrane Protein Extraction Kits
- Enables tissue-specific proteome analysis



Western blot analysis of subcellular protein fractions derived from dissociated tissues
Pancreas tissue from rat (left) and human (right) samples were dissociated according to the user protocol. Protein fractions from 1.3 × 10^7 cells were prepared using the ProteoExtract Subcellular Proteome Extraction Kit. Proteins from the cytosolic, membrane, nuclear, and the cytoskeletal fractions were separated by SDS-PAGE, transferred to PVDF membrane, and probed with antibodies to STAT3, calnexin, phosphorylated STAT3, and vimentin.

NEW ProteoExtract® Cytosol/Mitochondria Fractionation Kit

Selectively enrich for mitochondrial and cytosolic proteomes

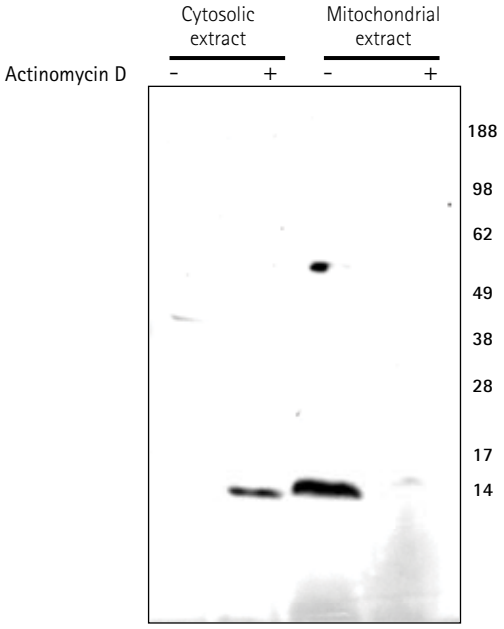
The ProteoExtract® Cytosol/Mitochondria Fractionation Kit is designed for extraction of cellular proteomes from mammalian cells. The simple procedure utilizes unique extraction buffers to deliver two distinct protein fractions: a mitochondrial fraction and a cytosolic fraction. The procedure is easy to perform and does not rely upon ultracentrifugation. Protein fractions are suitable for many downstream applications including 1D and 2D gel electrophoresis, immunoblotting, and apoptotic- and signal transduction-related translocation studies. Each kit contains sufficient reagents to process 100 samples (5×10^7 cells).

Product	Size	Cat. No.	Price
NEW ProteoExtract® Cytosol/Mitochrondria Fractionation Kit	1 kit	QIA88	

Components

Cat. No. QIA88

- 10 ml Mitochondria Extraction Buffer
- 20 ml 5X Cytosol Extraction Buffer
- 100 µl DTT (1 M)
- 250 µl Protease Inhibitor Cocktail



Apoptosis-associated relocalization of cytochrome *c* from mitochondria to the cytoplasm.

Jurkat cells (10^7) were untreated (-) or treated (+) for 7 h with 1 µM Actinomycin D (Cat. No. 114666). Cells were harvested and processed using the ProteoExtract Cytosol/Mitochondria Fractionation Kit. 50 µg of total protein from each fraction was loaded onto a denaturing 8-12% SDS PAGE gel. The proteins were transferred to nitrocellulose and detected with Anti-Cytochrome *c* Mouse mAb (Cat. No. AP1029) at 1 µg/ml.

NEW ProteoExtract® Formalin Fixed Tissue Kit

Extract full-length soluble proteins from formalin-fixed and paraffin embedded tissues

calbiochem.com/proteoextract

The ProteoExtract® Formalin Fixed Tissue Kit is a detergent-free method designed to extract full-length, soluble proteins directly from archived formalin-fixed (FF) or formalin-fixed, paraffin-embedded (FFPE) tissue sections. Formalin fixation effectively preserves the protein content within cells and tissues by cross-linking proteins. These cross-linking events provide excellent preservation of cellular morphology, but render the proteins insoluble. Using the kit's reagents and procedure, proteins can be extracted from various types of tissues (different species, organs, normal, diseased) and from simply fixed (e.g., formalin, paraformaldehyde) or FFPE samples. Use archived tissues, blocks, or slides, which can be stained or unstained. For comparative studies, the Formalin Fixed Tissue Kit can be used to prepare proteins from fresh and fresh frozen tissue. FFPE tissues need to be treated initially with xylene (not included in the kit) to remove paraffin. To extract proteins, FFPE tissue is pretreated with the supplied Pretreatment Reagent, homogenized, and centrifuged. The pellet is incubated with Protein Extraction Reagent, and soluble proteins are precipitated with the Protein Precipitation Reagent. Precipitated proteins can be resuspended in an appropriate buffer for downstream applications, such as Western blot analysis and mass spectrometry (ESI LC/MS and MALDI MS). Each kit contains sufficient reagents to process 10 x 30 mg tissue or 60 slide samples (70 mm^2 x 5 -15 µm thick sections).

Product	Size	Cat. No.	Price
NEW ProteoExtract® Formalin Fixed Tissue Kit	1 kit	539721	

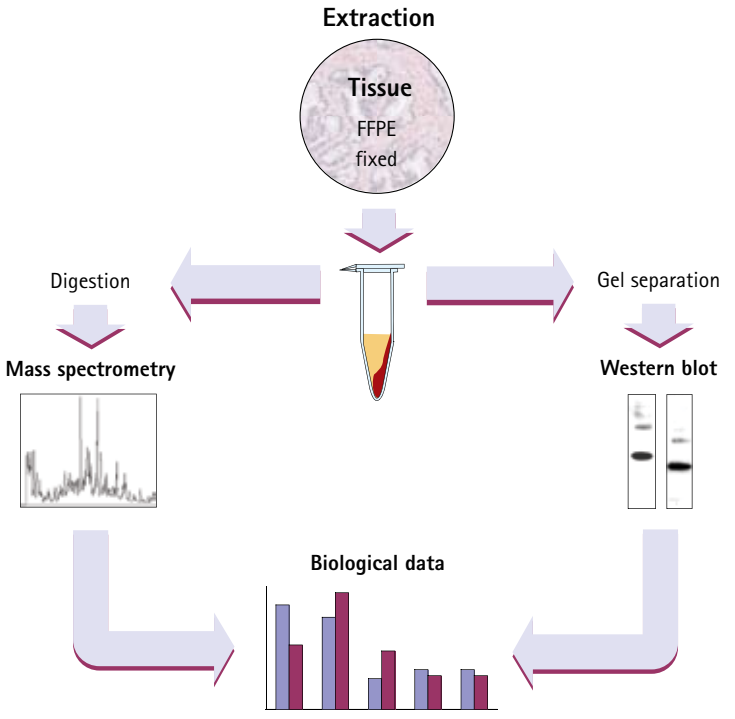
Components

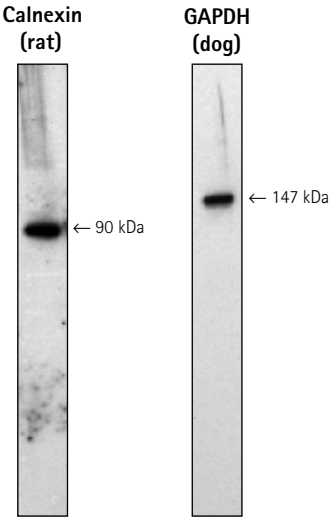
Cat. No. 539721

- 10 ml Pretreatment Reagent
- 3 ml Protein Extraction Reagent
- 1.5 ml Protein Precipitation Reagent

Features

- Use archived FF or FFPE tissues, blocks, or slides to reproducibly extract full-length soluble proteins
- Detergent-free reagents
- Use stained or unstained tissue samples
- Fully scalable procedure
- Identify protein biomarkers or do whole proteome analysis from FFPE tissues
- High protein recovery, sufficient for different downstream applications
- Extracted proteins are compatible with Western blot, ESI LC/MS, and MALDI MS





Western blot analysis of proteins extracted from formalin-fixed tissue

Formalin-fixed liver tissue (30 mg; rat or dog) was pretreated and incubated as described in the protocol. Proteins were precipitated and the pellet was dissolved in 200 µl urea buffer (7M urea, 2M thiourea). Samples (10 µl each) were diluted with 5 µl of 4X Laemmli buffer, separated by SDS-PAGE, transferred to PVDF membrane, and probed with antibodies to calnexin, and GAPDH.

ProteoExtract® Abundant Protein Removal Kits

Enhancing resolution of low-abundance proteins

calbiochem.com/proteoextract

A major challenge for disease marker identification in human serum or plasma is the extraordinary range of protein abundance present in the sample. Proteins in plasma differ in concentration by a factor of one billion. Serum albumin can constitute 55% of total serum proteins and IgG can be as much as 25%. The presence of these proteins makes the resolution of lower-abundance proteins difficult. The ProteoExtract® Abundant Protein Removal Kits provide highly specific and efficient depletion of either albumin alone or albumin and IgG from serum, plasma, or cerebrospinal fluid (CSF). Removal of serum albumin and IgG eliminates approximately 75% of the total protein present in serum. The gravity-flow depletion procedure offered with these kits is simple: equilibrate and load column, collect and pool flow-through and wash, concentrate the proteins, and use enriched samples for additional analysis. After significantly reducing the sample complexity by depleting albumin and IgG, detection and analysis of low-abundance proteins and peptides is improved for applications such as biomarker discovery, toxicological studies for new pharmaceuticals, and protein profiling using SELDI analysis and other applications.

ProteoExtract® Albumin Removal Kits

Highly specific albumin removal

The ProteoExtract Albumin Removal Kits are based on a new affinity resin which is highly specific for albumin. Depletion of albumin from a typical human serum sample is consistently higher than 80% without binding significant amounts of other serum proteins. The remarkable selectivity provided by this resin and the optimized design of these columns result in less than 10% background binding of other serum proteins. The gravity-flow and cartridge-style column formats minimize hands-on time, making them highly suitable for processing multiple samples in parallel. The high capacity, reusable cartridges are specifically designed for use either on an LC-instrument or manually (attached to a syringe). The albumin depletion procedure is convenient and straightforward: equilibrate, load the diluted sample, run through column, wash the column, combine collected flow-through and wash, and concentrate the proteins. Depleted samples are compatible with 1DGE, 2DGE, LC/MS, and MALDI-TOF MS. These kits have been optimized to bind human serum albumin, but are also compatible with rabbit, rat, and mouse samples.

Each ProteoExtract Albumin Removal Kit contains 12 disposable columns pre-packed with 300 µl albumin-binding resin and a binding buffer for optimal resin performance. Resin binding capacity is 2 mg albumin per column at a flow rate of 0.1 ml/min with 10% breakthrough. Sample volumes of up to 60 µl can be processed without any loss of selectivity.

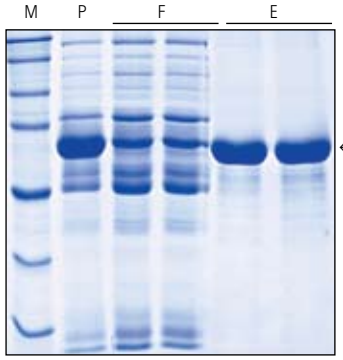
Each ProteoExtract Albumin Removal Kit, Maxi contains 2 cartridges pre-packed with 1 ml albumin-binding resin, a concentrated albumin-binding buffer, and an albumin-removal buffer for optimal resin performance. The resin binding capacity is 6.1 mg albumin per cartridge at a flow rate of 0.1 ml/min with 10% breakthrough. Sample volumes from 90 to 180 µl can be processed without any loss of selectivity. Each cartridge can be reused for 10 samples (for a total of 20 samples).

Product	Size	Cat. No.	Price
ProteoExtract® Albumin Removal Kit	1 kit	122640	
ProteoExtract® Albumin Removal Kit, Maxi	1 kit	122641	

Components	
Cat. No. 122640	
• 12	ProteoExtract Albumin Removal Columns
• 55 ml	ProteoExtract Albumin Binding Buffer
Cat. No. 122641	
• 2	Albumin Removal Column, Maxi with Luer Lock Adapters
• 90 ml	Albumin Binding Buffer, 10X (250 mM sodium phosphate, pH 7.4)
• 70 ml	Albumin Removal Buffer (25 mM sodium phosphate, pH 8.0; 2 M NaCl)

Features

- Efficient removal of albumin with albumin-specific resin (not based on Cibacron™ technology)
- Highly specific, exhibiting little non-specific binding
- Increased loading of enriched sample on 2DGE or LC
- Fast, 20–30 minute protocols
- Easy, column-based procedure to process multiple samples in parallel

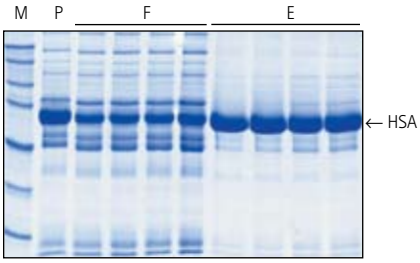


Lane	Sample
M	Markers
P	Human plasma
F	Flow-through
E	Eluate fraction

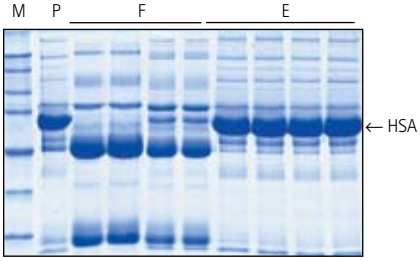
Efficient removal of albumin from human serum samples

Human plasma (35 µl) was processed with ProteoExtract® Albumin Removal Kit. From each fraction, 15 µg protein was separated on SDS-PAGE and visualized by Coomassie blue staining. Densitometric analysis of stained bands demonstrated that more than 80% of albumin was removed from the serum using the ProteoExtract Albumin Removal Kit. HSA: human serum albumin.

A. Kit from Supplier I

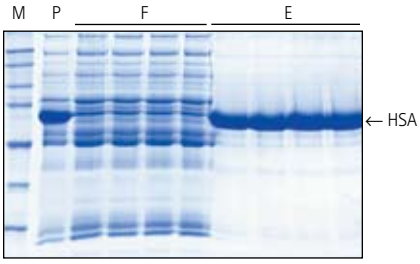


B. Kit from Supplier II



Lane	Sample
M	Markers
P	Human plasma
F	Flow-through
E	Eluate fraction

C. ProteoExtract® Albumim Removal Kit



Lower background binding

Depletion of albumin from human plasma using the ProteoExtract Albumin Removal Kit is more specific and more efficient than with products from other suppliers. The same experiment was performed in parallel using the ProteoExtract Albumin Removal Kit and albumin depletion products from Supplier I and Supplier II. From each fraction, 15 µg of protein was separated on SDS-PAGE and visualized by Coomassie blue staining. The absence of proteins other than albumin in the eluate fraction demonstrates the low background binding using the ProteoExtract Albumin Removal Kit (panel C). High background binding of proteins and less efficient removal of albumin is seen using products from Supplier I (panel A) and Supplier II (panel B).

ProteoExtract® Albumin/IgG Removal Kits

Enhancing resolution of low-abundance proteins

calbiochem.com/proteoextract

The ProteoExtract® Albumin/IgG Removal Kits use a combination of the albumin-specific resin (as in the ProteoExtract Albumin Removal Kits) and a unique immobilized protein A polymeric resin. Depletion of albumin and IgG from typical human serum samples is consistently higher than 80% without binding significant amounts of other serum proteins. The excellent selectivity provided by these resins and the optimized design of the columns result in less than 10% background binding of other serum proteins. The gravity-flow and cartridge-style column formats minimize hands-on time, allowing the parallel processing of multiple samples. The high capacity, reusable cartridges are specifically designed for use either on an LC-instrument or manually (attached to a syringe). The kit procedure is similar to the procedure described for the ProteoExtract Albumin Removal Kits (see preceding page). Depleted samples are compatible with downstream proteomics methods such as 1DGE, 2DGE, LC/MS, and MALDI-TOF MS. These kits have been optimized to bind human serum albumin and IgG, but will also deplete rabbit, rat, mouse, or pig samples effectively.

Each ProteoExtract Albumin/IgG Removal Kit contains 12 disposable gravity-flow columns pre-packed with 450 µl of a resin mix (albumin-removal resin plus the protein A IgG-removal resin) and an optimized binding buffer that promotes selective binding of albumin and IgG. The resin binding capacity of each column is 0.7 mg IgG and 2 mg albumin. Sample volumes up to 60 µl can be processed without any loss of selectivity.

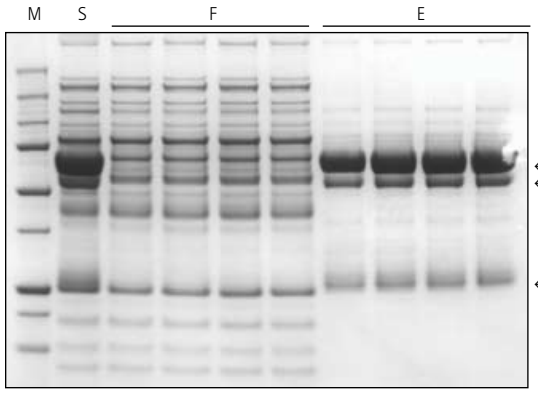
Each ProteoExtract Albumin/IgG Removal Kit, Maxi contains 2 cartridges pre-packed with 1 ml albumin-binding resin, 1 cartridge pre-packed with the IgG removal resin, an albumin/IgG-binding buffer concentrate, an albumin-removal buffer, and an IgG-removal buffer for optimal resin performance. The resin binding capacities are 6.1 mg albumin per albumin-removal cartridge and 4.5 mg IgG per IgG-removal cartridge at a flow rate of 0.1 ml/min with 10% breakthrough. Sample volumes up to 90 to 180 µl can be processed per albumin-removal cartridge and up to 400 µl per IgG-removal cartridge without any loss of selectivity. Each albumin-removal cartridge can be reused for 10 samples (for a total of 20 samples) and each IgG-removal cartridge can be reused for 20 samples. These two types of cartridges can be used either independently or connected in series, taking the flow-through from one cartridge and loading it directly onto the second cartridge.

Features

- Efficient removal of albumin and IgG from serum samples
- Highly specific, exhibiting very little non-specific binding
- Compatible with human, rabbit, rat, mouse, and pig serum samples
- Increased loading of enriched sample on 2D gel electrophoresis or LC
- Fast, 20–30 minute protocols
- Easy, column-based procedure to process multiple samples in parallel

Product	Size	Cat. No.	Price
ProteoExtract® Albumin/ IgG Removal Kit	1 kit	122642	
ProteoExtract® Albumin/ IgG Removal Kit, Maxi	1 kit	122643	

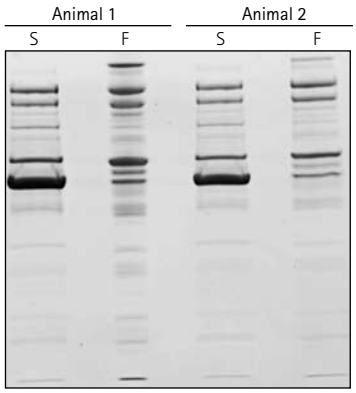
Components	
Cat. No. 122642	
• 12	ProteoExtract Albumin/IgG Removal Columns
• 55 ml	ProteoExtract Albumin/IgG Binding Buffer
Cat. No. 122643	
• 2	Albumin Removal Column, Maxi with Luer Lock Adapters
• 1	IgG Removal Column, Maxi with Luer Lock Adapter
• 90 ml	Albumin/IgG Binding Buffer 10X (250 mM sodium phosphate, pH 7.4)
• 70 ml	Albumin Removal Buffer (25 mM sodium phosphate, pH 8.0; 2 M NaCl)
• 70 ml	IgG Removal Buffer (250 mM citric acid)



Efficient and reproducible removal of albumin and IgG from serum samples

Using the ProteoExtract® Albumin/IgG Removal Kit, 35 µl human serum was processed, analyzed on 10% SDS-PAGE, and visualized by Coomassie blue staining. Densitometric analysis of stained bands of the eluate (E) fractions demonstrates that 88% ±5% albumin and 90% ±4% immunoglobulin are removed from the crude serum (S) using the ProteoExtract Albumin/IgG Removal Kit. HSA: human serum albumin.

Lane	Sample
M	Markers
S	Crude human serum
F	Flow through: depleted serum in four replicates
E	Eluate fraction: proteins eluted from depleting columns in four replicates

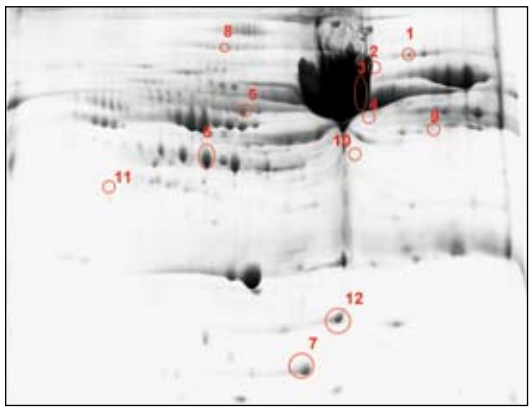


Lane	Sample
S	Crude rat serum
F	low through

Removal of albumin and IgG from rat serum

Using the ProteoExtract Albumin/IgG Removal Kit, 35 µl rat serum from two different animals was processed. Equal protein amounts were separated by 10% SDS-PAGE gels and visualized by Coomassie blue staining. Densitometric analysis of stained flow-through demonstrates that the ProteoExtract Albumin/IgG Removal Kit removes more than 78% of rat albumin.

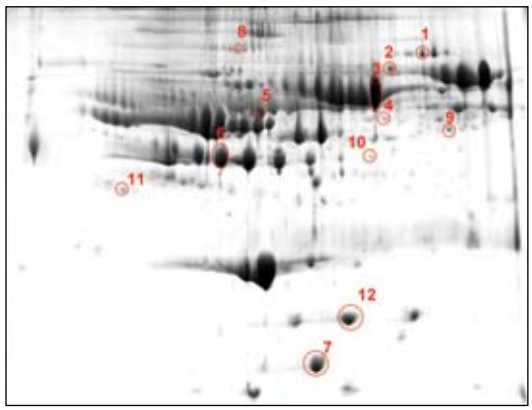
A.



Unprocessed human serum

Spot	Identity
1	Complement Factor B precursor
2	Human serum albumin fragment
3	Human serum albumin
4	Human serum albumin
5	Antithrombin III, chain L
6	Haptoglobin chain β
7	Transthyretin
8	Inter-α trypsin inhibitor—related protein precursor
9	Human serum albumin precursor
10	Non-identified
11	Non-identified
12	Haptoglobin chain α 2

B.



Albumin- and IgG-depleted serum

Spot	Identity
1	Complement Factor B precursor
2	Gelsolin precursor
3	Human serum albumin
4	Transferrin n-terminal lobe
5	Antithrombin III, chain L
6	Haptoglobin chain β
7	Transthyretin
8	Inter-α trypsin inhibitor – related protein precursor
9	Transferrin fragment HUMTF12 NID
10	Apolipoprotein L1 precursor
11	α-1-antitrypsin chain A
12	Haptoglobin chain α 2

Improving gel resolution

Human serum (35 µl) was either used directly (panel A) or subjected to albumin/IgG depletion (panel B) using the ProteoExtract Albumin/IgG Removal Kit. From each fraction 1.5 mg was precipitated, resolubilized in IEF buffer, subjected to 2DGE, and visualized by Coomassie blue staining. Selected spots were excised from the gel and proteins were digested with trypsin and identified by peptide mapping using nano-LC/MS and the Mascot® search algorithm. The identification of five additional proteins (spots 2, 4, 9, 10, and 11) in the depleted serum sample (panel B) demonstrates that removal of albumin and IgG allows visualization and identification of proteins otherwise obscured by albumin and IgG.

ProteoEnrich™ ATP-Binders™ Kit

Affinity enrichment of ATP-binding proteins

calbiochem.com/proteoextract

The ProteoEnrich™ ATP-Binders™ Kit allows group separation of protein kinases and other ATP binding proteins, yielding partially purified cell extracts enriched in active protein kinases that retain enzymatic activity. Based on an affinity resin containing immobilized ATP, the method is compatible with 2D gel electrophoresis, SDS-PAGE/tandem mass spectrometry, Western blot analysis, and activity assays.

The unique affinity resin contains ATP covalently linked through its γ-phosphate and a unique, flexible 13-atom linker to a polyacrylamide-based resin. The structure of the ATP-Binders Resin presents an ideal configuration for recognition by the conserved ATP-binding pocket of kinases. This resin packs more tightly than agarose beads and so facilitates binding and washing steps. The resin is compatible with mild detergents and suitable for isolation of cytosolic and solubilized membrane proteins. The 200 µm resin particles have protein binding capacity up to 250 µg per ml of packed resin. The gentle elution conditions (20 mM ATP) enable recovery of active proteins, including interacting partners such as scaffolding proteins, from crude cell or tissue lysates. The resin-based, batchwise purification scheme is well suited for multiple separations and can be used to selectively enrich for functional protein groups in proteomic studies or in individual kinase purification schemes. Further, this kit can remove interfering non-ATP–binding proteins and can be used as a "polishing" step in an ATP-binding protein purification strategy.

The kit contains the ATP-polyacrylamide–based resin, spin filters, and optimized buffers required to process extracts prepared from crude mammalian cell or tissue lysates by a standard protocol. The kit provides sufficient reagents to perform enrichments from up to 17.5 mg crude protein.

Features

- Based on novel resin for sample enrichment with functionally active protein kinases and other ATP-binding proteins
- Contains optimized buffers for maximum active protein recovery
- Compatible with 1D and 2D gel electrophoresis, LC/MS, and MALDI-TOF MS
- Compatible with ProteoExtract® All-in-One Trypsin Digestion Kit (see page 45)
- Compatible with protein tyrosine kinase activity assays

Reference:

Bartnicki, D., et al. 2004. *inNovations* **19**, 6–9.

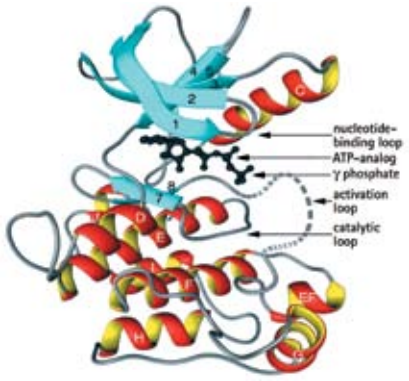
Product	Size	Cat. No.	Price
ProteoEnrich™ ATP-Binders™ Kit	1 kit	71438-3	
ATP-Binders™ Resin	100 mg	71445-3	

Components

Cat. No. 71438

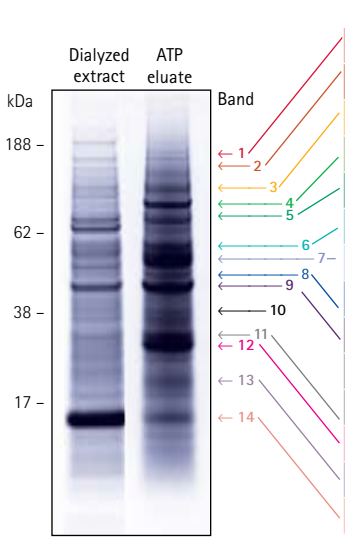
• 100 mg	ATP-Binders Resin
• 1	PBS Tablet
• 2 × 5 ml	100 mM EDTA
• 1.6 ml	1 M DTT
• 2 ml	ATP-Binders 6X Resin Conditioning Buffer
• 7 ml	ATP-Binders 5X Bind Buffer
• 5 ml	ATP-Binders Wash Buffer Concentrate
• 1 ml	ATP-Binders Elution Buffer Concentrate
• 5 ml	ATP-Binders 10X Nucleotide Mix (10 mM ADP, 10 mM AMP, 10 mM NADH, pH 7.0)
• 1 ml	Protease Inhibitor Cocktail Set V, EDTA-Free, Lyophilized
• 2 × 250 µl	100 mM DTT
• 100 µl	Activated Sodium Vanadate (200 mM Na_3VO_4, pH 10.0)
• pkg/10	Spin Filter, 2-ml capacity

Related application note on page 100



Ribbon diagram of the protein tyrosine kinase domain of the insulin receptor bound to an ATP analog

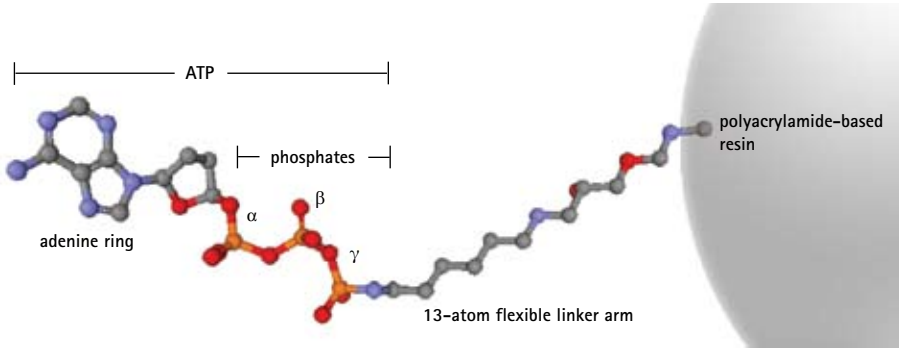
β-strands (numbered) are shown in cyan (•) and α-helices (lettered) in red (•) and yellow (•). An ATP analog is shown in ball and stick representation (black). The dashed gray line indicates the portion of the A-loop that is disordered (residues 1155–1171). Adapted from Till, J. H., Ablooglu, A. J., Frankel, M., Bishop, S. M., Kohanski, R. A., and Hubbard, S. R. (2001) *J. Biol. Chem.* **276**, 10049–10055, with permission from the American Society for Biochemistry and Molecular Biology.



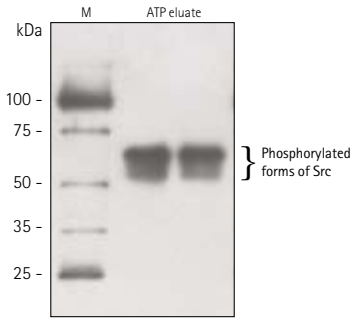
Band	Assignment	Function
1	Ca-ATPase, guanylate cyclase precursor	ATPase, nucleotide binding
2	Protein kinase splicing protein, guanylate cyclase	Protein kinase, nucleotide binding
3	Actinin, NOSIII, xylosyl transferase	Scaffolding protein, ATPase, nucleotide binding
4	ErbB kinase, DNA topoisomerase, HSP 86	Protein kinase, nucleotide binding, ATPase
5	HSP 70	ATPase
6	Chaperonin s3, Ser/Thr kinase 10	Nucleotide binding, protein kinase
7	Calreticulin	Activator ATPase
8	Protein disulfide isomerase	ATP binder
9	Actin-β	ATP binder
10	Actin-γ fragment	ATP binder
11	60S ribosomal protein L6	Related to histone H1
12	14-3-3 Protein (θ)	Scaffolding protein
13	14-3-3 Protein (ζ)	Scaffolding protein
14	Hemoglobin	Respiratory protein (very abundant)

Identification of enriched proteins from rat spleen

A rat spleen homogenate was processed with the ProteoEnrich™ ATP-Binders™ Kit. The crude dialyzed extract and the ATP eluate were subjected to SDS-PAGE and stained with colloidal Coomassie blue. The indicated bands from the ATP eluate were excised from the gel and washed several times with 100–250 µl of 50 mM ammonium hydrogen carbonate in 30% acetonitrile until completely colorless. These samples were digested with trypsin using the ProteoExtract® All-in-One Trypsin Digestion Kit according to the standard protocol and analyzed by reverse phase liquid chromatography (RPLC) combined with tandem mass spectrometry. Assignments of numbered bands are shown in the table.



Structure of ATP-Binders Resin



Detection of phosphorylated Src

Cell extract from the A431 cell line was prepared by the standard PhosphoSafe™ Extraction Reagent protocol and dialyzed to remove endogenous ATP. Dialyzed extract was processed according to the standard ProteoEnrich ATP-Binders Kit protocol. Duplicate extract samples (5 µg) were separated on SDS-PAGE (4–20% gradient gel). After transfer to nitrocellulose, phosphorylated Src was detected with a 1:500 dilution of primary antibody for rabbit anti-human Src, followed by an Anti-Rabbit IgG, H & L Chain Specific (Goat) Peroxidase Conjugate. Detection was by chemiluminescence using SuperSignal® HRP Substrate. (Cat. No. 69059)

Cation Exchange and Size-exclusion Chromatography Kits

Highly specific, reproducible, convenient methods to reduce proteome complexity

The ProteoEnrich™ CAT-X and CAT-X SEC Kits provide highly specific, reproducible, and convenient methods to reduce proteome complexity, maximizing the opportunity to visualize low-abundance proteins in a sample. The total charge of the protein, or the combination of the charge and the molecular weight, enable separation by cation exchange or cation exchange with size exclusion chromatography, and after a desalting step, samples are ready for mass spectrometry, SELDI, SDS-PAGE, and array-based analyses.

The ProteoEnrich CAT-X Kit enables separation of basic and neutral proteins that bind to a strong cation exchange resin, Fractogel® EMD SO_3^-, by reversible electrostatic interactions. The ProteoEnrich CAT-X SEC Kit enables separation of proteins and polypeptides by combining the capabilities of cation exchange with size exclusion chromatography, retaining biomolecules with a globular size of less than 20 kDa.

Each of these two kits contains two resin-packed cartridges, two Luer Lock Adaptors, and optimized buffers. A cartridge can be used with a syringe or as a liquid chromatography device. Cartridges also can be connected in series (up to three cartridges) for larger sample volumes, and can be reused at least 10 times.

NEW ProteoEnrich™ CAT-X Kit

The ProteoEnrich CAT-X Kit provides a convenient and reproducible method to fractionate the proteome of a given biological sample under non-denaturing conditions prior to downstream analysis by one- and two-dimensional gel electrophoresis, liquid chromatography, protein arrays, functional tests, such as activity assays and ELISA. The ProteoEnrich CAT-X Kit procedure fractionates proteins based on their binding to the strong cation exchange resin, Fractogel EMD SO_3^-. Nearly the complete proteome binds to the matrix under slightly acidic conditions, and partial proteomes can be eluted with a salt gradient. Excess salt should be removed prior to 2D gel electrophoresis. The kit contains two CAT-X cartridges with Luer Lock Adaptors, and optimized buffers. The CAT-X cartridges can be used manually with a syringe or as liquid chromatography devices. Each cartridge binds up to 140 mg of a single protein or up to 5 mg of a complex protein mixture and can be reused at least 10 times.

Product	Size	Cat. No.	Price
NEW ProteoEnrich™ CAT-X Kit	1 kit	71532-3	
NEW ProteoEnrich™ CAT-X SEC Kit	1 kit	71539-3	

Components

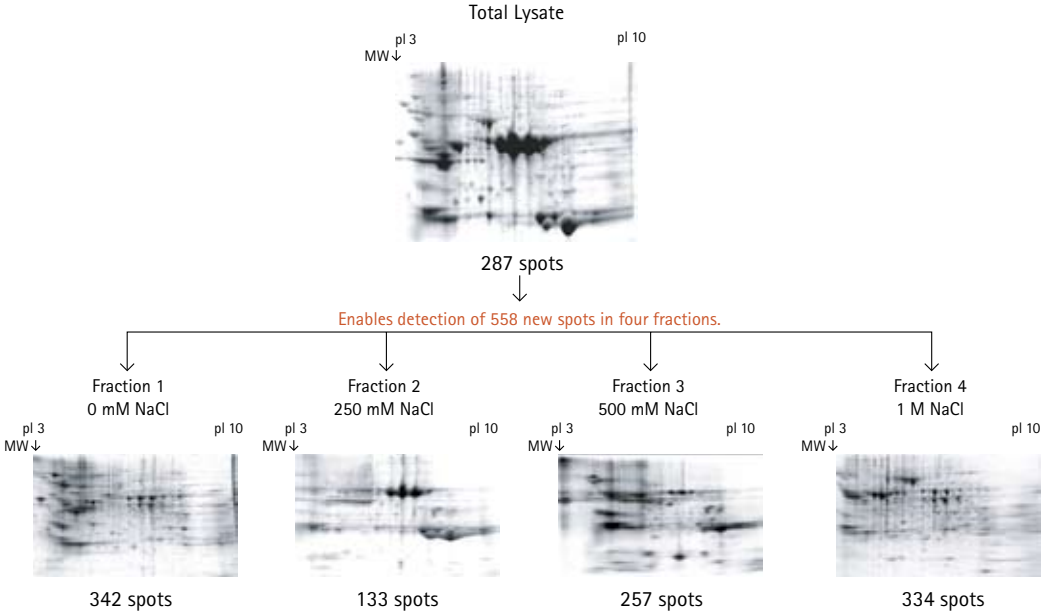
Cat. No. 71532

• 2	CAT-X Cartridge
• 50 ml	10X CAT-X Buffer
• 90 ml	5 M NaCl
• 1 pkg	Luer Lock Adaptors

Cat. No. 71539

• 2	CAT-X SEC Cartridge
• 90 ml	10X CAT-X SEC Bind/Wash Buffer
• 90 ml	CAT-X SEC Elute Buffer
• 3 ml	BSA, 100 mg/ml
• 1 pkg	Luer Lock Adaptors

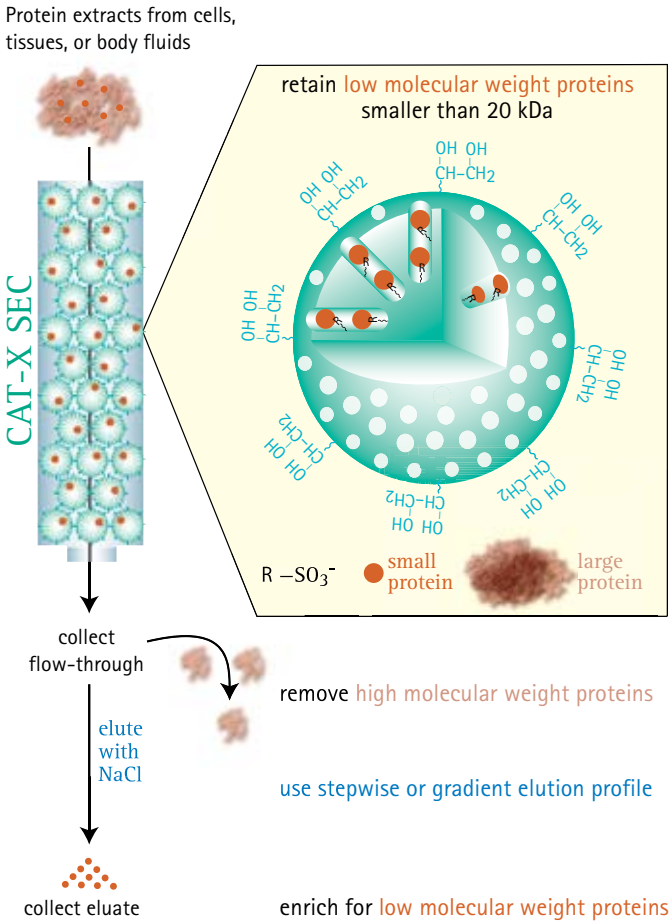
Fractionation of pig pancreatic extract using the ProteoEnrich CAT-X Kit



For more information or to place an order, contact your local office (see back cover).

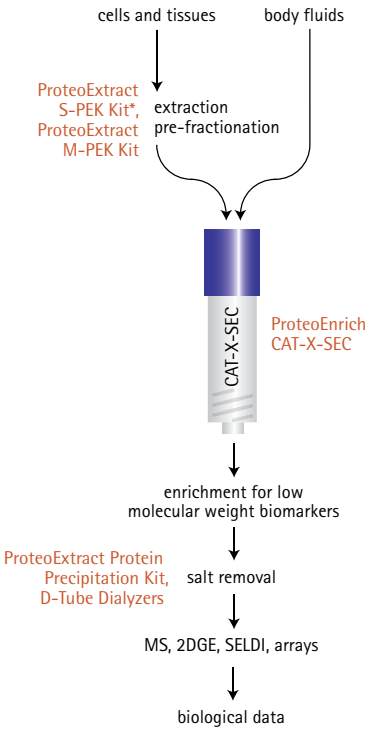
NEW ProteoEnrich™ CAT-X SEC Kit

The ProteoEnrich™ CAT-X SEC Kit provides a highly specific multidimensional chromatography method to enrich the proteome of a biological sample, such as body fluids and crude tissue extracts, for low molecular weight proteins. The method is based on a unique resin that allows proteins and polypeptides with a globular size of less than 20 kDa to penetrate the resin pores and bind to the surface of the inner pores according to their net charge, while larger proteins flow through the resin-filled cartridge. After a salt gradient or single step elution and excess salt removal, the sample is ready for downstream analysis by mass spectrometry, SELDI, or array-based analysis. The kit contains two CAT-X SEC cartridges with Luer Lock Adaptors and optimized buffers. The cartridges can be used manually with a syringe or as liquid chromatography devices. Each cartridge binds up to 5 mg complex protein mixture and can be reused at least 10 times.



CAT-X-SEC multidimensional chromatography resin

Ion-exchange and size-exclusion chromatography separate smaller biomolecules from larger, more abundant ones. The inset shows the porous external hydrophilic surface, which allows efficient exclusion of high molecular weight proteins, and an internal, cation exchange surface, which retains low molecular weight proteins by electrostatic interactions.



Processing samples for acquision of biological data

2DGE: two-dimensional gel eletrophoresis; M-PEK: Native Membrane Protein Extraction Kit; MS: mass spectrometry; SEC: size exclusion chromatography; SELDI: surface enhanced laser desorption ionization; S-PEK; Subcellular Proteome Extraction Kit

* Before the cytoskeletal fraction from an S-PEK extraction is applied to a CAT-X SEC cartridge, perform a salt removal step (ProteoExtract® Protein Precipitation Kit or D-Tube™ Dialyzers)

ProteoExtract® Phosphopeptide Capture Kit

Specific capture of phosphorylated peptides combined with high yields

calbiochem.com/proteoextract

The ProteoExtract® Phosphopeptide Capture Kit is a tool for the analysis of the phosphoproteome. The kit enables the user to isolate both specifically and quantitatively phosphorylated peptides derived from cleaved or digested protein samples or kinase reactions designed for phosphorylation site identification. Utilizing the specific interaction of phosphate groups with immobilized zirconium ions on the surface of MagPrep® PhosphoBind magnetic particles, phosphopeptides are captured and highly purified for identification by LC-MS or MALDI-MS. This kit provides sufficient reagents for isolating 100 samples of up to 2.5 nanomoles of phosphopeptide derived from digests of gels bands or isolated proteins.

Product	Size	Cat. No.	Price
ProteoExtract® Phosphopeptide Capture Kit	1 kit	525250	

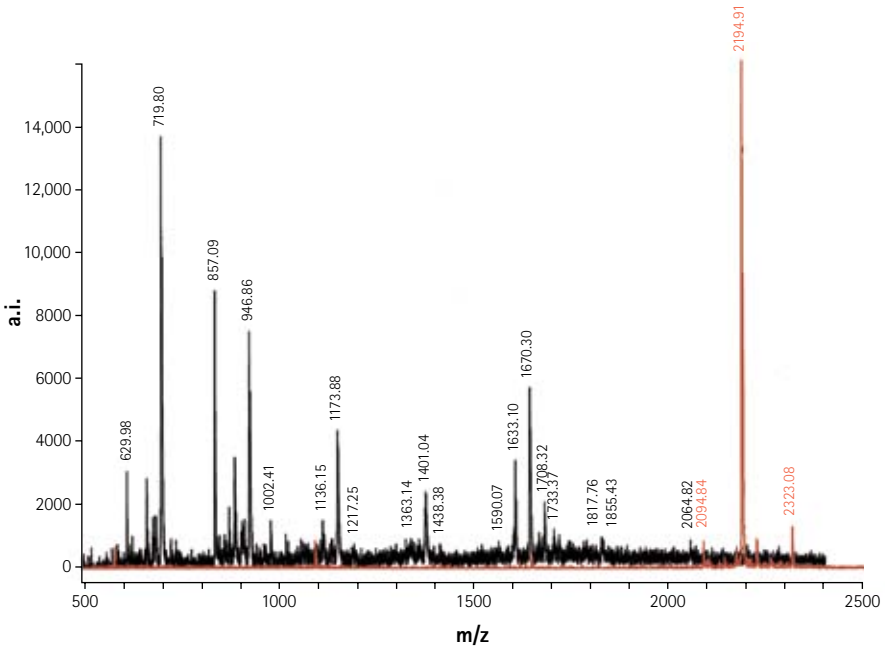
Components

Cat. No. 525250

- 25 ml Phospho Binding buffer
- 28 ml Wash buffer 1
- 28 ml Wash buffer 2 (buffer containing acetonitrile)
- 14 ml Elution buffer
- 6 ml MagPrep PhosphoBind (black particle suspension)

Features

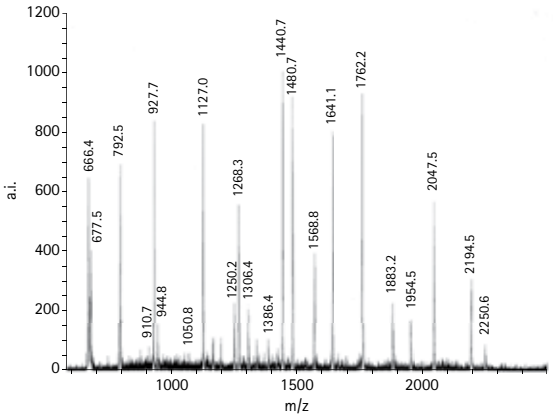
- Isolate phosphorylated species from complex mixtures in just 30 minutes
- Specific, high-affinity capture
- Scalable, adaptable to various sample quantities
- Solid phase extraction (SPE) for desalting not required
- Compatible with LC/ESI- and MALDI-MS platforms



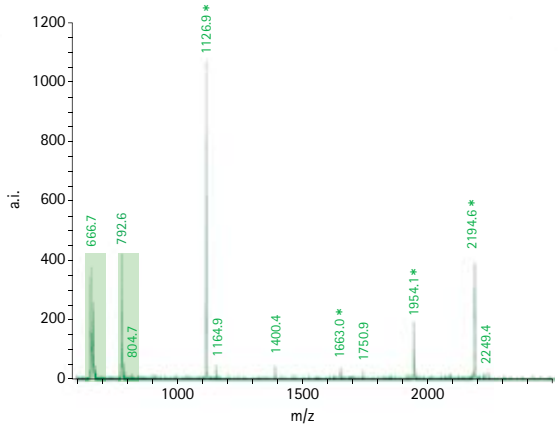
Specific isolation of phosphopeptides for MALDI-MS

A mixture of various peptides spiked with low amounts of one monophosphate peptide (M.W. 2194 Da) was processed according to the protocol of the ProteoExtract Phosphopeptide Capture Kit. The unprocessed sample (black) as well as the elution fraction (red) were directly applied on the MALDI target. The resulting peak pattern shows a single signal corresponding to the 2194-Da peptide in the elution fraction.

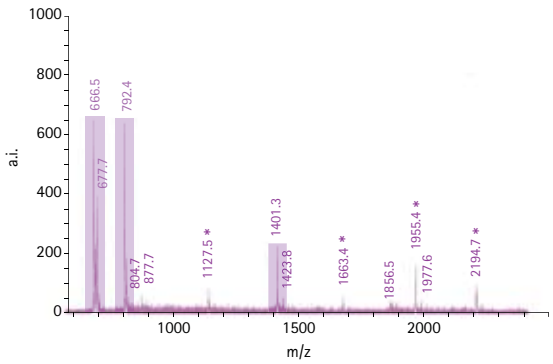
A. Unprocessed sample



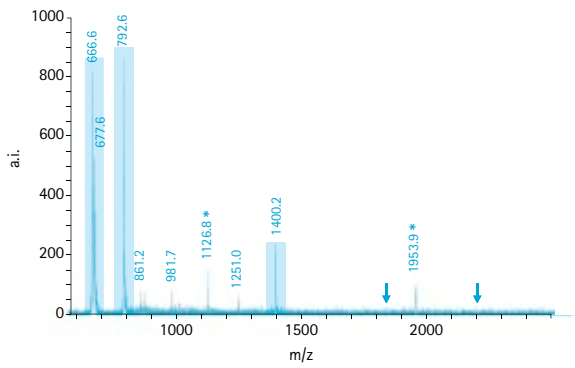
B. ProteoExtract® Phosphopeptide Capture Kit



C. Kit from Supplier I



D. Kit from Supplier II



MALDI-MS spectra from various phosphopeptide isolation methods

BSA, casein alpha, and histone type IIB1 were mixed, digested with trypsin, and supplemented with a serine-phosphopeptide and a tyrosine-phosphopeptide. Analysis was performed on a MALDI-TOF instrument in linear mode, positive ion selection, with 4-hydroxy-α-cyanocinnamic acid as the matrix. Resulting spectra show phosphopeptide ions, identified by asterisks (*), and contaminating peptides, identified by shading. Panel A shows an unprocessed sample. Panel B shows a sample processed using the ProteoExtract® Phosphopeptide Capture Kit, which detected four phosphopeptides. Panel C shows a sample processed using a kit from Supplier I, which also detected four phosphopeptides, but at reduced signal intensity and with contaminating non-phosphorylated peptides. Panel D shows a sample processed using a kit from Supplier II that detected only two of the phosphopeptides (positions of the missing phosphopeptide ions are marked by arrows); the most intense peaks represent contaminating non-phosphorylated peptides.

NEW ProteoExtract® Phosphoproteome Profiler Kit

Selectively enrich for phosphorylated peptides to measure changes in phosphoproteome profiles

www. calbiochem.com/proteoextract

The ProteoExtract® Phosphoproteome Profiler Kit provides a method to determine the protein phosphorylation patterns that result from external and internal signaling, which is accomplished by selectively enriching for phosphorylated peptides through affinity purification with MagPrep® PhosphoBind particles. This method is optimized for monitoring signal transduction from receptor to the nuclear response, but also is an optimal means for measuring the response to a drug treatment (e.g., comparing a new pharmaceutical with unknown function to ones with known functions). This procedure also can be used to measure the effect of overexpression or gene silencing on a signal transduction pathway. Each kit contains all reagents and components needed for 25 reactions, which include protein precipitation, trypsin digestion, and enrichment for phosphopeptides.

Product	Size	Cat. No.	Price
NEW ProteoExtract® Phosphoproteome Profiler Kit	1 kit	539181	

Components

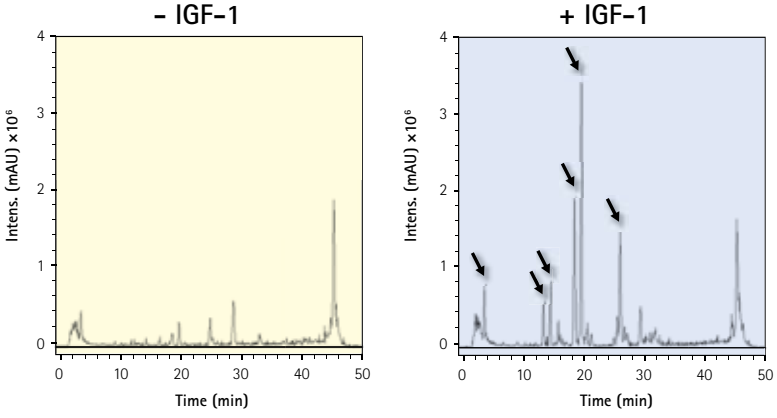
Cat. No. 539181

• 2.5 ml	Protein Precipitation Reagent
• 5 ml	Digest Buffer Solvent (buffer solution containing Acetonitrile)
• 1 vial	Digest Buffer (dry powder)
• 1 vial	Reducing Agent (lyophilisate)
• 1 vial	Blocking Agent (lyophilisate)
• 50 µg	Trypsin (lyophilisate)
• 1.3 ml	MagPrep® PhosphoBind (black particle suspension)
• 12.5 ml	Phospho Binding Buffer
• 5 ml	Wash Buffer I
• 5 ml	Wash Buffer II
• 1.0 ml	Elution Buffer

Features

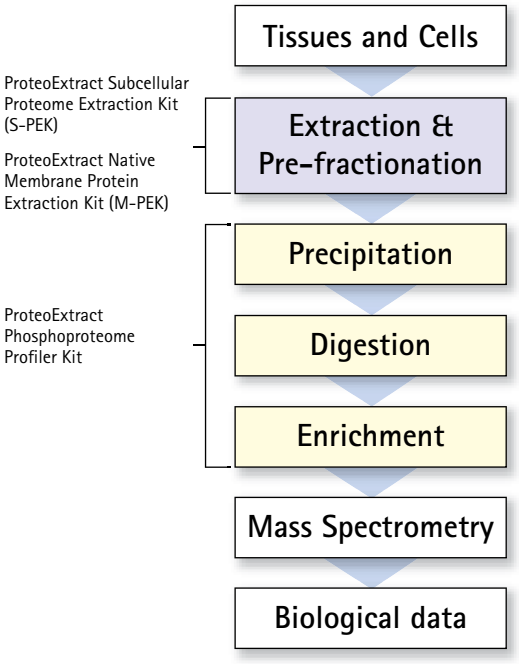
- Includes all reagents necessary for protein precipitation, trypsin digestion, and phosphopeptide enrichment
- Specific zirconium-based magnetic particles selectively enrich for phosphopeptides providing increased signal-to-noise ratios in MS
- Provides high yield of tryptic peptides to improve LS-MS peak patterns and sequence coverage
- Fractions compatible for direct use in MALDI and ESI-LC/MS analysis

Related application note on page 83



Phosphopeptide signal patterns of MCF-7 cells (membrane fraction) induced by IGF-1

MCF-7 human breast carcinoma cells were seeded at 3 x 10^6 cells per 75 cm^2 flask in RPMI 1640. Cells were serum-starved for 24 h and treated for 5 min with 100 ng/ml IGF-1. Membrane fractions were isolated from treated and untreated cells using the ProteoExtract Native Membrane Protein Extraction Kit (M-PEK). Each protein fraction (100 µg) was precipitated, digested with trypsin, and phosphopeptide enriched (40 µl) using the ProteoExtract Phosphoproteome Profiler Kit. Phosphopeptide-enriched fractions were analyzed by ESI LC/MS. Results were analyzed with Matrix Science's Mascot® software and MSDB protein database. Arrows indicate IGF-induced phosphorylation of specific peptides.



ProteoExtract® Phosphoproteome Profiler method

Cells are processed to give subcellular fractions using the ProteoExtract Subcellular Proteome Extraction Kit (S-PEK) or the ProteoExtract Native Membrane Protein Extraction Kit (M-PEK). The resulting fractions are digested with trypsin and enriched for phosphopeptides using the ProteoExtract Phosphoproteome Profiler Kit prior to mass spectometry (MS) analysis.

ProteoExtract® All-in-One Trypsin Digestion Kit

High yield of tryptic peptides from many sample types

calbiochem.com/proteoextract

The ProteoExtract® All-in-One Trypsin Digestion Kit contains a set of optimized reagents that allows tryptic digests of various protein sample types: spots or bands excised from polyacrylamide gels, protein solutions, or cell and tissue extracts. The kit includes affinity-purified trypsin, which guarantees efficient digestion of protein samples regardless of their origin. High digestion efficiency results in a high yield of tryptic peptides—even with hard-to-digest proteins—leading to improved LC-MS peak patterns and sequence coverage after MS analysis. This allows a comprehensive analysis of post-translational modifications such as phosphorylation site identification. Kit contains reagents sufficient for 100 reactions in samples containing up to 4 mg/ml protein. Includes Extraction Buffers, Extraction Buffer Solvent, Digest Buffer Reagent, Digest Buffer Reducing Agent, Blocking Agent, Trypsin, and Wash Buffer.

Product	Size	Cat. No.	Price
ProteoExtract® All-in-One Trypsin Digestion Kit	1 kit	650212	

Components

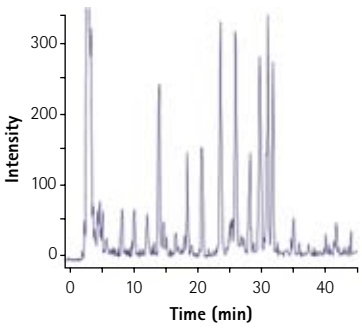
Cat. No. 650212

- 1 vial — Extraction Buffer 1
- 10 ml — E-Buffer Solvent 1 (buffer containing acetonitrile)
- 28 ml — Extraction Buffer 2 (buffer containing acetonitrile)
- 1 vial — D-Buffer Reagent (lyophilisate)
- 3 ml — Digest Buffer (buffer containing acetonitrile)
- 1 vial — Reducing agent (lyophilisate)
- 1 vial — Blocking agent (lyophilisate)
- 2 × 50 µg — Trypsin (lyophilisate)
- 28 ml — Wash buffer (buffer containing acetonitrile)

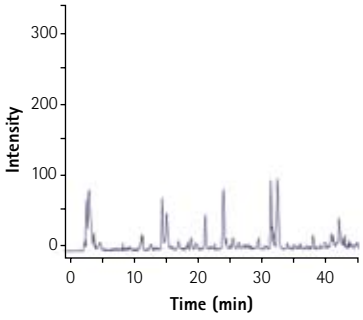
Features

- Compatible with a variety of protein sample types
- Digests proteins in gels or in solution
- Fast procedure, complete digestion in 3 hours
- 20–30% increase in sequence coverage

A. ProteoExtract® All-in-One Trypsin Digestion Kit

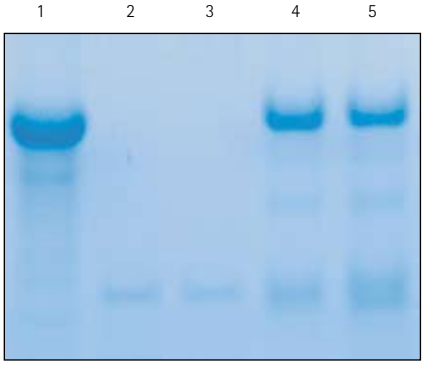


B. Kit from Supplier I



Enhanced detection allows comprehensive analysis

Ovalbumin was digested using ProteoExtract All-in-One Trypsin Digestion Kit (A) or according to Supplier I kit (B) protocols. Base peak chromatograms of nano-LC/MS analyses are shown.



Improved digestion of hard-to-digest proteins

Ovalbumin and ovalbumin digests were analyzed as follows: lane 1: ovalbumin, chicken egg, 43 kDa; lane 2: digest incubated 3 h using ProteoExtract All-in-One Trypsin Digestion Kit; lane 3: digest incubated 4 h using the same kit; lane 4: digest incubated 3 h using reagent from Supplier I; lane 5: digest incubated 3 h using kit from Supplier II.

NEW ProteoExtract® Tryptic Cleavage Modification Kit

Efficient trypsin, Arg-C- and Lys-C-like digestion of proteins for improved peptide profiling by MS

www. calbiochem.com/proteoextract

Proteolytic digestion of proteins is an important step for subsequent protein characterization and identification by mass-spectrometry (MS). Traditionally trypsin is the protease of choice and is used for its efficiency, substrate specificity, and reliability. To facilitate a detailed protein sequence characterization, digestion usually is repeated with different enzymes such as Arg-C, Lys-C, Glu-C proteases, etc. However, the efficiency, specificity, and reliability of these enzymes is by far inferior to that of trypsin. The ProteoExtract® Tryptic Cleavage Modification Kit is designed to utilize affinity purified trypsin in the ProteoExtract All-in-One Trypsin Digestion Kit and additionally to control its cleavage-site usage by specific chemical modification steps resulting in either trypsin, Arg-C-, or Lys-C-like digested peptide fragments for MS/MS analysis. Each kit contains the reagents needed for 100 reactions.

Product	Size	Cat. No.	Price
NEW ProteoExtract® Tryptic Cleavage Modification Kit	1 kit	539182	

Components

Cat. No. 539182

- 40 mg Lys Modifier (lyophilisate)
- 1.1 ml Lys Modifier Diluent
- 1 ml Arg Modifier 1
- 1 ml Arg Modifier 2

Features

- Specific chemical modification to control the trypsin cleavage site during in-gel digestion
- Modification efficiency typically >90%, depending on protein structure and composition
- Improved LC-MS peak patterns and sequence coverage
- Amenable for MS/MS and ESI-LC-MS/MS techniques

A

Query	Observed	Mr(expt)	Mr(calc)	Delta	Score	Rank	Peptide
14	395.29	783.57	783.66	0.10	13	1	LVTDLTK
18	424.23	846.45	846.50	-0.05	21	2	LSQKFPK
21	461.75	921.49	921.49	0.00	42	1	AEFVEVTK
23	464.26	926.51	926.49	0.02	33	1	YLYEIAR
28	495.53	989.05	989.55	-0.27	13	7	EKVLTSSAR
30	501.08	1001.71	1001.58	0.13	29	1	LVVSTQTALA
32	507.83	1013.65	1013.61	0.03	21	2	QTALVELLK
45	571.92	1141.83	1141.71	0.12	26	1	KQTALVELLK
88	582.32	1162.63	1162.62	0.00	41	1	LVNELTEFAK
90	587.43	1162.85	1162.59	0.25	32	1	DTHKSEIAHR
97	417.39	1248.88	1248.61	0.26	37	1	FKDLGEEHFK
75	473.88	1418.62	1418.69	-0.07	14	3	SLHTLFGDELCK + Carbamidomethyl (C)
78	720.39	1438.77	1438.80	-0.04	23	1	RHPEYAVSVLLR
87	511.84	1531.88	1531.77	0.12	12	8	LKECCDKPLLEK + 2 Carbamidomethyl (C)
83	784.50	1566.99	1566.74	0.25	49	1	DAFLGSFLYEYSR
103	547.58	1639.66	1639.93	-0.73	40	1	KVPQVSTPTLVEVSR
110	883.54	1724.07	1723.83	0.24	22	1	MPCTEDYLSLILNR + Carbamidomethyl (C)
122	630.55	1888.63	1887.92	0.71	21	1	HPYFYAPELLYYANK
130	682.38	2044.15	2044.02	0.13	28	1	RHPYFYAPELLYYANK
148	829.80	2486.38	2486.10	0.28	14	1	YNGVFQECCQAEDKGACLLPK + 3 Carbamidomethyl (C)

B

Mr	Dev.	Range	Sequence
724.3	-0.2	[581-587]	CCAADDK
757.4	0.5	[198-204]	GACLLPK + Carbamidomethyl (C)
817.4	0.2	[562-568]	ATEEQLK
885.4	0.5	[131-138]	DDSPDLPK
921.5	-0.1	[249-256]	AEFVEVTK
973.5	0.1	[37-44]	DLGEEHFK
991.5	0.3	[229-235]	FQERALK + Arg Modifier (1+2)
1001.6	-0.4	[598-607]	LVVSTQTALA
1013.6	0.1	[549-557]	QTALVELLK
1071.5	0.4	[310-318]	SHCIAEVEK + Carbamidomethyl (C)
1077.5	0.5	[205-211]	IETMREK + Arg Modifier (1+2)
1158.6	-0.7	[29-36]	SEIAHRFK + Arg Modifier (1+2)
1176.6	-0.0	[300-309]	ECCDKPLLEK
1293.7	0.7	[246-256]	FPKAEFVEVTK
1304.7	0.1	[402-412]	HLVDEPQNLIK
1398.7	0.3	[569-580]	TVMENFVAFVDK
1418.7	0.6	[89-100]	SLHTLFGDELCK + Carbamidomethyl (C)
1442.6	0.2	[286-297]	YICDNQDTISSK + Carbamidomethyl (C)
1501.6	-0.4	[375-386]	EYEATLEECCAK + 2 Carbamidomethyl (C)
1516.9	-0.9	[236-248]	AWSVARLSQKFPK
1575.8	-0.1	[139-151]	LKPDPNTLCDEFK + Carbamidomethyl (C)
1615.7	0.7	[118-130]	QEPERNECFLSHK
1759.9	-0.2	[198-211]	GACLLPKIETMREK + Arg Modifier (1+2)
2003.8	-0.6	[101-117]	VASLRETYGDMADCCEK + 2 Carbamidomethyl (C)
2235.0	0.3	[569-587]	TVMENFVAFVDKCCAADDK + 2 Carbamidomethyl (C), Oxidation (M)
2372.1	-0.4	[184-204]	YNGVFQECCQAEDKGACLLPK + Carbamidomethyl (C)
2371.2	0.5	[421-437]	LGEYGFQNALIVRYTRK + 2 Arg Modifier (1+2)
2493.2	-0.2	[66-88]	LVNELTEFAKTCVADESHAGCEK
2800.7	0.9	[545-568]	QIKKQTALVELLKHKPKATEEQLK
2829.3	-0.3	[131-155]	DDSPDLPKLKPDPNTLCDEFKADEK
2828.3	0.7	[499-523]	CCTESLVNRRPCFSALTPDETYVPK
2980.2	0.9	[375-399]	EYEATLEECCAKDDPHACYSTVFDK + 2 Carbamidomethyl (C)

C

Mr	Dev.	Range	Sequence
1164.5	-0.6	[460-468]	CCTKPESER + Carbamidomethyl (C), Lys Modifier
1248.6	0.1	[25-34]	DTHKSEIAHR + Lys Modifier
1250.6	0.1	[223-232]	CASIQKFGER + Carbamidomethyl (C), Lys Modifier
1438.8	0.1	[360-371]	RHPEYAVSVLLR
1462.8	0.1	[221-232]	LRCASIQKFGER + Lys Modifier
1638.9	0.2	[437-451]	KVPQVSTPTLVEVSR
1723.8	0.4	[469-482]	MPCTEDYLSLILNR + Carbamidomethyl (C)
1746.9	-0.0	[219-232]	QRLRCASIQKFGER + Lys Modifier
2289.2	-0.1	[223-241]	CASIQKFGERALKAWSVAR + Carbamidomethyl (C), 2 Lys Modifier

Assay Characteristics and Examples

BSA was digested using the ProteoExtract® All-in-One Trypsin Digestion Kit alone (A) and in combination with the Tryptic Cleavage Modification Kit according to the detailed protocols provided (B: Lys C-like) and (C: Arg C-like). Digests were analyzed by LC-MS/MS. The mass spectrometer was operated in the positive ion mode, peak width of less than 0.6 u, m/z range of 100 - 3000. The peptides were fragmented using auto-MS/MS, active exclusion, and two precursor ions. Protein identifications were made using the Matrix Science Mascot® algorithm. With these methods it is possible to generate either trypsin, Lys-C- or Arg-C -like peptide fragments leading to increased sequence coverage and improved MS profiling to facilitate protein identification.

PopCulture® Reagent

Protein extraction from *E. coli* cultures directly in the growth medium

PopCulture® Reagent* is a detergent-based concentrate that can be added directly to cultures of *E. coli* to effectively extract proteins without the need for cell harvest. Recombinant proteins can be directly screened in the crude extract or purified by adding an affinity matrix, washing the matrix-target protein complex to remove spent culture medium and cellular contaminants, and eluting the purified protein from the matrix. The entire culturing, extraction, and purification process can be performed in the original culture tube or multiwell plate. This "in-media" protein screening or purification procedure can be adapted to high-throughput robotic processing of samples for proteomics research and any application that would benefit from the increased speed and convenience it provides. Successful purification of intact fusion proteins from total culture extracts has been demonstrated using His•Bind® and GST•Bind™ Resins (Grabski 2001). The use of His•Mag™ or GST•Mag™ Agarose Beads enables the entire procedure to be carried out in a single tube without the need for columns or centrifugation. Addition of rLysozyme™ Solution or the use of pLysS hosts increases the efficiency of protein extraction with the procedure. Benzonase® Nuclease may also be added to reduce the viscosity of the extract.

PopCulture Reagent is supplied as a ready-to-use Tris-buffered liquid concentrate that is stable at room temperature.

Features

- No separation of cells from culture medium
- No mechanical disruption of cells
- No clarification of cell extracts prior to purification
- Direct affinity adsorption of target proteins to resin from the total culture extract
- Ability to rapidly perform the entire cell growth and purification process in a single tube or well

PopCulture® Purification Kits

PopCulture Reagent is available bundled with His•Mag and GST•Mag Agarose Beads and corresponding buffers, plus rLysozyme Solution, for convenient extraction and affinity purification using magnetic separation. These kits enable processing of 40 × 3–ml cultures with yields up to 375 µg His•Tag® or up to 150 µg GST•Tag™ fusion protein per 3 ml culture, based on bead binding capacity. For 96-well processing using PopCulture, please refer to RoboPop™ Purification Kits later in this section.

Reference:

Grabski, A. C., et al. 2001. *inNovations* **13**, 1.

* patent pending

Product	Size	Cat. No.	Price
PopCulture® Reagent	15 ml	71092-3	
	75 ml	71092-4	
	250 ml	71092-5	
PopCulture® GST•Mag™ Purification Kit	40 rxn	71113-3	
PopCulture® His•Mag™ Purification Kit	40 rxn	71114-3	
Benzonase® Nuclease, Purity >90%	2.5 KU	70746-4	
	10 KU	70746-3	
rLysozyme™ Solution	300 KU	71110-3	
	1200 KU	71110-4	
	6000 KU	71110-5	

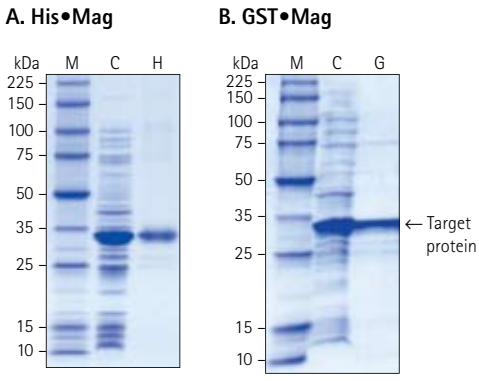
Components

Cat. No. 71113	
• 15 ml	PopCulture Reagent
• 3 × 1 ml	GST•Mag Agarose Beads
• 2 × 100 ml	10X GST•Bind™/Wash Buffer
• 40 ml	10X Glutathione Reconstitution Buffer
• 1 g	Glutathione, Reduced
• 300 KU	rLysozyme Solution
• 1 ml	rLysozyme Dilution Buffer
Cat. No. 71114	
• 15 ml	PopCulture Reagent
• 3 × 1 ml	His•Mag Agarose Beads
• 80 ml	8X Binding Buffer
• 2 × 25 ml	8X Wash Buffer
• 50 ml	4X Elute Buffer
• 300 KU	rLysozyme Solution
• 1 ml	rLysozyme Dilution Buffer

Note: 1 KU = 1000 units

Custom configurations BULK

PopCulture Reagent, rLysozyme Solution, GST•Mag Agarose Beads, and His•Mag Agarose Beads can be custom packaged in larger quantities to suit your needs. Call 800 854 2855 for details.



Lane	Sample
M	Perfect Protein™ Markers (10–225 kDa)
C	Crude extract (standard protocol)
H	PopCulture + His•Mag Agarose
G	PopCulture + GST•Mag Agarose

PopCulture His•Mag™ and GST•Mag™ purification

Induced cultures of *E. coli* strain BL21(DE3) containing pET-41b(+), which encodes a 35.6-kDa GST•Tag™/His•Tag® fusion protein, were processed using PopCulture Reagent and either His•Mag (panel A) or GST•Mag (panel B) Agarose Beads. Samples of a crude extract prepared with BugBuster® Reagent and the purified proteins were analyzed by SDS-PAGE and Coomassie blue staining.

RoboPop™ Solubility Screening Kit

Expression and solubility screening in a 96-well format

The RoboPop™ Solubility Screening Kit is designed for protein expression–level and solubility screening in a 96-well format and includes all reagents and plates necessary to perform parallel processing of 96 bacterial cultures. The kit contains PopCulture® Reagent and Lysonase™ Bioprocessing Reagent for efficient extraction of recombinant proteins from *E. coli* directly from the culture medium without cell harvest, mechanical disruption, or extract clarification. Lysonase significantly increases protein extraction efficiency and reduces sample viscosity, facilitating downstream processing and robotic pipetting.

The RoboPop Solubility Screening Kit also incorporates an innovative filtration plate capable of retaining insoluble inclusion bodies while allowing soluble proteins to be collected for rapid quantitation and analysis. Insoluble proteins retained by the filtration plate are solubilized with 4% SDS, collected, and quantified separately. The Filter Plate is compatible with standard vacuum manifolds for manual processing and the entire protocol has been validated for robotic sample processing with the Genesis® Freedom™ Workstation from Tecan and the MultiPROBE® II liquid handling work station from PerkinElmer Life Sciences. Two collection plates with sealers are provided for convenient storage of separated soluble and insoluble fractions.

Product	Size	Cat. No.	Price
RoboPop™ Solubility Screening Kit	1 plate	71255-3	
Solubility Screening 96-Well Filter Plate	1 plate	71258-3	
FRETWorks™ S•Tag™ Assay Kit	100 assays 1000 assays	70724-3 70724-4	
PopCulture® Reagent	15 ml 75 ml 250 ml	71092-3 71092-4 71092-5	
Lysonase™ Bioprocessing Reagent	0.2 ml 1 ml 5 ml	71230-3 71230-4 71230-5	
Overnight Express Instant TB Medium	1 × 60 g 5 × 60 g 1 kg	71491-3 71491-4 71491-5	

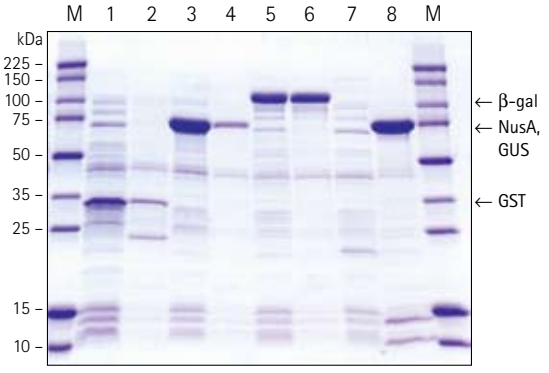
Components

Cat. No. 71255

- 15 ml PopCulture Reagent
- 0.2 ml Lysonase Bioprocessing Reagent
- 1 Solubility Screening 96-Well Filter Plate
- 20 ml 4% SDS
- 2 Collection Plate and Sealer

Related application note on page 102

A. SDS-PAGE analysis of soluble and insoluble fractions



Lane	Sample
M	Perfect Protein™ Markers, 10–225 kDa
1	GST, soluble
2	GST, insoluble
3	NusA, soluble
4	NusA, insoluble
5	β-gal, soluble
6	β-gal, insoluble
7	GUS, soluble
8	GUS, insoluble

B. FRETWorks™ S•Tag™ Assay of soluble and insoluble fractions

Construct	Fraction	pmol/ml	µg/ml	% in fraction
GST	soluble	116	209	65.1
GST	insoluble	62.2	112	34.9
NusA	soluble	33.2	121	74.7
NusA	insoluble	11.2	41	25.3
β-gal	soluble	33.7	202	44.7
β-gal	insoluble	41.7	250	55.3
GUS	soluble	1.0	3	0.7
GUS	insoluble	140	477	99.3

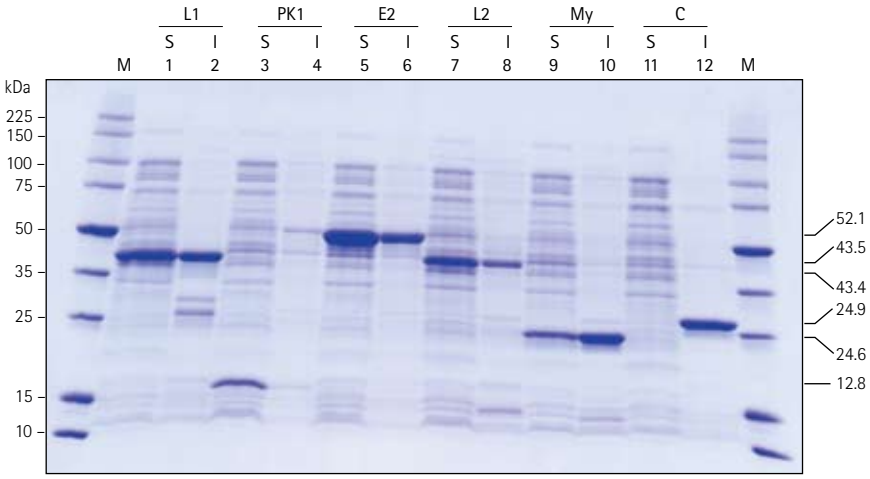
Solubility screening using four different vector constructs

Cultures of *E. coli* strain BL21(DE3) containing four separate pET vector constructs were incubated at 37°C to an OD_{600} = 1.5 and target protein expression was induced by adding IPTG to a final concentration of 1 mM. Following further incubation for approximately 3 h at 30°C (final OD_{600} = 5–6), the cultures were dispensed (1 ml/well) into sequential rows of 2-ml 96-well plates, and 100 µl PopCulture Reagent, containing 2 µl Lysonase Reagent, was added to each well. Soluble and insoluble protein fractions were generated according to the RoboPop Solubility Screening Protocol. Panel A shows SDS-PAGE analysis (Coomassie blue staining) of the indicated samples (15 µl extract). Panel B shows the results of the FRETWorks™ S•Tag™ Assay performed with the same fractions. For the assay, samples were serially diluted 1:25 to 1:2500, and the dilutions analyzed according to the standard protocol (20 µl diluted sample was used per assay). The S•Tag fusion proteins were quantified with a standard curve based on known amounts of S•Tag Standard.

A. FRETWorks™ S•Tag™ assays of soluble and insoluble fractions

	% in Fraction	
pET-30 Ek/LIC Construct	Soluble	Insoluble
Lipocortin I	51	49
PK1 alpha	74	26
Enolase 2	58	42
Lipocortin II	54	46
Myosin regulatory light chain 2	45	55
Casein alpha	34	66

B. SDS-PAGE analysis of soluble and insoluble fractions



Lane	Sample
M	Perfect Protein™ Markers, 10–225 kDa
L1	Lipocortin 1
PK1	Protein kinase inhibitor alpha
E2	Enolase 2
L2	Lipocortin II
My	Myosin regulatory light chain 2
C	Casein alpha
S	Soluble
I	Insoluble

Automated solubility screening using the RoboPop™ Kit and Tecan Genesis® Freedom™ Workstation

Cultures of *E. coli* strain BL21(DE3) containing the vector constructs listed in the table above were incubated at 30°C and target protein expression was induced by Overnight Express™ Autoinduction System 1. Following further incubation for approximately 16 h at 30°C, the cultures were processed robotically according to the RoboPop Solubility Screening protocol. Panel A shows the results of the FRETWorks™ S•Tag™ Assay performed with the same fractions. Panel B shows SDS-PAGE analysis (Coomassie blue staining) of the indicated samples (15 µl extract).

RoboPop™ Magnetic Purification Kits

PopCulture® extraction and His•Mag™ or GST•Mag™ purification in a 96-well format

The RoboPop™ Purification Kits are designed for 96-well format purification of soluble His•Tag® and GST•Tag™ fusion proteins directly from *E. coli* cultures without harvesting cells. The kits feature PopCulture® Reagent for extraction of proteins from total cultures without the need for centrifugation, and His•Mag™ or GST•Mag™ Agarose Beads for high-capacity magnetic affinity purification. The combination of PopCulture and magnetic agarose beads enables the entire procedure to be carried out in a single culture plate.

The kits contain one 96-well Deep Well Culture Plate (2-ml wells) with air-permeable sealing membranes for bacterial cell growth and protein purification, and one 96-well Collection Plate (450-µl wells) with an air-tight aluminum foil sealer for storage of the purified proteins. rLysozyme™ Solution, Benzonase® Nuclease, and purification buffers are also included.

The Culture Plate is compatible with the Novagen Magnetight™ HT96™ Separation Stand, which is recommended for efficient processing of magnetic affinity supports in deep-well plates.

The RoboPop His•Mag and GST•Mag Purification Kits will purify up to 12 mg of His•Tag fusion proteins per plate (up to 125 µg/well) or up to 4.8 mg of GST•Tag fusion proteins per plate (up to 50 µg/well). Stated yields are based on 1-ml cultures and binding capacities of the beads, and will vary with the folding properties, expression levels, and solubility of individual fusion proteins.

Product	Size	Cat. No.	Price
RoboPop™ His•Mag™ Purification Kit	1 plate	71103-3	
RoboPop™ GST•Mag™ Purification Kit	1 plate	71102-3	
Magnetight™ HT96™ Stand	1 each	71101-3	

Components

Cat. No. 71103

• 15 ml	PopCulture Reagent
• 1	Sterile 96-well Deep Well Culture Plate with Sealers (3)
• 1	Collection Plate with Sealer
• 3 × 1 ml	His•Mag Agarose Beads
• 80 ml	8X Binding Buffer
• 2 × 25 ml	8X Wash Buffer
• 2 × 25 ml	4X Elute Buffer
• 300 KU	rLysozyme™ Solution
• 1 ml	rLysozyme Dilution Buffer
• 2.5 KU	Benzonase® Nuclease, Purity > 90%

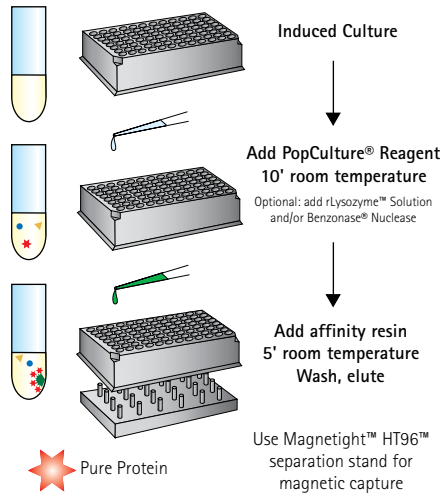
Cat. No. 71102

• 15 ml	PopCulture Reagent
• 1	Sterile 96-well Deep Well Culture Plate with Sealers (3)
• 1	Collection Plate with Sealer
• 3 × 1 ml	GST•Mag Agarose Beads
• 2 × 100 ml	10X GST•Bind/Wash Buffer
• 40 ml	10X Glutathione Reconstitution Buffer
• 1 g	Reduced Glutathione
• 300 KU	rLysozyme Solution
• 1 ml	rLysozyme Dilution Buffer
• 2.5 KU	Benzonase Nuclease, Purity > 90%

RoboPop Magnetic Purification Kits

Product	Culture scale	Processing method	Capacity*
RoboPop His•Mag Purification Kit	96 × 1 ml	Magnetic	125 µg/1 ml culture
RoboPop GST•Mag Purification Kit	96 × 1 ml	Magnetic	50 µg/1 ml culture
RoboPop Ni-NTA His•Bind® Purification Kit	96 × 5 ml	Filtration	1 mg/5 ml culture
RoboPop GST•Bind™ Purification Kit	96 × 5 ml	Filtration	0.8 mg/5 ml culture

* Capacities are based on 1- or 5-ml cultures and binding capacities of the resins. Yields will vary with the expression levels, folding properties, and solubility of individual fusion proteins.



RoboPop™ Filtration-based Purification Kits

High-throughput, milligram-scale purification of His•Tag® and GST•Tag™ fusion proteins

The RoboPop™ Ni-NTA His•Bind® and GST•Bind™ Purification Kits are designed for high-throughput (HT) purification of soluble His•Tag® and GST•Tag™ fusion proteins directly from *E. coli* cultures. Like the corresponding RoboPop His•Mag™ and GST•Mag™ Kits (Grabski 2001), the RoboPop Kits feature PopCulture® Reagent, rLysozyme™ Solution, and Benzonase® Nuclease for centrifugation-free cell lysis and extract preparation in one step. However, these kits are designed for larger-scale cultures (up to 5 ml) and include Ni-NTA His•Bind or GST•Bind Resin plus a filtration-based processing protocol. Magnetic-based GST•Mag and His•Mag Kits purify up to 50–125 µg target protein per 1 ml culture; filtration-based kits purify up to 1 mg His•Tag fusion protein or 0.8 mg GST•Tag fusion protein per well (see table on page 50).

Bacterial culture, cell lysis, and resin binding steps are carried out in standard 24-well plates (not supplied), which accommodate a maximum volume of 5 ml per well. The reaction slurry is then transferred to a 96-well Filter Plate (included) and the washing and elution steps are carried out on a vacuum manifold. The Filter Plate is compatible with standard filter manifolds for manual sample processing, and the entire purification has been validated for robotic sample processing with the MultiPROBE® II liquid handling workstation from PerkinElmer Life Sciences and with the Genesis® Freedom™ workstation from Tecan. A 96-well Collection Plate (1-ml wells) with an air-tight aluminum foil sealer is provided for storage of the purified proteins.

Reference:

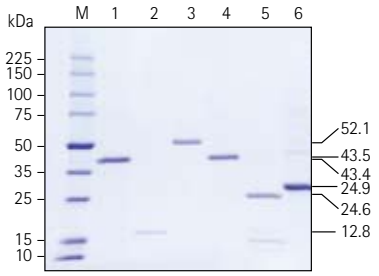
Grabski, A. C., et al. 2001. *inNovations* **14**, 2.

Product	Size	Cat. No.	Price
RoboPop™ Ni-NTA His•Bind® Purification Kit	1 kit	71188-3	
RoboPop™ GST•Bind™ Purification Kit	1 kit	71189-3	

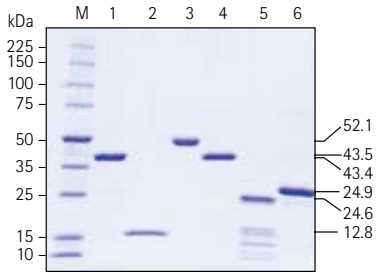
Components

Cat. No. 71188-3:	
• 75 ml	PopCulture Reagent
• 25 ml	Ni-NTA His•Bind Resin
• 125 ml	4X Ni-NTA Bind Buffer
• 2 x 125 ml	4X Ni-NTA Wash Buffer
• 50 ml	4X Ni-NTA Elute Buffer
• 1	2-ml 96-well Filter Plate
• 1	1-ml 96-well Collection Plate with Sealer
• 300 KU	rLysozyme Solution
• 1 ml	rLysozyme Dilution Buffer
• 10 KU	Benzonase® Nuclease, Purity > 90%
Cat. No. 71189-3	
• 75 ml	PopCulture Reagent
• 25 ml	GST•Bind Resin
• 100 ml	10X GST•Bind/Wash Buffer
• 1 g	Glutathione, reduced
• 40 ml	10X Glutathione Reconstitution Buffer
• 1	2-ml 96-well Filter Plate
• 1	1-ml 96-well Collection Plate with Sealer
• 300 KU	rLysozyme Solution
• 1 ml	rLysozyme Dilution Buffer
• 10 KU	Benzonase Nuclease, Purity > 90%

A. Magnetic-based affinity purification



B. Filtration-based affinity purification



Automated purification of His•Tag® fusion proteins

Cultures of *E. coli* strain BL21(DE3) containing the vector constructs described in the table above were grown at 30°C for16 h with target protein expression induced by Overnight Express™ Autoinduction System 1. The cultures were processed robotically (Tecan Genesis® 200) according to the RoboPop purification protocols. Panel A (approximately 1 µg protein load) and panel B (approximately 2 µg protein load) show purified protein samples analyzed by SDS-PAGE (10–20% gradient gel with Coomassie blue staining). Protein assays were performed by the Bradford method and purity was determined by densitometry of the scanned gel. Lane M: Perfect Protein™ Markers, 10–225 kDa.

RoboPop Filtration-based Purification Kits

			Magnetic (His•Mag Agarose Beads)		Filtration (Ni-NTA His•Bind Resin)	
Lane	Target protein	Expected size (kDa)	Yield (µg/ml culture)	% Purity	Yield (µg/ml culture)	% Purity
1	Lipocortin I	43.5	61	> 98	61	> 98
2	Protein kinase inhibitor alpha	12.8	26	> 98	20	> 98
3	Enolase	52.1	40	> 98	146	> 98
4	Lipocortin II	43.4	47	> 98	36	> 98
5	Myosin regulatory light chain 2	24.6	56	90	35	72
6	Casein alpha	24.9	122	> 98	54	> 98

Insect PopCulture® Reagent

Protein extraction directly from insect cell cultures

Insect PopCulture® Reagent is a detergent-based lysis reagent specifically formulated for total insect cell culture extraction without the need for centrifugation. The improved method recovers both protein released into the medium and intracellular protein, increasing processing efficiency and target protein yields (Loomis 2002). It is amenable for automated expression-level screening and is fully compatible with the Ni-NTA His•Bind® affinity purification method. Insect PopCulture reagent can be used for protein extraction from insect cells grown in suspension and adherent cells grown on tissue culture plates.

Features

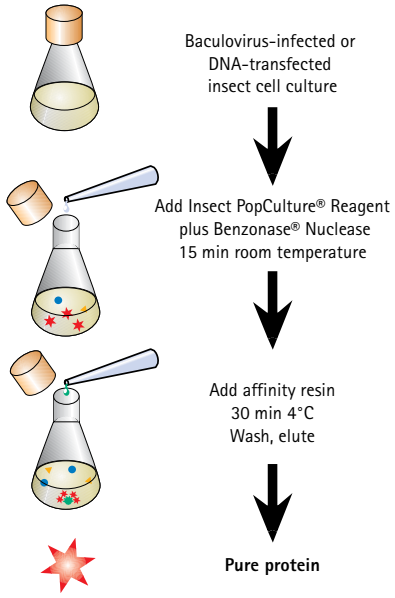
- No need to separate cells from culture media
- No need to clarify cell extracts prior to purification
- Higher protein yield due to target protein recovery from both medium and cells
- Direct affinity adsorption of target proteins to IMAC affinity resin from the total culture extract
- Compatible with protease inhibitor cocktails
- Ideal for high-throughput, expression-level screening and protein purification
- Compatible with transfected and baculovirus-infected insect cell cultures

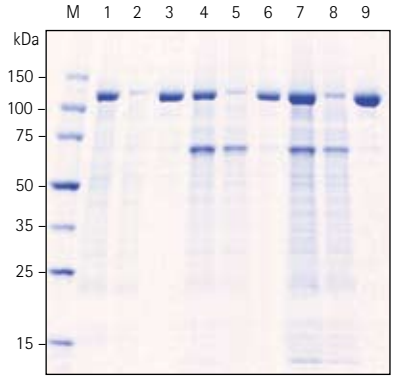
Reference:

Loomis, K., et al. 2002. *inNovations* **15**, 16.

Product	Size	Cat. No.	Price
Insect PopCulture® Reagent	50 ml 250 ml	71187-3 71187-4	
Benzonase® Nuclease, Purity > 99%	10 KU	70664-3	
Benzonase® Nuclease, Purity > 90%	2.5 KU 10 KU	70746-4 70746-3	

Note: 1 KU = 1000 units





Lane	Sample
M	Perfect Protein™ Markers
1	Cell pellet, crude
2	Cell pellet, flow-through
3	Cell pellet, eluate
4	Medium, crude
5	Medium, flow-through
6	Medium, eluate
7	Insect PopCulture, crude
8	Insect PopCulture, flow-through
9	Insect PopCulture, eluate

Insect PopCulture® Reagent

Sample	Purified protein
Cell pellet	56 µg/ml culture
Medium	64 µg/ml culture
Insect PopCulture	131 µg/ml culture

Purification of His•Tag® β-galactosidase from baculovirus-infected insect cell cultures

The bacterial β-galactosidase coding sequence, *lacZ*, was PCR amplified and cloned into the pTriEx™-4 Ek/LIC Vector. Recombinant baculoviruses were generated by cotransfection using BacVector®-3000 Triple Cut Virus DNA according to the recommended Novagen protocol. For protein expression, TriEx Sf9 cells in a shaker culture in TriEx Insect Cell Medium were infected with baculovirus at an MOI of 5. At 72 h post infection, 1 ml of the culture was used for direct in-media cell lysis with Insect PopCulture Reagent, and 1 ml was processed by standard extraction with CytoBuster™ Extraction Reagent. The His•Tag β-galactosidase fusion protein was purified with the Ni-NTA His•Bind affinity purification method. The crude extract, flow-through, and pooled eluates were analyzed by SDS-PAGE and Coomassie blue staining. Protein concentration of the pooled eluates was determined by the BCA method (see table, above right).

Insect RoboPop™ Ni-NTA His•Bind® Purification Kit

Filtration-based, 96-well format purification directly from transfected cultures of insect cells

The Insect RoboPop™ Ni-NTA His•Bind® Purification Kit is designed for filtration-based, 96-well format purification of His•Tag® fusion proteins directly from insect cell cultures. This kit includes Insect PopCulture® Reagent for protein extraction from total cultures, Benzonase® Nuclease for viscosity reduction, Ni-NTA His•Bind Resin and buffers, 2-ml 96-well Filter Plate, and Collection Plate with Sealer. The kit is configured for robotic processing of 10-ml suspension cultures and purifies up to 400 µg His•Tag fusion protein per culture based on binding capacity of the resin. Protein yields ranging from 60 to 140 µg per 10 ml culture were obtained with β-gal, Fluc, MAP kinase, and cdc2 kinase expressed in the pIEx™ transient protein expression system (Loomis 2002).

In practice, Insect PopCulture Reagent is added directly to the cell culture, followed by the addition of Benzonase Nuclease. The lysate is transferred to a tube of appropriate size or deep-well plate and Ni-NTA His•Bind resin is added. After mixing, the lysate-resin slurry is transferred to the Filter Plate, and washing and elution steps are carried out on a vacuum manifold. The Filter Plate is compatible with standard vacuum manifolds for either manual or automated processing, and the entire purification has been validated for robotic sample processing with the MultiPROBE® II liquid handling workstation from PerkinElmer Life Sciences and with the Genesis® Freedom™ workstation from Tecan.

Also use the Insect RoboPop Ni-NTA His•Bind Purification Kit with centrifugation when a vacuum manifold is not available.

Product	Size	Cat. No.	Price
Insect RoboPop™ Ni-NTA His•Bind® Purification Kit	1 plate	71257-3	

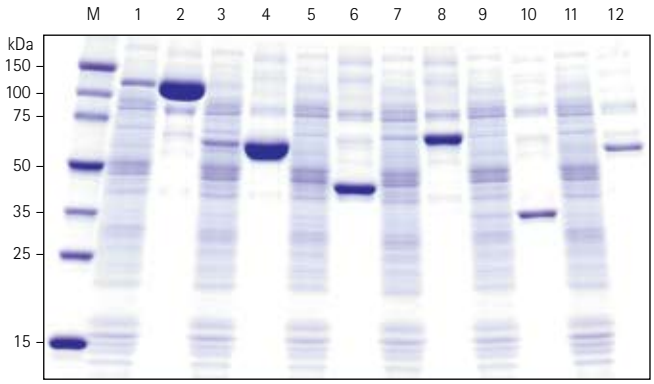
Components

• 50 ml	Insect PopCulture Reagent
• 10 KU	Benzonase Nuclease
• 10 ml	Ni-NTA His•Bind Resin
• 125 ml	4X Ni-NTA Bind Buffer
• 2 × 125 ml	4X Ni-NTA Wash Buffer
• 50 ml	4X Ni-NTA Elute Buffer
• 1	2-ml 96-well Filter Plate
• 1	Collection Plate with Sealer

Note: 1 KU = 1000 units

Reference:

Loomis, K., et al. 2002. *inNovations* **15**, 16.



Lane	Sample	Protein yield (µg)
M	Perfect Protein™ Markers, 15–150 kDa	
1	pIEx-1/β-gal, total cell protein	
2	pIEx-1/β-gal, purified	137
3	pIEx-1/Fluc, total cell protein	
4	pIEx-1/Fluc, purified	123
5	pIEx-1/MAP kinase, total cell protein	
6	pIEx-1/MAP kinase, purified	91
7	pIEx-2/MAP kinase, total cell protein	
8	pIEx-2/MAP kinase, purified	89
9	pIEx-1/cdc2 kinase, total cell protein	
10	pIEx-1/cdc2 kinase, purified	63
11	pIEx-2/cdc2 kinase, total cell protein	
12	pIEx-2/cdc2 kinase, purified	63

Target protein expression levels and purification from transfected Sf9 cells

Sf9 cells in 10-ml suspension cultures (1 × 10^6 cells/ml) were transfected with 20 µg of the indicated pIEx™ recombinants using Insect GeneJuice® Transfection Reagent. Total culture extracts were prepared 48 h later by the addition of Insect PopCulture Reagent (500 µl) followed by the addition of Benzonase Nuclease (5 µl). Samples were taken at this point to represent the total cell protein. Ni-NTA His•Bind® Resin (50 µl per culture) was then added to the extracts. Samples were processed robotically using a Genesis® Freedom™ workstation from Tecan. Target protein was eluted in a volume of 150 µl. 10 µl crude and purified fractions were loaded in adjacent lanes of a 10–20% SDS polyacrylamide gel, which was stained with Coomassie blue. Purified protein yields were determined by BCA assay.

Benzonase® Nuclease

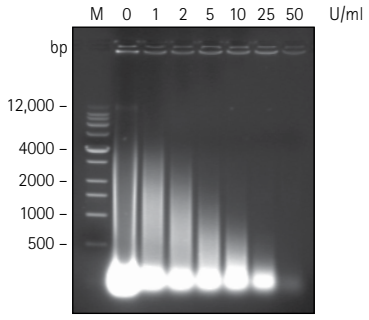
Effective viscosity reduction by removing nucleic acids from protein solutions

Benzonase® Nuclease is a genetically engineered endonuclease from *Serratia marcescens*. It degrades all forms of DNA and RNA (single-stranded, double-stranded, linear, and circular) while having no proteolytic activity. It is effective over a wide range of conditions and possesses an exceptionally high specific activity. The enzyme completely digests nucleic acids to 5′-monophosphate terminated oligonucleotides 2 to 5 bases in length (below the hybridization limit), which is ideal for removal of nucleic acids from recombinant proteins, enabling compliance with FDA guidelines for nucleic acid contamination. The ability of Benzonase to rapidly hydrolyze nucleic acids makes the enzyme an excellent choice for viscosity reduction, to reduce processing time and to increase yields of protein. For example, the enzyme is compatible with BugBuster® and PopCulture® Protein Extraction Reagents and can be added along with these reagents to eliminate viscosity by removing nucleic acids from *E. coli* extracts.

The enzyme consists of two subunits of 30 kDa each. It is functional between pH 6 and 10 and from 0-42°C and requires 1–2 mM Mg^{2+} for activation. The enzyme is also active in the presence of ionic and non-ionic detergents, reducing agents, PMSF (1 mM), EDTA (1 mM) and urea (relative activity depends on specific conditions). Activity is inhibited by >150 mM monovalent cations, >100 mM phosphate, >100 mM ammonium sulfate, or >100 mM guanidine hydrochloride. Benzonase Nuclease is available in ultrapure (> 99% by SDS-PAGE) and pure (>90%) grades at a standard concentration of 25 U/µl and at a high concentration (HC) of 250 U/µl. Both preparations are free of detectable protease and have specific activity >1 × 10^6 U/mg protein. The >99% purity grade is tested for endotoxins and contains <0.25 EU/1000 units. The product is supplied as a 0.2-µm filtered solution in 50% glycerol. Store at –20°C.

Unit definition: one unit is defined as the amount of enzyme that causes a ΔA_{260} of 1.0 in 30 minutes, which corresponds to complete digestion of 37 µg DNA.

Product	Size	Cat. No.	Price
Benzonase® Nuclease, Purity >99%	10 KU	70664-3	
Benzonase® Nuclease HC, Purity >99%	25 KU	71206-3	
Benzonase® Nuclease, Purity >90%	2.5 KU 10 KU	70746-4 70746-3	
Benzonase® Nuclease HC, Purity >90%	25 KU	71205-3	



Nucleic acid digestion by Benzonase

E. coli BL21(DE3) cells containing a pET construct were suspended in BugBuster Reagent (5 ml/g wet weight). Samples of the suspension were treated with the indicated amounts of Benzonase for 30 min at room temperature. Samples were clarified by centrifugation and analyzed by agarose gel electrophoresis and ethidium bromide staining.

Viscosity reduction by Benzonase

E. coli BL21(DE3) cells containing a pET construct were suspended in BugBuster Reagent (5 ml/g wet weight). Samples of the suspension were treated with the indicated amounts of Benzonase for 10 min at room temperature, centrifuged at 350 × g for 3 min and photographed.

Protease Inhibitor Cocktails

Protective inhibitor mixtures prevent proteolysis during purification

calbiochem.com/inhibitorcocktails

Protease inhibitor mixtures (cocktails) contain highly specific inhibitors, which are important tools for proteomic studies such as biomarker discovery, quantitative measurement of proteins, mapping of post-translational modifications, and protein expression, characterization, and profiling. Adding a protease inhibitor cocktail (or protease inhibitor) ensures the integrity of proteins for downstream analysis and further characterization. Endogenous proteases rapidly begin to degrade protein samples after cell lysis or tissue disaggregation. To prevent proteolysis, which can drastically reduce the protein sample quality and quantity that is required for downstream analysis, complex protein solutions are treated with a mixture of protease inhibitors to help preserve the activity and nature of proteins. Specific combinations help protect samples from the most common proteases: serine proteases, metalloproteases, cysteine proteases, aminopeptidases, and aspartic proteases. Our high-quality cocktails provide reproducible inhibition over a range of protease classes and offer:

- Convenient, ready-to-use or ready-to-reconstitute vials
- Protection for most tissue or cell type extracts from mammalian, bacterial, yeast, fungal, and plant cells
- Formulations without EDTA for compatibility with metal ion chelating chromatography and 2D gel electrophoresis
- Recombinant aprotinin to meet animal-free requirements

Properties of Selected Protease Inhibitors

Protease Inhibitor	MW	Target Protease Class and Mechanism of Action	Solubility	Suggested Concentration
AEBSF, Hydrochloride	239.5	Water soluble, non-toxic alternative to PMSF. Irreversible inhibitor of serine proteases. Reacts covalently with a component of the active site. Inhibits chymotrypsin, kalikrein, plasmin, trypsin, and related thrombolytic enzymes.	H_2O	0.1–1 mM
ALLN	383.5	Inhibits plasmin, thrombin, calpain I (K_i = 190 nM), calpain II (K_i = 220 nM), Cathepsin B (K_i = 150 nM), and cathepsin L (K_i = 600 pM).	Methanol, Ethanol, DMSO	0.2–2 µM
Aprotinin, Bovine Lung; Aprotinin, Recombinant	6512	A serine protease inhibitor that acts as a competitive and reversible inhibitor of proteolytic and esterolytic activity. In cell cultures, extends the life of cells and prevents proteolytic damage to intact cells.	H_2O	0.6–2.0 µg/ml
Bestatin	308.4	Binds to cell surfaces and inhibits cell surface aminopeptidases, notably aminopeptidase B and leucine aminopeptidase. Activates macrophages and T lymphocytes. Has antitumor properties.	Methanol	1–10 µM
Cathepsin Inhibitor I	475.5	Selectively inhibits cathepsin B ($k_2/K_i = 6.9 \times 10^3$ M^{-1} sec^{-1}), cathepsin L ($k_2/K_i = 3.1 \times 10^5$ M^{-1} sec^{-1}), cathepsin S ($k_2/K_i = 6.6 \times 10^4$ M^{-1} sec^{-1}), and papain ($k_2/K_i = 1.8 \times 10^3$ M^{-1} sec^{-1}).	DMSO, Ethanol	100–200 µM
E-64 Protease Inhibitor	351.4	An irreversible cysteine protease inhibitor that has no action on cysteine residues in other proteins. Specific active site titrant.	H_2O, DMSO	1–10 µM
EDTA, Disodium Salt, Molecular Biology Grade	372.2	A reversible metalloprotease inhibitor. A chelator that may interfere with other metal ion-dependent biological processes.	H_2O	1–10 µM
Elastatinal	512.6	A competitive inhibitor of elastase (K_i = 240 nM).	H_2O, DMSO, Ethanol	0.5–2 µg/ml
EST	342.4	A membrane-permeable calpain inhibitor.	Ethanol	20–50 µg/ml
GGACK	392.8	An irreversible inhibitor of Urokinase (IC_{50} <1 mM).	H_2O	1–10 µM
Leupeptin, Hemisulfate	475.6	A reversible inhibitor of trypsin-like proteases and cysteine proteases, including endoproteinase Lys-C, papain, cathepsin B, trypsin, kallikrein, and thrombin.	H_2O	10–100 µM
Pepstatin A	685.9	A reversible inhibitor of aspartic protease. Inhibits cathepsin D, cathepsin G, pepsin, and renin.	DMSO, Methanol	~ 1 µM
o-Phenanthroline	198.2	A metalloprotease inhibitor.	DMSO, Ethanol, H_2O	
Phosphoramidon, Disodium Salt	587.5	A highly specific inhibitor of thermolysin. Also inhibits the conversion of big endothelin-1 to endothelin-1.	H_2O, DMSO, Methanol	1–10 µM
TLCK, Hydrochloride	369.3	An irreversible inhibitor of trypsin-like serine proteases. Inactivates trypsin, specifically and irreversible. Does not have any significant inhibitory effect on chymotrypsin.	H_2O	10–100 µM
TPCK	351.1	An irreversible inhibitor of chymotrypsin. Useful for inhibiting chymotrypsin activity in trypsin preparations.	Ethanol, Methanol	10–100 µM

More online... www.calbiochem.com/proteaseinhibitors

Protease Inhibitor Cocktails *continued*

Selection Guide for the Use of Specialized Protease Inhibitor Cocktails

Product	Cat. No.	Recommended Application
Protease Inhibitor Cocktail Set I	539131	General use
NEW Protease Inhibitor Cocktail Set I, Animal-Free	535142	General use and for applications that require the use of animal-free reagents
Protease Inhibitor Cocktail Set II	539132	Bacterial cell extracts (except those intended for metal chelation chromatography)
Protease Inhibitor Cocktail Set III	539134	Bacterial cell extracts being used for metal chelation chromotography, mammalian cell and tissue extracts
NEW Protease Inhibitor Cocktail Set III, Animal-Free	535140	Bacterial cell extracts being used for metal chelation chromotography, mammalian cell and tissue extracts that require the use of animal-free reagents
Protease Inhibitor Cocktail Set IV	539136	Fungal and yeast cell extracts
Protease Inhibitor Cocktail Set V, EDTA Free	539137	Mammalian cell and tissue extracts purified using metal chelation chromatography; samples to be analyzed by 2-D gel electrophoresis
NEW Protease Inhibitor Cocktail Set V, Animal-Free	535141	Mammalian cell and tissue extracts for applications that require the use of animal-free reagents
Protease Inhibitor Cocktail Set VI	539133	Plant cell extracts
Protease Inhibitor Cocktail Set VII	539138	Purification of proteins containing His•Tag® sequences
Protease Inhibitor Cocktail Set VIII	539129	Broad range cysteine protease inhibition
Serine Protease Inhibitor Cocktail Set I	565000	Broad range serine protease inhibition
Protease Inhibitor Set	539128	Seven individual inhibitors for easy economical screening

Protease Inhibitors Cocktails

Protection against proteolysis during purification

calbiochem.com/inhibitorcocktails

Protease Inhibitor Cocktail Set I (with EDTA)

Protease Inhibitor Cocktail Set I is a specially formulated mixture of five protease inhibitors with broad specificity for the inhibition of various proteases and esterases. It is provided as a lyophilized solid, ready for reconstitution, and is available in either of two configurations: as a single vial or as a set of 10 vials. Each vial, when reconstituted with 1 ml of water, makes 1 ml of 100X stock solution.

Each reconstituted vial of Protease Inhibitor Cocktail Set I contains 50 mM AEBSF-HCl, 15 µM Aprotinin, 0.1 mM E-64, 50 mM EDTA, and 0.1 mM Leupeptin Hemisulfate. Note that the presence of EDTA may interfere with purification of His•Tag® fusion proteins if a cell extract is applied directly to IDA or Ni-NTA resins. For these applications we recommend Protease Inhibitor Cocktail Set III.

NEW Protease Inhibitor Cocktail Set I, Animal–Free

The animal-free formulation includes the same five protease inhibitors as the original version (Cat. No. 539131), but substitutes a recombinant Aprotinin (instead of Aprotinin prepared from bovine lung).

Protease Inhibitor Cocktail Set II (with EDTA)

This lyophilized cocktail includes five protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, serine, and metalloproteases, as well as aminopeptidases. It is recommended for use with bacterial cell extracts (except those being used for metal chelation chromatography). Reconstitute each vial with 1 ml DMSO and 4 ml water to obtain a 5-ml stock solution. Slight turbidity in the reconstituted solution is normal. When reconstituted, each vial contains 20 mM AEBSF-HCl, 1.7 mM Bestatin, 200 µM E-64, 85 mM EDTA, and 2 mM Pepstatin A. One set contains 1 vial of lyophilized inhibitors plus 1 vial DMSO. Five sets contain 5 vials of lyophilized inhibitors plus 5 vials DMSO, enough for 25 ml total after addition of water. Use of 5 ml is recommended for the inhibition of proteases extracted from 20 g *E. coli*.

Protease Inhibitor Cocktail Set III (EDTA-free)

This liquid cocktail includes six protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, and serine proteases, as well as aminopeptidases. It is recommended for use with bacterial cell extracts being used for metal chelation chromatography, mammalian cell and tissue extracts. Each 1 ml vial contains 100 mM AEBSF-HCl, 80 µM Aprotinin, 5 mM Bestatin, 1.5 mM E-64, 2 mM Leupeptin Hemisulfate, and 1 mM Pepstatin A as a solution in DMSO. Contains no metal chelators. Use of 1 ml is recommended for the inhibition of proteases extracted from 20 g of bovine liver or 20 g *E. coli*.

NEW Protease Inhibitor Cocktail Set III, Animal–Free (EDTA-free)

The animal-free formulation includes the same five protease inhibitors as the original version (Cat. No. 539134), but substitutes a recombinant Aprotinin (instead of Aprotinin prepared from bovine lung).

Product	Size	Cat. No.	Price
Protease Inhibitor Cocktail Set I	1 vial 10 vials	539131	
NEW Protease Inhibitor Cocktail Set I, Animal-Free	1 ml 1 set (10 × 1 ml)	535142	
Protease Inhibitor Cocktail Set II	1 set 5 set (5 × 1 ml)	539132	
Protease Inhibitor Cocktail Set III	1 ml 1 set (5 × 1 ml)	539134	
NEW Protease Inhibitor Cocktail Set III, Animal-Free	1 ml 1 set (5 × 1 ml)	535140	

Protease Inhibitor Cocktail Set I and Protease Inhibitor Cocktail Set I, Animal-Free

Inhibitor	M.W.	1X Concentration	Target protease
AEBSF, Hydrochloride	239.5	500 µM	Serine Proteases
Aprotinin, Bovine Lung or Aprotinin, Recombinant (Animal-Free)	6512	150 nM	Serine Proteases and Esterases
E-64 Protease Inhibitor	357.4	1 µM	Cysteine Proteases
EDTA, Disodium	372.2	500 µM	Metalloproteases
Leupeptin, Hemisulfate	475.6	1 µM	Cysteine Proteases and Trypsin-like Proteases

Protease Inhibitor Cocktail Set II

Inhibitor	M.W.	Concentration in the vial	Target protease
AEBSF, Hydrochloride	239.5	20 mM	Serine Proteases
Bestatin	308.4	1.7 mM	Aminopeptidase B and Leucine Aminopeptidase
E-64 Protease Inhibitor	357.4	200 µM	Cysteine Proteases
EDTA, Disodium	372.2	85 mM	Metalloproteases
Pepstatin A	685.9	2 mM	Aspartic Proteases

Protease Inhibitor Cocktail Set III and Protease Inhibitor Cocktail Set III Animal-Free

Inhibitor	M.W.	1X Concentration	Target protease
AEBSF, Hydrochloride	239.5	500 µM	Serine Proteases
Aprotinin, Bovine Lung or Aprotinin, Recombinant (Animal-Free)	6512	150 nM	Serine Proteases and Esterases
E-64 Protease Inhibitor	357.4	1 µM	Cysteine Proteases
Leupeptin, Hemisulfate	475.6	1 µM	Cysteine Proteases and Trypsin-like Proteases
Pepstatin A	685.9	1 mM	Aspartic proteases

Protease Inhibitor Cocktails *continued*

Protease Inhibitor Cocktail Set IV (EDTA-free)

This liquid cocktail includes four protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, metallo-, and serine proteases. It is recommended for fungal and yeast cell extracts. One set includes five 1-ml vials. Each 1-ml vial contains 100 mM AEBSF-HCl, 1.5 mM E-64, 2 mM Pepstatin A, and 500 mM 1,10-Phenanthroline as a solution in DMSO.

Protease Inhibitor Cocktail Set V, EDTA-Free

This cocktail includes four protease inhibitors for the inhibition of serine and cysteine proteases, but not metalloproteases. Reconstitute each vial with 1 ml H_2O to obtain 1 ml of 100X concentrated stock solution. A 1X stock solution contains 500 µM AEBSF-HCl, 150 nM Aprotinin, 1 µM E-64, and 1 µM Leupeptin Hemisulfate. **Note:** this product is hygroscopic.

NEW Protease Inhibitor Cocktail Set V Animal-Free (EDTA-free)

The animal-free formulation includes the same four protease inhibitors as the original version (Cat. No. 535137), but substitutes a recombinant Aprotinin (instead of Aprotinin prepared from bovine lung).

Protease Inhibitor Cocktail Set VI, EDTA-Free

This cocktail is recommended for use with plant cell extracts. Cocktail contains six protease inhibitors (in 1 ml DMSO) with broad specificity for the inhibition of aspartic, cysteine, metallo- and serine proteases, as well as aminopeptidases. Each vial contains the concentrations of inhibitors shown in the table. One ml is recommended for about 30 g of various plant tissues.

Product	Size	Cat. No.	Price
Protease Inhibitor Cocktail Set IV	1 ml 1 set (5 × 1 ml)	539136	
Protease Inhibitor Cocktail Set V, EDTA-free	10 vials	539137	
NEW Protease Inhibitor Cocktail Set V, Animal-Free	1 ml 1 set (5 × 1 ml)	535141	
Protease Inhibitor Cocktail Set VI	1 ml 1 set (5 × 1 ml)	539133	

Protease Inhibitor Cocktail Set IV

Inhibitor	M.W.	Concentration after reconstitution	Target protease
AEBSF, Hydrochloride	239.5	100 mM	Serine Proteases
E-64	357.4	1.5 mM	Cysteine Proteases
Pepstatin A	685.9	2 mM	Aspartic Proteases
o-Phenanth-roline	198.2	500 mM	Metalloproteases

Protease Inhibitor Cocktail Set V, EDTA-Free and Protease Inhibitor Cocktail Set V, Animal-Free

Inhibitor	M.W.	1X concentration	Target protease
AEBSF, Hydrochloride	239.5	500 µM	Serine Proteases
Aprotinin, Bovine Lung or Aprotinin, Recombinant (Animal-Free)	6512	150 nM	Broad Spectrum, Serin Proteases
E-64	357.4	1 µM	Cysteine Proteases
Leupeptin Hemisulfate	475.6	1 µM	Cysteine Proteases and Trypsin-like Proteases

Protease Inhibitor Cocktail Set VI

Inhibitor	M.W.	Concentration in the vial	Target protease
AEBSF, Hydrochloride	239.5	200 mM	Serine Proteases
Bestatin	308.4	10 mM	Aminopeptidase B and Leucine Aminopeptidase
E-64 Protease Inhibitor	357.4	3 mM	Cysteine Proteases
Leupeptin, Hemisulfate	475.6	2 mM	Cysteine Proteases and Trypsin-like Proteases
o-Phenanth-roline	198.2	500 mM	Metalloproteases
Pepstatin A	685.9	2 mM	Aspartic Proteases

Protease Inhibitor Cocktail Set VII (EDTA-free)

This cocktail is recommended for purification of proteins containing His•Tag® sequences. Cocktail contains five protease inhibitors (in 1 ml DMSO) with broad specificity for the inhibition of cysteine, serine, aspartic, and thermolysin-like proteases and aminopeptidases. Each vial contains the concentrations of inhibitors shown in the table on the right. One ml is sufficient for about 10 g cells.

Protease Inhibitor Cocktail Set VIII (EDTA-free)

A DMSO solution of three protease inhibitors with selective specificity for the inhibition of cysteine proteases, including calpains, cathepsins, and papain. Each vial contains the concentrations of inhibitors shown in the table on the right.

Serine Protease Inhibitor Cocktail Set I (EDTA-free)

A cocktail of four protease inhibitors that is useful for the inhibition of a broad range of serine proteases. Reconstitute each vial with 1 ml H_2O to obtain a 100X stock solution. When diluted to 1X stock solution, the set will contain the concentrations of inhibitors shown in the table on the right.

Protease Inhibitor Set (EDTA-free)

This unique set, consisting of seven different inhibitors, allows you to perform easy, economical screening for a suitable inhibitor for your application.

Protease Inhibitor Set

Inhibitor	M.W.	Quantity Supplied	Target Proteases
AEBSF, Hydrochloride	239.5	50 mg	Serine Proteases
E-64 Protease Inhibitor	357.4	1 mg	Cysteine Proteases
EST	342.4	1 mg	Calpains
Leupeptin, Hemisulfate	475.6	5 mg	Cysteine Proteases and Trypsin-like Proteases
Pepstatin A	685.9	5 mg	Aspartic Proteases
TLCK, Hydrochoride	369.3	50 mg	Trypsin-like Serine Proteases
TPCK	351.1	250 mg	Chymotrypsin-like Proteases

Product	Size	Cat. No.	Price
Protease Inhibitor Cocktail Set VII	1 ml 1 set (5 × 1 ml)	539138	
Protease Inhibitor Cocktail Set VIII	1 ml 1 set (5 × 1 ml)	539129	
Serine Protease Inhibitor Cocktail Set I	1 vial 5 vials	565000	
Protease Inhibitor Set	1 set	539128	

Protease Inhibitor Cocktail Set VII

Inhibitor	M.W.	Concentration in the vial	Target Proteases
AEBSF, Hydrochloride	239.5	100 mM	Serine Proteases
Bestatin	308.4	5 mM	Aminopeptidase B and Leucine Aminopeptidase
E-64 Protease Inhibitor	357.4	1.5 mM	Cysteine Proteases
Pepstatin A	685.9	2 mM	Aspartic Proteases
Phosphoramidon, Disodium Salt	587.5	200 µM	Metalloendopeptidases

Protease Inhibitor Cocktail Set VIII

Inhibitor	M.W.	Concentration in the vial	Target Proteases
ALLN	383.5	1.56 mM	Calpain I/II, Cathepsin B, Cathepsin L, Cysteine Proteases
Cathepsin Inhibitor I	475.5	0.5 mM	Cathepsin B, Cathepsin L, Cathepsin S, Papain
E-64 Protease Inhibitor	357.4	1.5 mM	Cysteine Protease

Serine Protease Inhibitor Cocktail Set I

Inhibitor	M.W.	Concentration in the vial	Target Proteases
AEBSF, Hydrochloride	239.5	500 µM	Serine Proteases
Aprotinin, Bovine Lung	6512	420 nM	Serine Proteases and Esterases
Elastatinal	512.6	20 µM	An inhibitor of elastase-like serine protease.
GGACK	392.8	1 µM	An irreversible inhibitor of Urokinase and Factor Xa.

Selection Guide for the Use of Specialized Phosphatase Inhibitor Cocktails

calbiochem.com/inhibitorcocktails

Phosphatase Inhibitor Cocktails

Phosphorylation and dephosphorylation of proteins mediate signal transduction events that control many cellular processes such as cell division, cell proliferation, apoptosis, and others. Protein kinases phosphorylate target proteins by transfering a phosphate group to a specific protein, typically at serine, threonine, or tyrosine residues. Covalent attachment of the phosphoryl group changes protein conformation and ligand interaction capabilities. Protein phosphatases can remove phosphate groups and restore the original dephosphorylated state of the protein. During extraction from cell and tissue lysates, it is highly critical to preserve the phosphorylation state of the proteins with products like the Phosphatase Inhibitor Cocktails shown here and PhosphoSafe™ Extraction Reagent (p 25).

Product	Cat. No.	Recommended Application
Phosphatase Inhibitor Cocktail Set I	524624	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A
Phosphatase Inhibitor Cocktail Set II	524625	Protection against acid and alkaline phosphatases and Protein Tyrosine Phosphatases (PTPs)
Phosphatase Inhibitor Cocktail Set III	524627	Protection against Ser/Thr phosphatases and Protein Tyrosine Phosphatases (PTPs)
Phosphatase Inhibitor Cocktail Set IV	524628	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A
PhosphoSafe™ Extraction Reagent	71296	Protection against Ser/Thr phosphatases and Protein Tyrosine Phosphatases (PTPs)

Phosphatase Inhibitors

Convenient phosphatase inhibitor cocktails

Phosphatase Inhibitor Cocktail Set I

Phosphatase Inhibitor Cocktail Set I is a mixture of three inhibitors that will inhibit alkaline phosphatase as well as serine/threonine protein phosphatases such as PP1 and PP2A. The inhibitors are provided at the following concentrations: 2.5 mM (–)-*p*-Bromotetramisole Oxalate, 500 µM Cantharidin, and 500 nM Microcystin-LR in DMSO. Dilute 1:100 immediately before use.

Not available for sale outside of the United States.

Phosphatase Inhibitor Cocktail Set II

Phosphatase Inhibitor Cocktail Set II is an aqueous solution of five phosphatase inhibitors for the inhibition of acid and alkaline phosphatases as well as protein tyrosine phosphatases (PTPs). It is suitable for use with tissue and cell extracts, including extracts containing detergents. The inhibitors are provided at the following concentrations: 200 mM Imidazole, 100 mM Sodium Fluoride, 115 mM Sodium Molybdate, 100 mM Sodium Orthovanadate, and 400 mM Sodium Tartrate Dihydrate. Dilute 1:100 immediately before use.

Product	Size	Cat. No.	Price
Phosphatase Inhibitor Cocktail Set I*	1 set	524624	
Phosphatase Inhibitor Cocktail Set II	1 set	524625	

Phosphatase Inhibitor Cocktail Set I

Inhibitor	M.W.	Concentration in vial	Target Phosphatase
(–)-*p*-Bromotetramisole Cxalate	373.2	2.5 mM	Alkaline Phosphatase
Cantharidin	196.2	500 µM	PP1 and PP2A
Microcystin-LR	995.2	500 nM	PP1 and PP2A

Phosphatase Inhibitor Cocktail Set II

Inhibitor	M.W.	Concentration in vial	Target Phosphatase
Imidazole	68.1	200 mM	Alkaline Phosphatase
Sodium Fluoride	42.0	100 mM	Acid Phosphatase
Sodium Molybdate	205.9	115 mM	Acid Phosphatase
Sodium Orthovanadate	183.9	100 mM	Protein Tyrosine Phosphatase Alkaline Phosphatase
Sodium Tartrate Dihydrate	230.1	400 mM	Acid Phosphatase

*Not for sale outside of the U.S.

NEW Phosphatase Inhibitor Cocktail Set III

The Phosphatase Inhibitor Cocktail Set III contains four phosphatase inhibitors for broad-spectrum inhibition of both serine/threonine and protein tyrosine phosphatases, and is available as a 1-ml vial or as a set of five 1-ml vials. Each vial contains 1 ml aqueous solution with the concentrations of the individual inhibitors shown in the table.

NEW Phosphatase Inhibitor Cocktail Set IV

The Phosphatase Inhibitor Cocktail Set IV contains three phosphatase inhibitors for the inhibition of both serine/threonine and alkaline phosphatases, and is available as a 1-ml vial or as a set of five 1-ml vials. Each vial contains 1 ml solution (in DMSO/water) with the concentrations of the individual inhibitors are shown in the table.

Product	Size	Cat. No.	Price
NEW Phosphatase Inhibitor Cocktail Set III	1 ml 1 set (5 × 1 ml)	524627	
NEW Phosphatase Inhibitor Cocktail Set IV	1 ml 1 set (5 × 1 ml)	524628	

Phosphatase Inhibitor Cocktail Set III

Inhibitor	M.W.	Concentration in vial	Target Phosphatases
Sodium Fluoride	42.0	50 mM	Acid Phosphatases
Sodium Orthovanadate	183.9	1 mM	Protein tyrosine Phosphatase, Alkaline Phosphatase
Sodium Pyrophosphate, decahydrate	416.1	10 mM	PP1 and PP2A
β-Glycerophosphate	306.1	10 mM	Ser/Thr Phosphatases

Phosphatase Inhibitor Cocktail Set IV

Inhibitor	M.W.	Concentration in vial	Target Phosphatases
(-)-*p*-Bromotetramisole oxalate	373.2	2.5 mM	Alkaline Phosphatases
Cantharidin	196.2	500 µM	PP1 and PP2A
Calyculin A, *Discodermia calyx*	1009.2	1 µM	PP1 and PP2A

ProteoExtract® Protein Precipitation Kit

For concentration and clean up of proteins from aqueous samples

calbiochem.com/proteoextract

Sample analysis in proteomics can be affected by the presence of non-protein impurities, such as buffers, salts, and detergents, that interfere with eletrophoretic separation. Further, proteins in solution are often too dilute for direct downstream applications. Precipitation of proteins can both concentrate the proteins and remove interfering substances in just one step.

The ProteoExtract® Protein Precipitation Kit offers a fast, efficient means for concentrating proteins and removing impurities from a variety of sources. Interfering substances, such as detergents, chaotropes, buffer reagents, salts, and other impurities, remain in solution and can easily be separated from the precipitated proteins. This procedure is compatible with virtually any aqueous protein sample containing between 50 µg to 10 mg per milliliter protein. Precipitated proteins can be resuspended for applications such as IEF, 1DGE, 2DGE, or tryptic digestion before mass spectrometry and peptide separation. Kit contains reagents sufficient for 200 reactions for samples up to 200 µl. Includes 4 precipitants and Wash Solution.

Product	Size	Cat. No.	Price
ProteoExtract® Protein Precipitation Kit	1 kit	539180	

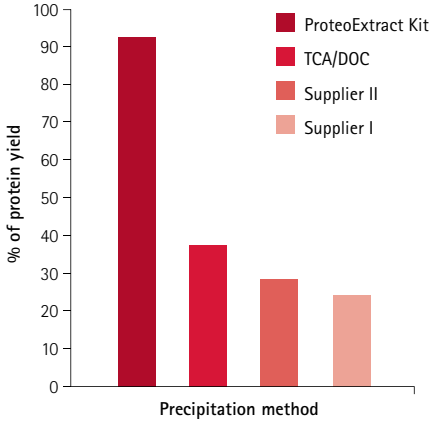
Components

Cat. No. 539180

- 5 × 29 ml Precipitant 1
- 10 ml Precipitant 2
- 10 ml Precipitant 3
- 10 ml Precipitant 4
- 65 ml Wash Solution

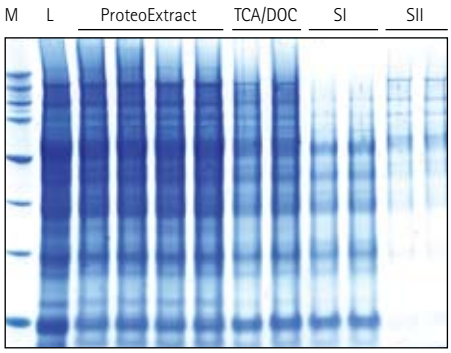
Features

- Highest yields and quantitative protein recovery including low–molecular weight proteins
- High solubility of protein pellets



Protein methods by various methods

Protein at a concentration of 2 mg/ml was precipitated using the ProteoExtract® Kit; protein pellets were redissolved in IEF buffer prior to determination of protein concentration. The same experiment was performed in parallel using TCA/deoxycholate (DOC) and products from Supplier I and Supplier II. All experiments were performed in duplicate. The ProteoExtract Protein Precipitation Kit delivers up to 3 times greater protein yields as compared to other precipitation methods.



Quantitative recovery of proteins

Equal volume equivalents from samples precipitated with different methods were separated by 1D SDS-PAGE and visualized by Coomassie blue staining. Protein patterns from the ProteoExtract-precipitated proteins (P) are identical to the pattern from the liver extract (L), clearly demonstrating the quantitative recovery of proteins. Weaker staining is achieved by TCA/DOC indicating protein loss. Selective protein loss is observed using products from Supplier I (SI) and Supplier II (SII). M: molecular weight markers.

BCA Protein Assay Kit

Simple and reliable protein quantification

The BCA protein assay is based on a biuret reaction, which is the reduction of Cu^{2+} to Cu^{+} by proteins in an alkaline solution with concentration-dependent detection of the monovalent copper ions. Bicinchoninic acid is a chromogenic reagent that chelates the reduced copper, producing a purple complex with strong absorbance at 562 nm. This assay can be used to quantify protein concentration with a wide variety of samples and can be performed in minutes.

The BCA Protein Assay Kit can be used to determine protein concentration in the range of 20-2000 µg/ml in either a standard assay or microassay configuration. Kit components are sufficient to complete 500 standard-size reactions (50 µl protein sample plus 1 ml reagent) or 2500 micro-scale reactions (25 µl protein sample plus 200 µl reagent). A BSA standard (2 mg/ml) is provided for convenient preparation of standard curves.

This assay is robust and can be performed in the presence of many compounds. Some reagents, including chelating agents, strong acids or bases, and reducing agents, interfere with the reduction and chelating reactions on which this assay depends. The BCA assay is compatible with the following protein extraction and lysis reagents: BugBuster® Protein Extraction Reagent, PopCulture® Reagent, CytoBuster™ Protein Extraction Reagent, Reportasol™ Extraction Buffer, and Insect PopCulture Reagent. Options for the removal or dilution of interfering substances are described in the kit literature.

References:

Smith, P.K., et al. 1985. *Anal. Biochem.* **150**, 76. Wiechelman, K., et al. 1988. *Anal. Biochem.* **175**, 231. Brown, R., et al. 1989. *Anal. Biochem.* **180**, 136.

Product	Size	Cat. No.	Price
BCA Protein Assay Kit	500 assays	71285-3	
BugBuster® Protein Extraction Reagent	100 ml 500 ml	70584-3 70584-4	
PopCulture® Reagent	15 ml 75 ml 250 ml	71092-3 71092-4 71092-5	
CytoBuster™ Protein Extraction Reagent	50 ml 250 ml	71009-3 71009-4	
Reportasol™ Extraction Buffer	25 ml 125 ml	70909-3 70909-4	
Insect PopCulture® Reagent	50 ml 250 ml	71187-3 71187-4	

Components

Cat. No. 71285	
• 500 ml	BCA Solution
• 15 ml	4% Cupric Sulfate
• 3 × 1 ml	BSA Standard, 2 mg/ml

Non-Interfering Protein Assay™ Kit

Accurate protein quantification from solutions containing interfering compounds

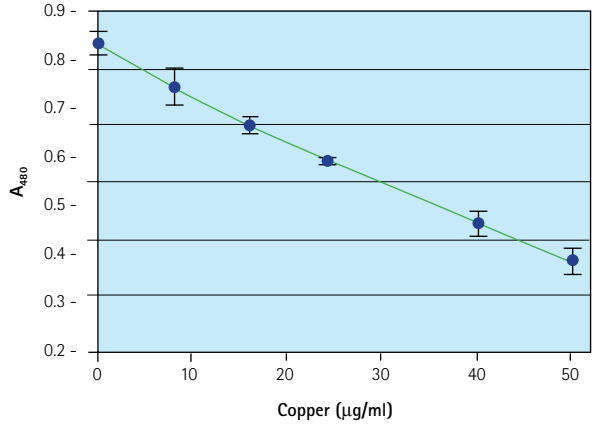
The easy-to-use Non-Interfering Protein Assay™ Kit overcomes interference of agents found in protein solutions including detergents, chelating agents, reducing agents, amines, sugars, urea, and others. The Universal Protein Precipitating Agent (UPPA™ Reagent) is used to precipitate and immobilize the protein in the tube while the interfering reagents are removed. Protein concentration is based on the specific binding of copper to the peptide backbone. As the protein concentration increases, the concentration of unbound copper ions decreases, and the color density is inversely related to the amount of protein present in solution. The kit includes UPPA reagents I and II, Copper Solution I, Color Agents A and B, and BSA standard. One kit is sufficient for 500 individual protein determinations.

Product	Size	Cat. No.	Price
Non-Interfering Protein Assay™ Kit	1 kit	488250	

Components

Cat. No. 488250

- 250 ml UPPA-I
- 250 ml UPPA-II
- 50 ml Copper Solution-Reagent-I
- 2 × 250 ml Color Agent-A
- 5 ml Color Agent-B
- 5 ml Protein Standard BSA (2 mg/ml)



Standard curve generated with the BSA standard and the Non-Interfering Protein Assay Kit

Assay measures copper ions in solution. A predetermined concentration of copper reagent binds to the peptide backbone of proteins. Higher concentrations of protein in the standards or sample bind more copper, leaving less unbound copper in solution to react with the colorimetric reagent and resulting in lower absorbance readings at 480 nm.

Perfect Protein™ Markers

Precisely sized, conveniently spaced for accurate protein size determination

novagen.com/markers

The Perfect Protein™ Markers are a novel set of recombinant proteins with defined sizes at convenient intervals. Designed for routine use in SDS-polyacrylamide gel electrophoresis, the Perfect Protein Markers enable highly accurate size determination of unknown samples. Unlike many conventional markers (e.g., ovalbumin, serum albumin, etc.), the Perfect Protein Markers contain no oligosaccharides that cause anomalous migration, heterogeneous "fuzzy" bands, or inaccurate size estimation. The known mass of each Perfect Protein Marker band also enables estimation of concentration of sample proteins. The markers are optimized for use with Coomassie blue staining, but adjusted amounts can also be used with other gel staining methods (e.g., silver staining, fluorescent dyes, etc.).

The Perfect Protein Markers, 15–150 kDa, include protein sizes of 15, 25, 35, 50, 75, 100, and 150 kDa. Each vial contains 400 µg protein. A 5-µl load contains 0.5 µg for each of the protein sizes, except for the 50-kDa band, which contains twice as much protein (1.0 µg).

The Perfect Protein Markers, 10–225 kDa, include the protein sizes listed above and two additional proteins, 10 kDa and 225 kDa, for applications requiring a broader size range. Each vial contains 500 µg protein. A 5-µl load contains 0.5 µg for each of the protein sizes, except for the 50-kDa band, which contains twice as much protein (1.0 µg).

The 4X SDS Sample Buffer is a standard formulation commonly used for SDS-PAGE analysis of proteins. The solution includes DTT for complete denaturation of disulfide bonds. The buffer can be used at a final concentration of 2X for most applications.

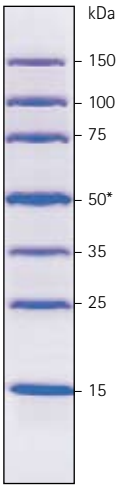
Features:

- Markers are supplied at the working dilution in gel loading buffer; concentration optimized for Coomassie blue staining
- Recombinant, unmodified markers produce sharp, accurately sized bands
- Available in two size ranges: 15-150 kDa and10-225 kDa

Product	Size	Cat. No.	Price
Perfect Protein™ Markers,15-150 kDa	100 lanes	69149-3	
Perfect Protein™ Markers,10-225 kDa	100 lanes	69079-3	
4X SDS Sample Buffer	2 ml	70607-3	

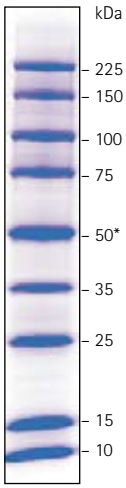
Perfect Protein Markers

15–150 kDa



Coomassie blue 4–20% SDS-polyacrylamide gel

10–225 kDa



Coomassie blue 4–20% SDS-polyacrylamide gel

*high-intensity reference band

Perfect Protein™ Western Markers & Blot Kits

Accurate size markers detectable on any Western blot

The Perfect Protein™ Western Markers consist of seven recombinant S•Tag™/His•Tag® fusion proteins that can be detected on any Western blot using S-protein AP or HRP Conjugates (or the His•Tag Monoclonal Antibody with AP or HRP conjugated secondary antibody). These markers serve as precise size standards that appear simultaneously on blots with target proteins, eliminating the uncertainty and imprecision associated with other methods. The Perfect Protein Western Blot Kits include markers and either S-protein AP or HRP Conjugate. For tracking electrophoresis and Western transfer, please see Trail Mix™ Western Markers and Blot Kits.

Features

- Detect markers simply by adding the S-protein (AP or HRP Conjugate) or His•Tag Monoclonal Antibody to the same incubation used for sample detection.
- S-protein conjugate (included) does not interfere with antibodies or streptavidin detection.
- Can be used with colorimetric and chemiluminescent AP or HRP substrates.
- Recombinant, unmodified markers give sharp, accurately sized bands.
- Markers are supplied at the working dilution in gel loading buffer; concentration optimized for Western detection.
- Protein sizes are 15, 25, 35, 50, 75, 100, and 150 kDa.

Product	Size	Cat. No.	Price
Perfect Protein™ Western Markers	25 lanes	69959-3	
Perfect Protein™ AP Western Blot Kit	25 blots	69965-3	
Perfect Protein™ HRP Western Blot Kit	25 blots	69078-3	

Components	
Cat. No. 69965	
• 125 µl	Perfect Protein Western Markers
• 50 µl	S-protein AP Conjugate
Cat. No. 69078	
• 125 µl	Perfect Protein Western Markers
• 50 µl	S-protein HRP Conjugate

Trail Mix™ Protein Markers

Novel protein markers for visible tracking and accurate sizing in stained gels

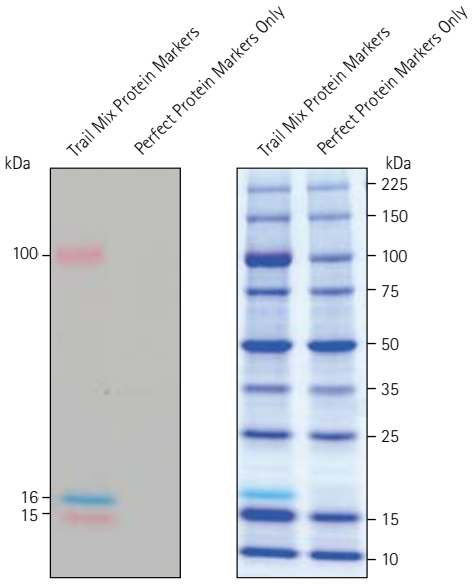
novagen.com/markers

Trail Mix™ Protein Markers are a mixture of the Novagen Perfect Protein™ Markers supplemented with a group of three prestained indicator proteins to allow direct visualization of protein migration during electrophoresis. Unlike other marker sets in which the entire ladder is prestained, Trail Mix uses only three reference bands (at 100, 16, and 15 kDa) to confirm separation and indicate gel orientation. Prestaining can cause band broadening or affect mobility, reducing the precision with which mobility and molecular weight determinations can be made. The migration or band sharpness of the Perfect Protein Markers is unaffected by the presence of the prestained bands in Trail Mix.

When stained with Coomassie blue, 10 bands are visible, ranging from 10 to 225 kDa. In addition to the prestained bands, the 50-kDa marker serves as a landmark on stained gels because it is present at a higher concentration in the mixture relative to the adjacent bands.

The 4X SDS Sample Buffer is a standard formulation commonly used for SDS-PAGE analysis of proteins. The solution includes DTT for complete denaturation of disulfide bonds. The buffer can be used at a final concentration of 2X for most applications.

Product	Size	Cat. No.	Price
Trail Mix™ Protein Markers	100 lanes	70980-3	
4X SDS Sample Buffer	2 ml	70607-3	



Unstained gel and Western transfer 4–20% SDS-PAGE

Coomassie blue 4–20% SDS-PAGE

Features:

- Trail Mix™ Protein Markers contain three prestained indicator proteins mixed with protein markers.
- Allows direct visualization of protein migration during electrophoresis.
- Markers are supplied at the working dilution in gel loading buffer; concentration optimized to Coomassie blue staining.
- Prestained proteins migrate at 15, 16, and 100 kDa. Protein sizes detected after staining are 10,15, 16, 25, 35, 50, 75, 100, 150, and 225 kDa

Trail Mix™ Western Markers & Blot Kits

Novel protein markers for visible tracking and accurate sizing on any Western blot

Trail Mix Western Markers consist of the Perfect Protein Western Markers supplemented with a group of three prestained indicator proteins to allow direct visualization of protein mobility during electrophoresis. The Trail Mix Western Markers and Western Blot Kits (AP, HRP) are supplied at the optimal concentration for detection by Western blot. Detection of Trail Mix Western Markers is not recommended with the His•Tag® Antibody and Goat Anti-Mouse IgG HRP Conjugate (H+L).

Product	Size	Cat. No.	Price
Trail Mix™ Western Markers	25 lanes	70982-3	
Trail Mix™ AP Western Blot Kit	25 blots	71047-3	
Trail Mix™ HRP Western Blot Kit	25 blots	71048-3	

Components

Cat. No. 71047
- 125 µl Trail Mix Western Markers
- 50 µl S-protein AP Conjugate

Cat. No. 71048
- 125 µl Trail Mix Western Markers
- 50 µl S-protein HRP Conjugate

Features

- Trail Mix Western Markers contain three prestained indicator proteins plus eight unstained Perfect Protein Western Markers.
- The Trail Mix markers are supplied at concentrations optimized for detection by Western blot.
- Kits contain S-protein AP or HRP Conjugate for convenient Western blot detection of markers.
- Conjugates can be added together with secondary antibody or streptavidin conjugates for simultaneous detection of target proteins and markers.
- Markers are supplied at the working dilution in gel loading buffer.
- Prestained proteins migrate at 15, 16, and 100 kDa. Proteins detected on Western blots are 15, 25, 35, 50, 75, 100, 150, and 225 kDa.

NEW iFOLD™ Protein Refolding System 1

Purify inclusion bodies and determine optimal protein refolding conditions in a 96-well plate

novagen.com/ifold

Structural and functional proteomics requires large amounts of very pure and correctly folded protein. Due to simplicity, low cost, and potential to express large quantities of target protein, *E. coli* expression systems are frequently used. However, production of foreign proteins in *E. coli* may result in the formation of inclusion bodies. Although inclusion body formation is typically not desirable, the aggregates are resistant to proteolysis, easily purified, and can be denatured with chaotropes. Unfortunately, defining conditions that promote refolding of the target protein into its native conformation is empirical and difficult.

The iFOLD™ Protein Refolding System 1 is designed to determine optimal protein refolding conditions by a systematic evaluation of 92 different buffers covering a range of pH and different salt, cyclodextrin, redox agent, and refolding additive concentrations. The iFOLD system matrix design is based on an extensive literature review of successful refolding experiments and on information in the REFOLD database (http://refold.med.monash.edu.au). The system includes inclusion body purification reagents combined with a 96-well plate-based protein refolding matrix. Following cell lysis, membrane components and contaminating proteins trapped within inclusion body pellets are removed by a series of detergent and buffer washes. The purified inclusion bodies are denatured by addition of TCEP [Tris(2-carboxyethyl)phosphine] and N-Lauroylsarcosine and refolded by rapid dilution into the iFOLD 96-well buffer matrix. Each kit is sufficient for screening up to 96 refolding conditions (92 experimental and four control wells) for a single protein.

Product	Size	Cat. No.	Price
NEW iFOLD™ Protein Refolding System 1	1 system	71552-3	

Components	
• 30 ml	10X IB-Prep™ Buffer
• 0.5 ml	1M TCEP
• 1.5 ml	TRITON® X-100
• 0.1 ml	Lysonase™ Bioprocessing Reagent
• 10 ml	30% N-Lauroylsarcosine
• 50 ml	50X iFOLD Dialysis Buffer
• 1	iFOLD Protein Refolding Plate 1
• 2	Aluminum Plate Sealers

Related application note on page 105

Features

- All reagents for inclusion body purification and pre-dispensed iFOLD 96-well protein refolding buffer matrix
- Comprehensive set of optimized screening conditions for protein refolding
- Refolding conditions based on extensive literature review
- 96-well refolding plate compatible with HT methodology

Protein Refolding Kit

Simple solubilization and refolding of inclusion body proteins

The Protein Refolding Kit provides the basic reagents to solubilize and refold recombinant proteins that accumulate as inclusion bodies in *E. coli* by a patented procedure (Burgess 1996, Frankel 1993).

References:

Burgess, R.R. 1996. *Meth. Enzymol.* **273**, 145. Frankel, S.A. and Leinwand, L.A. 1993. U.S. Patent No. 5,240,834.

Product	Size	Cat. No.	Price
Protein Refolding Kit	1 kit	70123-3	

Components	
• 100 ml	10X IB Wash Buffer
• 50 ml	10X IB Solubilization Buffer
• 2 × 100 ml	50X Dialysis Buffer
• 2 × 1.6 ml	1M DTT
• 10 ml	30% N-lauroylsarcosine

Features

- Provides easy isolation and solubilization of inclusion bodies under mild denaturing conditions.
- Solubilized fraction is dialyzed against a neutral pH buffer containing a reducing agent to encourage correct disulfide bond formation.
- A second dialysis step removes excess reducing agent and transfers the protein into the buffer of choice
- Additional separate components such as NDSBs can be added to increase refolding efficiency of individual proteins.
- Sufficient buffers are provided to process up to 2 g inclusion bodies.

NDSB Refolding Agents

Non-detergent sulfobetaines for protein refolding

The NDSBs are a group of zwitterionic compounds that can reduce aggregation and aid in refolding proteins found in inclusion bodies in bacterial expression systems (Goldberg 1996, Ochem 1997, Benetti 1998, Vuillard 1998). These compounds carry a sulfobetaine hydrophilic group and a short hydrophobic group that cannot aggregate to form micelles. It is hypothesized that the short hydrophobic group on sulfobetaines interacts with the hydrophobic regions of the protein to prevent aggregation during renaturation. The NDSBs can be used in conjunction with the Protein Refolding Kit to improve refolding efficiency. They all are soluble in water and easily removed by dialysis. The type of NDSB and conditions of use should be determined empirically for optimal refolding of individual proteins.

References:

Goldberg, M.E., et al. 1996. *Fold. Des.* **1**, 21. Ochem, A.E., et al. 1997. *J. Biol. Chem.* **272**, 29919. Benetti, P.H., et al. 1998. *Prot. Expr. Purif.* **13**, 283. Vuillard, L., et al. 1998. *Eur. J. Biochem.* **256**, 128.

NDSB-195

A non-detergent sulfobetaine. Reported to prevent protein aggregation and facilitate the renaturation of chemically and thermally denatured proteins. Zwitterionic over a wide pH range. Does not absorb significantly in the near-UV range. Easily removed by dialysis.

NDSB-201

A non-detergent sulfobetaine. Reported to prevent protein aggregation and facilitate the renaturation of chemically and thermally denatured proteins. Zwitterionic over a wide pH range. Easily removed by dialysis.

NDSB-211

A non-detergent sulfobetaine useful for protein solubilization and stabilization of halophilic proteins. NDSB-211 has been successfully employed to separate halophilic bacteria elongation factor Tu and halophilic malate dehydrogenase by high-performance ion-exchange chromatography without significant loss of activity.

NDSB-221

A non-detergent sulfobetaine useful for the renaturation of unfolded proteins. At 1.8 M, NDSB-221 yields about 35% native hen egg white lysozyme (HEWL), which is about 10-fold higher than in the absence of NDSB-221. At about 1.5 M, NDSB-221 efficiently increases the renaturation of β-D-galactosidase.

NDSB-256

A non-detergent sulfobetaine. Reported to prevent protein aggregation and facilitate the renaturation of chemically and thermally denatured proteins. Zwitterionic over a wide pH range. Easily removed by dialysis. At 1 M, NDSB-256 will restore 30% of the enzymatic activity of denatured egg white lysozyme; at 800 mM it will restore 16% of the enzymatic activity of denatured β-galactosidase.

NDSB-256-4T

A non-detergent sulfobetaine (NDSB) that prevents protein aggregation and facilitates protein-folding, presumably by interacting with the early folding intermediates. Shown to improve the *in vitro* renaturation efficiency of reduced hen lysozyme (60% enzymatic activity at 0.6 M) and chemically unfolded tryptophan synthase β_2 subunit (100% enzymatic activity at 1 M).

NDSB Set

Contains the following non-detergent sulfobetaines: 5 g of NDSB-195 (Cat. No. 480001), 25 g of NDSB-201 (Cat. No. 480005), and 5 g of NDSB-256 (Cat. No. 480010). Supplied with an informational insert.

Non-detergent Sulfobetaines

Product	Cat. No.	M.W.	Size	Price
NDSB-195	480001	195.3	5 g 25 g	
NDSB-201	480005	201.2	25 g 250 g	
NDSB-211	480013	211.3	1 g 5 g	
NDSB-221	480014	221.3	5 g 25 g	
NDSB-256	480010	257.4	5 g 25 g	
NDSB-256-4T	480011	257.4	5 g 25 g	
NDSB Set	480012		1 set	

D-Tube™ and D-Tube96™ Dialyzers

Dialysis and electroelution from polyacrylamide or agarose gels

novagen.com/dtube

D-Tube™ Dialyzers

The D-Tube™ Dialyzers* can be used for dialysis and electroelution of proteins, RNA, DNA, and oligonucleotides from polyacrylamide or agarose gels. The disposable, single-use tubes require no syringes, microcentrifuge, or laborious steps to manipulate small sample volumes. The sample is added and removed using a standard laboratory pipet. Available with molecular weight cutoffs (MWCO) from 3.5 to 14 kDa, the D-Tube Dialyzers are designed in three volume capacities: mini (10–250 µl), midi (50–800 µl), and maxi (100–3000 µl). The membrane is ultra-clean, EDTA-treated regenerated cellulose, sulfur- and heavy metal-free. Each kit contains 10 D-Tube Dialyzers and one floating rack that can hold up to four D-Tube Dialyzers.

Features:

- Easy-to-handle dialyzers for buffer exchange and removal of urea and detergents
- Ideal for electroelution of proteins, protein-DNA complexes, oligonucleotides, DNA, and RNA from polyacrylamide and agarose gels
- One-step procedure that does not require syringes or any special equipment
- Sample volume recovery >97%
- Endotoxin, Protease, RNAse, and DNAse free

Typical recoveries using D-Tube Dialyzer Kits

Sample Type	Method	Typical Recovery
Sample in solution	Dialysis	>97%
DNA or RNA in agarose gel slice	Electroelution	>90%
Oligonucleotides, DNA, or RNA in polyacrylamide gel slice	Electroelution	>90%
Protein in polyacrylamide gel slice	Electroelution	60%

NEW D-Tube96™ Dialyzers

The D-Tube96™ Dialyzer*, 6–8 kDa and D-Tube96 Dialyzer, 12–14 kDa offer the same great features as our D-Tube Dialyzers Mini in convenient, 96-tube configurations. Each kit contains a floatable rack with 96 D-Tube Dialyzers, 96 caps (for storage of unused D-Tube96 Dialyzers), and an adhesive aluminum plate sealer. Select the most appropriate cut-off value and prepare up to 96 samples (10–250 µl each) by floating the tube rack in a large beaker for buffer exchange or removal of salt, detergents, or urea. The D-Tube96 Dialyzers are ideal for protein, oligonucleotide, RNA, and DNA samples that require dialysis for downstream applications in a high-throughput format. Extracted proteins are compatible with most downstream applications, such as MALDI-MS, antibody production (animal immunization), HPLC, peptide mapping, and functional assays.

* Sold under license of U.S. Patent 7,074,313. For licensing information, contact Gene Bio-Application.

Product	Size	Cat. No.	Price
D-Tube™ Dialyzer Mini, MWCO 6-8 kDa	1 kit	71504-3	
D-Tube™ Dialyzer Mini, MWCO 12-14 kDa	1 kit	71505-3	
D-Tube™ Dialyzer Midi, MWCO 3.5 kDa	1 kit	71506-3	
D-Tube™ Dialyzer Midi, MWCO 6-8 kDa	1 kit	71507-3	
D-Tube™ Dialyzer Maxi, MWCO 3.5 kDa	1 kit	71508-3	
D-Tube™ Dialyzer Maxi, MWCO 6-8 kDa	1 kit	71509-3	
D-Tube™ Dialyzer Maxi, MWCO 12-14 kDa	1 kit	71510-3	
NEW D-Tube96™ Dialyzer, 6–8 kDa	1 kit	71712-3	
NEW D-Tube96™ Dialyzer, 12–14 kDa	1 kit	71713-3	

D-Tube Dialyzer Size	Volume (µl)	MW Cutoff (MWCO) (kDa)
Mini	10-250	6-8
	10-250	12-14
Midi	50-800	3.5
	50-800	6-8
Maxi	1000-3000	3.5
	1000-3000	6-8
	1000-3000	12-14

Chemical Compatibility with D-Tube and D-Tube96 Membranes

Compatible

Solvents: acetonitrile, benzene benzyl alcohol, *n*-butanol, *n*-butyl acetate, carbon tetrachloride, cellosolve, chloroform, cyclohexane, cyclohexanone, diethyl ether, dimethyl formamide, dimethylsulfoxide, dioxane, ethanol (98%), ethyl acetate, ethylene glycol, gasoline, glycerol, *n*-heptane, *n*-hexane, isobutanol, isopropanol, isopropyl acetate, methanol (98%), methyl acetate, methylene chloride, methyl ethyl ketone, methyl isobutyl ketone, monochlorobenzene, nitrobenzene, *n*-pentane, perchloroethylene, pyridine, tetrahydrofuran, toluene, trichloroethane, trichloroethylene, xylene

Acids: acetic acid (25% and 96%), trichloracetic acid (25%)

Aqueous Solutions: ammonium persulfate, ferric chloride (25%), sodium hypochlorite (5%)

Limited compatibility (swelling or shrinkage may occur)

Solvents: diethylacetamide

Acids: hydrofluoric acid (25%), perchloric acid (25%), phosphoric acid (85%), sulfuric acid (25%)

Bases: ammonium hydroxide (1N and 25%), potassium hydroxide (32%), sodium hydroxide (32% and 1N)

Aqueous Solutions: ammonium fluoride (20%), formaldehyde (30%), hydrogen peroxide (35%)

Incompatible

Acids: hydrochloric acid (25% and 37%), hydrofluoric acid (50%), nitric acid (25% and 65%), phosphoric acid (85%), sulfuric acid (98%)

D-Tube™ Electroelution Accessory Kit

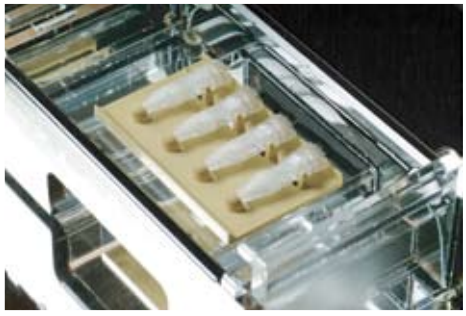
Optimized reagents for protein and nucleic acid precipitation following electroelution

www. novagen.com/dtube

The D-Tube™ Electroelution Accessory Kit provides support trays for the D-Tube Dialyzers* (compatible with most commercially-available horizontal electrophoresis units) and optimized reagents for protein and nucleic acid precipitation following electroelution. The kit also contains an optimized buffer for the removal of SDS from extracted protein samples, making them compatible with MALDI-MS analysis. The combination of D-Tube Dialyzers and D-Tube Electroelution Accessory Kit provides a unique tool for extraction of any protein, protein-protein and protein-DNA complexes from non-denaturing and denaturing (SDS) polyacrylamide gels with 60% recovery yield in less than 2 hours. The procedure efficiently recovers proteins and simultaneously removes ampholytes from proteins run on 2D gels. Extracted proteins are compatible with most downstream applications such as MALDI-MS, animal immunization for antibody production, HPLC analysis, peptide mapping, and functional assays. In addition, D-Tube Dialyzers can be used for oligonucleotides, RNA and DNA extraction from both polyacrylamide and agarose gels. Efficient extraction (>90%) is achieved for 15 nt oligos and for up to 80 kbp DNA fragments.

Features:

- Efficient extraction of protein, protein-DNA complexes, oligonucleotides, DNA, and RNA from 1D and 2D polyacrylamide and agarose gels
- Greater than 60% protein recovery
- Greater than 90% recovery of oligonucleotides , RNA, and DNA from 15 nt to 80 kbp in size
- Procedure compatible with variety of downstream applications including MALDI-MS, functional assays, and HPLC
- High-throughput electroelution from multiple samples simultaneously



D-Tube Electroelution Accessory Kit

Product	Size	Cat. No.	Price
D-Tube™ Electroelution Accessory Kit	1 kit	71511-3	

Components

• 1	Supporting Tray, Mini
• 1	Supporting Tray, Midi
• 1	Supporting Tray, Maxi
• 1 ml	MS Precipitation Buffer
• 10 ml	Trichloroacetic acid (TCA), 20% w/v
• 2 x 1 ml	3M Sodium acetate, pH 5.2

Minimum electroelution times to extract samples from polyacrylamide and agarose gels

	Polyacrylamide Gels	
	Protein Size (kDa)	Elution Time* (min)
Protein	14	35–45
	19–26	45–55
	29	55–65
	40	60–70
	45	65–75
	50	75–85
	66	85–95
	81	105–115
	116	120–130
	128	140–150
	DNA Fragment Size (bp)	Elution Time† (min)
DNA	100	10–20
	300	15–25
	500	20–30
	822	25–35
	1044	30–40
	2700	45–55
	RNA Fragment Size (nt)	Elution Time† (min)
RNA	100	15–25
	400	25–35
	600	35–45
	1000	45–55
	Oligonucleotide Size (nt)	Elution Time† (min)
Oligos	15-100	10-20
	AGAROSE GELS	
	DNA Fragment Size (bp)	Elution Time‡ (min)
DNA	100–200	10–20
	500–700	15–20
	1000	20–30
	4361	25–35
	6557	45–55
	9416	55–65
	23130	70–80

Note: electroelution times shown in this table are for D-Tube Midi; optimum times depend on the sample contents. For each D-Tube Midi, the gel piece should not exceed 0.5 cm × 1 cm. Additional information about optimum electroelution times for D-Tube Mini and Maxi varieties is available in TB422 at www.novagen.com

*Minimum time recommended for elution of protein from a 10% SDS-polyacrylamide gel at 100 V

† Minimum time recommended for elution of DNA or RNA fragments from a native or denaturing 4% polyacrylamide gel at 100–150 V

‡ Minimum time recommended for elution of DNA fragments from a 1% agarose gel at 80–110 V

* Sold under license of U.S. Patent 7,074,313.
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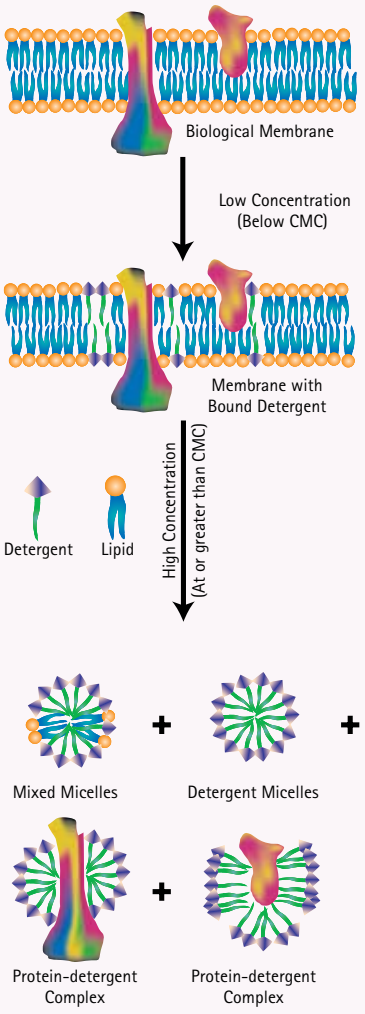
Biological Properties and Uses of Detergents

Solubilization of Cell Membranes with Detergents



Biological membranes are complex assemblies of lipids and proteins that serve as physical barriers in the cell and are sites for many cellular signaling events. Most membrane lipids contain two hydrocarbon tails (hydrophobic) connected to a polar head group (hydrophilic), allowing the formation of lipid bilayer structures where the polar heads face the aqueous environment and the hydrophobic tails are sandwiched between two layers of hydrophilic head groups.

To understand the function and structure of membrane proteins, which interact hydrophobically with the hydrocarbon chains of the lipids, it is necessary to carefully isolate highly purified, native forms of these proteins. Approximately one third of all membrane-associated proteins are integral membrane proteins, but their solubilization and purification is more challenging because most of these proteins are present at very low concentrations. Amphiphilic detergents can solubilize membrane proteins, but many of these proteins are susceptible to denaturation during the isolation process, which affects their biological and functional activities.

Detergents are amphipathic: they contain a polar group at one end and long hydrophobic carbon chain at the other end. The polar group forms hydrogen bonds with water molecules and the hydrocarbon chains interact by hydrophobic reactions and aggregate. Detergents solubilize membrane proteins by mimicking the lipid bilayer environment and forming micelles, thermodynamically stable, non-covalent aggregates, with which proteins can interact. The hydrophobic regions of a membrane protein, which normally are embedded in the membrane lipid bilayer, are surrounded by a layer of detergent molecules, which exposes the hydrophilic portions to the aqueous medium and allows these hydrophobic proteins to stay in solution.

Detergent molecules exist as monomers at low concentrations, but when the detergent concentration increases above a critical concentration, the detergent molecules self-associate and form micelles. This critical micelle concentration (CMC) is important for selecting an appropriate detergent. At the CMC, detergents begin to accumulate in the membrane. Other components of the biological system, such as lipids, proteins, pH, ionic strength, and temperature of the medium can affect the effective CMC. Because increased ionic strength tends to reduce the repulsion between charged head groups, the addition of salts to ionic detergents may reduce the CMC, allowing micelles to form at a lower concentration.

At low concentrations, detergents merely bind to the membrane by partitioning into the lipid bilayer. When the concentration of detergent increases, it disrupts and lyses the membrane bilayer, producing lipid-protein-detergent mixed micelles. Further increases in detergent concentration produce a heterogeneous complex of detergent, lipid-detergent, and protein-detergent mixed micelles. In the protein-detergent mixed micelles, the hydrophobic chains of micelle-forming lipids surround the hydrophobic regions of the membrane proteins.

Often excessive amounts of detergent are used to solubilize membrane proteins, ensuring complete dissolution and creating a population of micelles that will allow one micelle per protein molecule. For further physiochemical and biochemical characterization, the unbound detergent should be removed, either by dialysis, which is more effective for high-CMC detergents, or by hydrophobic, gel filtration, or ion-exchange chromatography.

More information about detergents is available at www.calbiochem.com/detergents and detergent removal at www.calbiochem.com/removal.

Types of Detergents: Main Features

Ionic Detergents	Non-ionic Detergents	Zwitterionic Detergents
Examples: Anionic: Sodium dodecyl sulfate (SDS) Cationic: Cetyl methyl ammonium bromide (CTAB) • Contain head group with a net charge. • Either anionic (- charged) or cationic (+ charged). • Micelle size is determined by the combined effect of hydrophobic attraction of the side chain and the repulsive force of the ionic head group. • Neutralizing the charge on the head group with increasing counter ions can increase micellar size. • Useful for dissociating protein-protein interactions. • The CMC of an ionic detergent is reduced by increasing the ionic strength of the medium, but is relatively unaffected by changes in temperature.	Examples: TRITON®-X-100 *n*-octyl-β-D-glucopyranoside • Uncharged hydrophilic head group. • Better suited for breaking lipid-lipid and lipid-protein interactions. • Considered to be non-denaturants. • Salts have minimal effect on micellar size. • Solubilize membrane proteins in a gentler manner, allowing the solubilized proteins to retain native subunit structure, enzymatic activity and/or non-enzymatic function. • The CMC of a non-ionic detergent is relatively unaffected by increasing ionic strength, but increases substantially with rising temperature.	Examples: CHAPS ZWITTERGENT® Detergents • Offer combined properties of ionic and non-ionic detergents. • Lack conductivity and electrophoretic mobility. • Do not bind to ion-exchange resins. • Suited for breaking protein-protein interactions.

Guidelines for Selecting a Detergent

A membrane protein is considered "solubilized" if it is present in the supernatant of a lysate or a homogenate after a 1-hour centrifugation at 100,000 × g. Any detergent selected for solubilization should yield the maximum amount of protein and preserve its biological activity. Use these guidelines to help selecting a detergent suitable for your protein of interest.

Survey the literature. Try a detergent that has been used previously for the isolation and characterization of a protein with similar biochemical or enzymatic properties first.

Know the solubility of the detergent at working temperature. For example, ZWITTERGENT® 3-14 Detergent is insoluble in water at 4°C while TRITON® X-114 undergoes a phase separation at room temperature.

Remove the detergent. If you plan on using dialysis to remove the detergent, select a detergent with a high CMC. If you plan on using ion exchange chromatography, choose a ZWITTERGENT® or non-ionic detergent.

Preserve biological or enzymatic activity. Preservation of activity may require experimenting with several detergents: different types of detergents and detergent concentrations. Sometimes biological activity is preserved over a very narrow concentration range: below the range, the protein may not solubilize, and above the range, the protein may become inactivated.

Consider compatibility with downstream applications. Avoid TRITON® X-100 for protocols require UV monitoring of protein concentration—it contains aromatic rings that absorb at 260-280 nm. Similarly, avoid ionic detergents if isoelectric focusing will be used to separate the proteins. For gel filtration of proteins, consider detergents with smaller aggregation numbers.

Consider detergent purity. Use ultra high purity detergents: some detergents may contain contaminants (TRITON® X-100 contains peroxides). Try our PROTEIN GRADE® and ULTROL® GRADE detergents, which have been purified to minimize oxidizing contaminants.

Try Molecular Biology Grade detergents. For any research where contaminants such as DNase, RNase, and proteases are problematic, select Molecular Biology Grade detergents.

Use non-toxic detergents. Select a non-toxic detergent instead of a toxic one such as digitonin, which is a cardiac glycoside and should be handled with special care.

Expect some "trial and error." Some detergents often work better for particular isolation procedures. Hence, some "trial and error" may be required for determining optimal conditions for isolation of a membrane protein in its biologically active form. For example, EMPIGEN® BB (Cat. No. 324690) is the most efficient detergent for solubilizing keratins and preserving their antigenicity, and *n*-Dodecyl-β-D-maltoside (Cat. No. 324355) is the detergent of choice for the isolation of cytochrome c oxidase.

Include an NDSB. Sometimes adding non-detergent sulfobetaines (NDSBs) to the detergents in the isolation buffer dramatically improves the yields of solubilized membrane proteins.

ProteoExtract® Detergent Set

Set of 8 detergents for solubilizing proteins

calbiochem.com/detergents

The ProteoExtract® Detergent Set contains eight detergents that can be used alone or in combination to solubilize proteins. The set includes two non-ionic detergents (*n*-Dodecyl-β-D-maltoside and TRITON® X-100) and six zwitterionic detergents (ASB-14, ASB-14-4, ASB-16, ASB-C8Ø, CHAPS, and ZWITTERGENT® 3-10). Each detergent is conveniently packaged in a 1-g bottle, except for the 10-g bottle of TRITON X-100.

Product	Size	Cat. No.	Price
ProteoExtract® Detergent Set	1 set	539751	

Detergent Properties

	Name	Cat. No.	MW	CMC* (mM)	Agg. No. (mM)	Avg. Micellar Wt.**	Solubility (in H_2O, mg/ml)	Storage Stability***	Size	Price
Ionic	Sodium *n*-Dodecyl Sulfate, 20% Solution (w/v)	428018	288.4	7-10	62	18,000	-	2 years at 20°C (as supplied)	200 ml	
	Taurocholic Acid, Sodium Salt, ULTROL® Grade	580218	537.7	3-11	4	2,100	-	2 months at 4°C	1 g 5 g	
	Tauroursodeoxycholic Acid, Sodium Salt	580549	521.7	-	-	-	10	3 years at 20°C (as supplied)	1 g 5 g	
	Ursodeoxycholic Acid, Sodium Salt	672305	414.6	-	-	-	200	2 months at 4°C	1 g	
Non-Ionic	*n*-Dodecyl-β-D-maltoside ULTROL® Grade	324355	510.6	0.1-0.6	98	50,000	100	2-3 months at 4°C	1 g 5 g	
	NP-40 Alternative	492016	-	0.05-0.3	-	-	25	2 years at 20°C (as supplied)	100 ml 500 ml 1000 ml	
	n-Octyl-β-D-glucopyranoside, ULTROL® Grade	494459	292.4	20-25	84	25,000	100	3 months at 4°C (under sterile conditions)	500 mg 1 g 5 g 25 g	
	n-Octyl-β-D-maltopyranoside	494465	454.5	23.4	84	38,000	100	3 years at 4°C (as supplied)	1 g	
	TRITON® X-100	648462	625 (avg)	0.2-0.9	100-155	80,000	100	2-3 months at 4°C	1 kg 3 kg	
	TRITON® X-100 Detergent, Hydrogenated	648465	631 (avg.)	0.25	100-155	80,000	-	3 years at 4°C (as supplied)	10 g	
Zwitterionic	ASB-14	182750	434.7	-	-	-	50	1 year at -20°C	5 g 25 g	
	ASB-14-4	182751	448.7	-	-	-	5	6 months at 4°C	1 g 5 g	
	ASB-16	182755	462.7	-	-	-	10	1 year at -20°C	5 g 25 g	
	ASB-C8Ø	182730	440.6	-	-	-	50	6 months at 4°C	1 g 5 g	
	CHAPS	220201	614.9	6-10	4-14	6150	615	2-3 months at 4°C	1 g 5 g 10 g 25 g	
	ZWITTERGENT® 3-10	693021	307.6	25-40	41	12,500	10	up to 1 month at 4°C	5 g 25 g 100 g	
	ZWITTERGENT® 3-12	693015	335.6	2-4	55	18,500	-	up to 1 month at 4°C	5 g 25 g	
	ZWITTERGENT® 3-14	693017	363.6	0.1-0.4	83	30,000	50	several months at 4°C	5 g 25 g 100 g	

* Average molecular weights are given for detergents composed of mixtures of different chain lengths.
** Temperature = 20-25°C.
*** Storage stability shown after reconstitution unless otherwise noted.

Removal of Unbound Detergent

calbiochem.com/removal

Excess detergent is normally employed in solubilization of membrane proteins. This helps to ensure complete dissolution of the membrane and to provide a large number of micelles such that only one protein molecule is present per micelle. However, for further physicochemical and biochemical characterization of membrane proteins, it is often necessary to remove the unbound detergent. Several methods have been used for detergent removal that take advantage of the general properties of detergents: hydrophobicity, CMC, aggregation number, and the charge.

Hydrophobic adsorption

CALBIOSORB™ Adsorbent is a hydrophobic, insoluble resin that can be used in batchwise applications to remove excess detergent and unbound organic contaminants, salts, and heavy metal ions.

Size exclusion chromatography

Size exclusion gel chromatography takes advantage of the difference in size between protein-detergent, detergent-lipid, and homogeneous detergent micelles, where most protein-detergent micelles elute in the void volume.

Dialysis

When detergent solutions are diluted below the CMC, the micelles are dispersed into monomers. The size of the monomers is usually an order of magnitude smaller than that of the micelles and thus can be easily removed by dialysis. If a large dilution is not practical, micelles can be dispersed by other techniques such as the addition of bile acid salts. For detergents with high CMC, dialysis is usually the preferred choice.

Ion exchange chromatography

This method exploits the differences in charge between protein-detergent micelles and protein-free detergent micelles. When non-ionic or zwitterionic detergents are used, conditions can be chosen so that the protein-containing micelles are adsorbed on the ion-exchange resin and the protein-free micelles pass through. Adsorbed protein is washed with detergent-free buffer and is eluted by changing either the ionic strength or the pH. Alternatively, the protein can be eluted with an ionic detergent thus replacing the non-ionic detergent.

Detergent Removal

Product	Cat. No.	Description	Removal Method	Size	Price
CALBIOSORB™ Adsorbent	206550	Off-white beads slurried in 100 mM sodium phosphate buffer, 0.1% NaN_3, pH 7.0. Designed for the removal of detergents from protein solutions and other biological mixtures in aqueous medium.	Hydrophobic adsorption	50 ml	
CALBIOSORB™ Adsorbent, Prepacked Columns	206552	Designed for the removal of detergents from protein solutions and other biological mixtures in aqueous medium. Each set contains three columns. Each column has a 10 ml total volume (5 ml resin bed in 100 mM sodium phosphate, 0.1% NaN_3, pH 7.0 with a 5 ml buffer reservoir) and an upper frit to protect the resin bed from disruption.	Hydrophobic adsorption	1 set	
Detergent-OUT™, Detergent Removal Kit	263455	A simple and rapid column based method to remove detergents such as TRITON® X-100 Detergent, NP-40, CTAB, CHAPS, Lubrol, TWEEN® Detergent, sodium deoxycholate, and others from protein solutions. Simply load protein solutions onto column and spin. Detergent is retained by the column matrix and the protein is collected in a small volume. Offered as a mini kit to process samples containing up to 3 mg of detergent, or as a medi kit to process samples containing up to 15 mg of detergent.	Size-exclusion chromatography	1 mini 1 medi	
Detergent-OUT™, SDS Removal Kit	263454	A simple and rapid column based method to remove free SDS from protein solutions. Simply load protein solutions onto column and spin. The detergent is retained by the column matrix and the protein is collected in a small volume. An SDS test kit is provided for determining detergent removal efficiency. Offered as a mini kit with the capacity to remove 2 mg of SDS from solution or as a medi kit with the capacity to remove up to 10 mg of SDS from the protein solution.	Size-exclusion chromatography	1 mini 1 medi	

Affinity Purification

Affinity purification is based on the specific interaction of a target molecule with an immobilized ligand. Affinity technology can be used to isolate specific molecules from a mixture (e.g., His•Bind® purification), capture a desired molecule for interaction studies (e.g., immobilization on GST•Bind™ Resin), or remove a component from a reaction (e.g., protease removal). For recombinant proteins, the addition of fusion tags using appropriate expression vectors enables affinity purification by a number of strategies. Our selection of immobilized metal affinity chromatography (IMAC) matrices for purification of His•Tag® fusion proteins is the widest available (see Table 1 below and Table 2 on p. 76). Other agarose-based affinity resins allow rapid purification of GST•Tag™, S•Tag™, Strep•Tag® II, and T7•Tag® fusion proteins.

His•Tag® Affinity Purification

NTA & IDA Chemistries

www.novagen.com/histag

With the His•Tag®/His•Bind® technology (see Table 3 on p. 77), purification is based on the affinity between the neighboring histidines of the His•Tag sequence and an immobilized metal ion (usually Ni^{2+} or Co^{2+}). The metal is held by chelation with reactive groups covalently attached to a solid support. The most commonly used chelators include nitriloacetic acid (NTA**) and iminodiacetic acid (IDA), which have four and three sites available for interaction with metal ions, respectively. The two chemistries confer different properties to the affinity support and conditions used for binding, washing, and elution of target proteins for both native and denaturing conditions. In practice, the additional chelation site available with NTA minimizes leaching of the metal during the purification and is compatible with

Table 1. Supports for Protein Purification

Support	Composition	Particle Size (µm)	Maximum Pressure (psi)	Products	Basis of Separation
Agarose-based affinity resins	Cross-linked beaded agarose	45–165	2.8	Ni-NTA His•Bind® Resin**	His•Tag® affinity (IMAC)
				His•Bind Resin	His•Tag affinity (IMAC)
				His•Bind Resin, Ni-charged	His•Tag affinity (IMAC)
				T7•Tag® Antibody Agarose	T7•Tag affinity
				Strep•Tactin® SpinPrep™ Column***	Strep•Tag® affinity
				S-protein Agarose	S•Tag™ affinity
				GST•Bind™ Resin	GST•Tag™ affinity
				Streptavidin Agarose	Biotin affinity
				EKapture™ Agarose	Enterokinase affinity
				Xarrest™ Agarose	Factor Xa affinity
				Protein G Plus Agarose	IgG affinity
				Protein A Agarose	IgG affinity
				Protein G Plus/Protein A Agarose	IgG affinity
				PreACT™ Agarose ALD	User-provided ligand affinity
		100–160	140	Ni-NTA His•Bind Superflow™ **	His•Tag affinity (IMAC)
		60–160	not determined*	Strep•Tactin Superflow products***	Strep•Tactin affinity
Polymer affinity resin	Methacrylate polymer	50	not determined*	Strep•Tactin MacroPrep® products***	Strep•Tactin affinity
Fractogel® EMD Tentacle resins	Methacrylate polymer	40–90	267	His•Bind Fractogel Resin	His•Tag affinity (IMAC)
				Co-MAC™ Purification Kit	
				Ni-MAC™ Purification Kit	
				u-MAC™ Cartridge	
Cellulose-based resins	Beaded cellulose	100–200	50	His•Bind Quick Cartridges/Columns	His•Tag affinity (IMAC)
Magnetic beads	Agarose embedded with ferric oxide	Average 3 µm		GST•Mag™ Agarose Beads	GST•Tag affinity
				His•Mag™ Agarose Beads	His•Tag affinity (IMAC)
	Polystyrene-coated ferric oxide	Polydisperse; average 1 µm		MagPrep® Anti-Mouse IgG Beads	IgG affinity
				MagPrep Anti-Mouse IgM Beads	IgM affinity
				MagPrep Anti-Rabbit IgG Beads	IgG affinity
				MagPrep Anti-Rabbit IgM Beads	IgM affinity
				MagPrep Anti-Human IgG Beads	IgG affinity
				MagPrep Anti-Human IgM Beads	IgM affinity
				MagPrep Streptavidin Beads	Biotin affinity
ATP-Binders™ Resin	Polyacrylamide	200	14.5	ProteoEnrich™ ATP-Binders Kit	ATP-binding protein affinity
				ATP-Binders Resin	
CAT-X	Methacrylate polymer	40-90		ProteoEnrich CAT-X Kit	Cation exchange
CAT-X SEC	Porous silica	25		ProteoEnrich CAT-X SEC Kit	Size exclusion and cation exchange

* Strep•Tactin Superflow and MacroPrep resins are suitable for gravity flow and FPLC applications.

** Manufactured by QIAGEN.

*** Manufactured by IBA GmbH

2-mercaptoethanol (up to 20 mM) for reduction of disulfide bonds. The higher metal leaching rates of IDA-based resins in the presence of other chelating or reducing components can produce poor purification results when these products are present in the buffer. However, IDA supports can be recycled many hundreds of times with no loss in performance. For both types of support the conditions can be modified to optimize the purification of individual target proteins expressed in specific systems. Most often, the imidazole concentrations of the wash and elution buffers under native conditions are adjusted to minimize copurification of non-specifically bound proteins.

The His•Bind family of products offers a wide selection of supports designed for rapid one-step purification of proteins containing the His•Tag sequence by immobilized metal affinity chromatography (IMAC). Several supports are provided uncharged or precharged with Ni^{2+} or Co^{2+}, and in either NTA or IDA chemistries. The His•Tag sequence (6, 8, or 10 consecutive histidine residues) binds to divalent cations (Ni^{2+}) immobilized on NTA- or IDA-based His•Bind and His•Mag™ resins. After unbound proteins are washed away, the target protein is eluted with either imidazole or slight reduction in pH. This versatile system enables protein purification under gentle, non-denaturing conditions, or in the presence of 6 M guanidine or urea.

Ni-MAC™, Co-MAC™, and u-MAC™ cartridges are also available, containing prepacked His•Bind® Fractogel® resin, precharged with nickel (Ni-MAC) or cobalt (Co-MAC), or uncharged (u-MAC). Each charged cartridge binds up to 30 mg His•Tag fusion protein and can be regenerated and reused at least 10 times. Cartridges can be used manually with a syringe or with liquid chromatography instruments at flow rates up to 4 ml/min and pressures up to 20 bar. See pages 78-79 for more information.

GST•Tag™ Affinity Purification

www.novagen.com/gsttag

The GST•Bind™ and GST•Mag™ purification systems are based on the affinity of glutathione-S-transferase (GST•Tag™) fusion proteins for immobilized glutathione. Glutathione-resin based purifications require a soluble, properly folded GST domain, and these systems use gentle, reduced glutathione elution conditions that prevent denaturation. GST•Bind Resin uses an 11-atom spacer arm to attach reduced glutathione to agarose by a covalent sulfide linkage, offers a high degree of glutathione substitution with high binding capacity, and can be reused several times without loss of capacity. For rapid purification of multiple samples with minimum handling time, GST•Mag Agarose Beads are easily collected with a magnet during binding, washing, and elution steps, which all can be carried out in a single tube or well. Using these systems, GST•Tag fusion proteins are quickly purified to near homogeneity in simple one-step chromatography processes.

Strep•Tactin® Affinity Purification

www.novagen.com/streptag

Strep•Tag† technology, using the Strep•Tag fusion tag, is based on strong biotin/streptavidin binding specificity. An 8-amino acid Strep•Tag II fusion tag binds to the biotin-binding pocket of streptavidin. The Strep•Tactin® protein, a streptavidin derivative, was developed for optimal Strep•Tag II binding. The binding affinity of Strep•Tag II for Strep•Tactin is nearly 100 times higher than for streptavidin. The Strep•Tactin† product family offers a variety of resins for rapid one-step affinity purification of recombinant proteins containing the Strep•Tag II tag. Purified target protein is gently eluted with desthiobiotin, an analog of biotin that reversibly binds Strep•Tactin, in PBS or other physiological buffers.

Table 2. Affinity Purification Kits

Product	Culture scale	Processing method	Capacity*	Throughput level
BugBuster® Ni-NTA His•Bind® Purification Kit	Any	Gravity flow column chromatography	5–10 mg/ml resin	Low
BugBuster His•Bind Purification Kit	Any	Gravity flow column chromatography	5–10 mg/ml resin	Low
PopCulture® His•Mag™ Purification Kit	3 ml	Magnetic	375 µg/culture	Low
RoboPop™ Ni-NTA His•Bind Purification Kit	96 × 5 ml	Filtration	1 mg/culture	High
Insect RoboPop Ni-NTA His•Bind Purification Kit	96 × 1 ml	Filtration	400 µg/culture	High
RoboPop His•Mag Purification Kit	96 × 1 ml	Magnetic	125 µg/culture	High
BugBuster GST•Bind™ Purification Kit	Any	Gravity flow column chromatography	5–8 mg/ml resin	Low
PopCulture GST•Mag™ Purification Kit	3 ml	Magnetic	150 µg/culture	Low
RoboPop GST•Bind Purification Kit	96 × 5 ml	Filtration	800 µg/culture	High
RoboPop GST•Mag Purification Kit	96 × 1 ml	Magnetic	50 µg/culture	High
Strep•Tactin HT96™ Purification Plate	96 × 1 ml	Vacuum manifold	100 µg/well	High

* Capacities are based on 1- or 5-ml cultures and binding capacities of the resins. Yields will vary with the expression levels, folding properties, and solubility of individual fusion proteins.

† Strep•Tag® technology for protein purification and detection is covered under US Patent No. 5,506,121, UK Patent No. 2,272,698 and French Patent No. 93 13 066. Strep•Tactin® is covered by US Patent No. 6,103,493. These products are provided only for research use. Information about licenses for commercial use is available from IBA GmbH, Rudolf-Wissell-Str. 28, D-37079 Goettingen, Germany.

Table 3. His•Bind and His•Mag Matrix Selection Guide

Product	Form	Capacity‡	Features	Applications
Ni-NTA His•Bind® Resin*	Ni-charged NTA agarose	5–10 mg/ml	Minimal Ni^{2+} leaching Compatible with 20 mM 2-ME and 1 mM THP Compatible with Ni-NTA Buffer Kit Reuse 2-3 times	Small to medium scale Gravity flow column Recommended for eukaryotic extracts
Ni-NTA His•Bind Superflow™ *	Ni-charged NTA Superflow agarose	5–10 mg/ml	Minimal Ni^{2+} leaching Compatible with 20 mM 2-ME and 1 mM THP Compatible with Ni-NTA Buffer Kit High flow rates and pressures Reuse 2-3 times	Small to production scale FPLC or gravity flow column Recommended for eukaryotic extracts
Co-MAC™ Purification Kit	Co-charged IDA methacrylate	up to 30 mg/ml	Minimal Co^{2+} leaching 40-90 µm particle size Compatible with 1 mM THP Fast flow rates up to 7 ml/min Pressures up to 20 bar High mechanical and chemical stability Reuse at least 10 times	Use with syringe or FPLC Rapid small-scale purification
Ni-MAC™ Purification Kit	Ni-charged IDA methacrylate	up to 30 mg/ml	Minimal Ni^{2+} leaching 40-90 µM particle size Compatible with 1 mM THP Fast flow rates up to 7 ml/min Pressures up to 20 bar High mechanical and chemical stability Reuse at least 10 times	Use with syringe or FPLC Rapid small-scale purification
u-MAC™ Cartridge	Uncharged† IDA methacrylate	up to 30 mg/ml	Easily charged with choice of metal ion 40-90 µM particle size Compatible with 1 mM THP Fast flow rates up to 7 ml/min Pressures up to 20 bar High mechanical and chemical stability Reuse at least 10 times	Use with syringe or FPLC Rapid small-scale purification
His•Bind Fractogel® Resin	Uncharged† tentacle IDA methacrylate	up to 30 mg/ml	40–90 µM particle size High flow rates and pressures Compatible with 1 mM THP	Small to production scale FPLC or gravity flow column
His•Bind Resin	Uncharged† IDA agarose	8 mg/ml	Reuse many times Compatible with His•Bind Buffer Kit Compatible with 1 mM THP	Small to medium scale Gravity flow column or batch mode
His•Bind Column	Ni-charged IDA agarose, pre-packed column	10 mg	Pre-packed column Compatible with His•Bind Quick Buffer Kit Compatible with 1 mM THP	Convenient purification Gravity flow column
His•Bind Resin, Ni-charged	Ni-charged IDA agarose	8 mg/ml	Pre-charged bulk resin Compatible with His•Bind Buffer Kit Compatible with 1 mM THP	Small to medium scale Gravity flow column or batch mode
His•Bind Quick 300 Cartridge	Ni-charged IDA cellulose packed cartridge	0.5 mg	Luer fitting on each end Compatible with His•Bind Quick Buffer Kit Compatible with 1 mM THP	Syringe-driven processing Vacuum manifold processing Rapid purification
His•Bind Quick 900 Cartridge	Ni-charged IDA cellulose packed cartridge	2 mg	Luer fitting on each end Compatible with His•Bind Quick Buffer Kit Compatible with 1 mM THP	Syringe-driven processing Vacuum manifold processing Rapid purification
His•Bind Quick Column	Ni-charged IDA cellulose packed cartridge	5 mg	Luer fitting on one end Compatible with His•Bind Quick Buffer Kit Compatible with 1 mM THP	Vacuum manifold processing Rapid purification of multiple samples
His•Mag™ Agarose Beads	Ni-charged IDA magnetic agarose	5 mg/ml	3 µm magnetic agarose beads Compatible with His•Bind Quick Buffer Kit Compatible with 1 mM THP	Rapid small scale purification Magnetic separation High-throughput-compatible

Note: As with any affinity matrix, the cleanest separations are achieved when a His•Bind resin is used near its binding capacity.

Abbreviations: 2-ME: 2-mercaptoethanol; IDA: iminodiacetic acid; NTA: nitriloacetic acid; THP: tris(hydroxypropyl) phosphine.

For more information on His•Tag® affinity purification, see the Novagen Catalog 2006/2007 p 278.

† The binding capacity for uncharged resins is for His•Tag proteins after charging with nickel or cobalt according to the manufacturer's protocol.

‡ The binding capacities are shown as X mg/ml for bulk resins and Y mg for fixed-volume cartridges.

* Manufactured by QIAGEN.

Fractogel® EMD Tentacle Resins

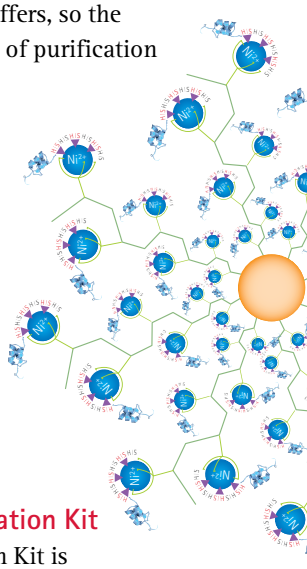
Unique matrix and accessible tentacles for affinity purification of His•Tag® proteins

The structure of the Fractogel® matrix is unique compared to other chromatographic resins, such as dextran, agarose, or cellulose. This Fractogel matrix is a very stable and durable synthetic methacrylate-based polymeric resin that provides excellent mechanical and chemical stability, particle size (40 to 90 µm), and an inert hydrophilic surface. These properties result in resins with high flow rates and low non-specific binding that can be regenerated and reused.

The resin tentacles are long polymers covalently bonded to hydroxyl groups on the Fractogel bead surface. Functional ligands are attached to the extended end of the tentacles. The steric accessibility of the ligands enables very high protein binding capacities. With low steric hinderance, biomolecules also bind more readily during the separation process, leading to higher purification yields. Like the bead surface, the tentacles and functional ligands are stable in the presence of cleaning and regeneration buffers, so the resins can be reused many times without loss of purification performance.

Features

- Pressure stability—up to 20 bar
- High flow rates—up to 7 ml/min
- High binding capacity—up to 30 mg/ml
- Efficient protein capture for high protein yields
- Stabile resin permits efficient regeneration
- Economical, reusable resin



His•Bind® Fractogel® Metal Affinity Chromatography (MAC) Resins

High yield chromatography for efficient purification of His•Tag® proteins

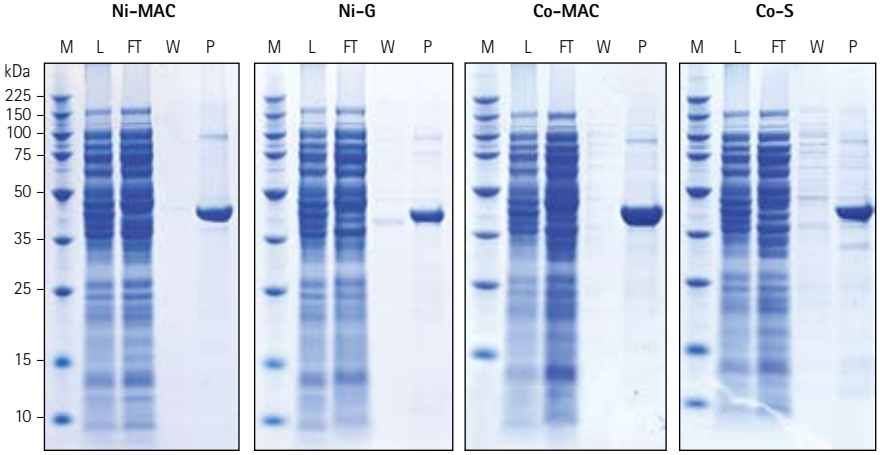
For Metal Affinity Chromatography (MAC) applications, the 40–90 µm methacrylate Fractogel beads have iminodiacetic acid (IDA) groups attached to the tentacles. IDA can be charged with different metal ions, providing a powerful tool for rapid, efficient one-step purification of His•Tag® fusion proteins. The Ni-MAC™, Co-MAC™, and u-MAC™ cartridges are pre-packed, ready-to-use cartridges that contain His•Bind® Fractogel Resin charged with nickel (Ni-MAC), cobalt (Co-MAC). or provided as uncharged (u-MAC). The high capacity and high flow rates of the resins provide a powerful tool for metal affinity protein purification. The high chemical resistance and mechanical stability of Fractogel allows the cartridges to be easily regenerated and reused.

Features

- Rapid affinity purification of His•Tag fusion proteins
- Cartridges precharged with Ni^{2+} or Co^{2+}, or uncharged to use with choice of metal ion
- High mechanical and chemical stability
- Compatible with a syringe or liquid chromatography systems, pressure up to 20 bar
- High flow rates—up to 7 ml/min
- High capacity—binds up to 30 mg protein per milliliter resin
- Reuse at least 10 times

NEW Ni-MAC™ Purification Kit

The Ni-MAC Purification Kit is designed for rapid affinity purification of His•Tag fusion proteins by metal affinity chromatography on a Ni^{2+}-charged resin. The kit contains a set of concentrated phosphate-based buffers and 5 ready-to-use cartridges. Each Ni-MAC cartridge is packed with 1 ml His•Bind Fractogel Resin, precharged with Ni^{2+}. The cartridges can be used manually with a syringe or with liquid chromatography instruments at flow rates up to 7 ml/min and pressures up to 20 bar. Each cartridge binds up to 30 mg protein and can be reused at least 10 times.



Comparison of metal affinity purification of an ERK2–His•Tag fusion protein with Ni-MAC, Co-MAC, and competitor cartridges

An ERK2–His•Tag fusion protein was expressed from BL21(DE3) and purified by metal affinity chromatography. The protein was purified according to manufacturers' protocols for Ni-MAC, nickel-affinity sepharose (Competitor G, Ni-G), Co-MAC, and cobalt-affinity cross-linked agarose (Competitor S, Co-S) cartridges. Crude load (L), flow-through (FT), wash (W), and elute (E) fractions (20 µl each) were collected and analyzed by 10% BIS TRIS gels after staining with Coomassie blue. M: molecular weight markers.

NEW Co-MAC™ Purification Kit

The Co-MAC™ Purification Kit is designed for rapid affinity purification of His•Tag® fusion proteins by metal affinity chromatography on a Co^{2+}-charged resin. The kit contains a set of concentrated Tris-based buffers and 5 ready-to-use cartridges. Each Co-MAC cartridge is packed with 1 ml of His•Bind® Fractogel® Resin, precharged with Co^{2+}. The Co-MAC cartridges can be used manually with a syringe or with liquid chromatography instruments at flow rates up to 7 ml/min and pressures up to 20 bar. Each cartridge binds up to 30 mg protein and can be reused at least 10 times.

NEW u-MAC™ Cartridges

The u-MAC™ Cartridges are designed for rapid affinity purification of His•Tag fusion proteins by immobilized metal affinity chromatography. Each u-MAC cartridge is packed with 1 ml of uncharged His•Bind Fractogel Resin. The u-MAC cartridges can be custom charged with different metal ions (Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}) depending on the protein characteristics and desired binding efficiency. The u-MAC cartridges can be used manually with a syringe or with liquid chromatography instruments at flow rates up to 7 ml/min and pressures up to 20 bar. After charging with Ni^{2+} or Co^{2+}, each cartridge binds up to 30 mg His•Tag fusion protein and can be regenerated and reused at least 10 times.

Product	Size	Cat. No.	Price
Ni-MAC™ Purification Kit	1 kit	71658-3	
Co-MAC™ Purification Kit	1 kit	71659-3	
u-MAC™ cartridge	5 cartridges	71651-3	

Components

Cat. No. 71659

• 5	Co-MAC Cartridges
• 2 × 80 ml	8X Bind Buffer
• 3 × 25 ml	8X Wash Buffer
• 3 × 25 ml	4X Elute Buffer

Cat. No. 71658

• 5	Ni-MAC Cartridges
• 2 × 100 ml	4X MAC Wash Buffer, Phosphate
• 2 × 75 ml	4X MAC Bind Buffer, Phosphate
• 75 ml	4X MAC Elute Buffer, Phosphate

His•Bind® Fractogel® Resin

Rapid affinity purification of His•Tag® fusion proteins

His•Bind® Fractogel® Resin is a 40–90 µm methacrylate bead matrix ideal for low to medium pressure chromatography, such as FPLC up to 20 bar. The bulk resin is supplied uncharged and can be charged with Co^{2+} using $CoSO_4$, Cu^{2+} using $CuSO_4$, or Ni^{2+} using $NiSO_4$. The resin can be regenerated and reused at least 10 times for protein purification under either gentle, non-denaturing conditions, or in the presence of up to 6 M guanidine or 8 M urea without reducing agents.

Product	Size	Cat. No.	Price
His•Bind® Fractogel® Resin	25 ml	70693-3	

Features

- High mechanical and chemical stabilities
- Compatible with a syringe or liquid chromatography instruments, pressures up to 20 bar
- Charged resin binds >30 mg protein per milliliter resin
- Reuse at least 10 times

A rapid procedure for protein extraction from filamentous fungi and plants using YeastBuster™ Protein Extraction Reagent*

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Efficient release of proteins is demonstrated using YeastBuster™ Protein Extraction Reagent with the mycelium of a fungus (*Aspergillus nidulans*) and the seeds of bambara groundnut (*Voandzeia subterrannea*).

The main methods for preparing protein extracts from filamentous fungi and plants involve the homogenization of mortar-pulverized material in liquid nitrogen and extraction under either non-denaturing or denaturing conditions. The simple non-denaturing method used in our laboratory with *Aspergillus nidulans* involves extraction with a buffer containing 50 mM Tris-HCl (pH 8.0), 1 mM PMSF, 2 mM EDTA, and 2 mM DTT under agitation at room temperature; proteins are recovered as a soluble fraction after centrifugation. The protein recovery is variable and, furthermore, most of the proteins remain in the pellet. Denaturing methods provide proteins in a single extract fraction; however the protein extracts are not fully compatible with a variety of downstream applications, such as enzyme assays.

Recently, YeastBuster™ Protein Extraction Reagent was developed for gentle release of soluble proteins from yeast cells (Drott 2002). The success in our laboratory with *in vitro* enzyme assays using *Saccharomyces cerevisiae* protein extracts obtained with YeastBuster reagent prompted us to investigate whether a similar approach could be used for filamentous fungi.

Here we present an optimized protocol for *A. nidulans* that also enables the processing of seeds of *Voandzeia subterrannea* (L) Thouars (bambara groundnut), widely used as a main source of dietary protein by rural communities in many African countries.

A. nidulans strain G191 (Keszenman-Pereya 2003) was cultured at 37°C in rich liquid medium (YEPD broth with supplements as required) and mycelia were collected by filtration through Miracloth. Both *Aspergillus* mycelial biomass and seeds of bambara groundnut (accession number 2, as described in Odeigah 1998) were ground to a fine powder with a mortar and pestle under liquid nitrogen. The pulverized materials were used immediately or after storage at –80°C.

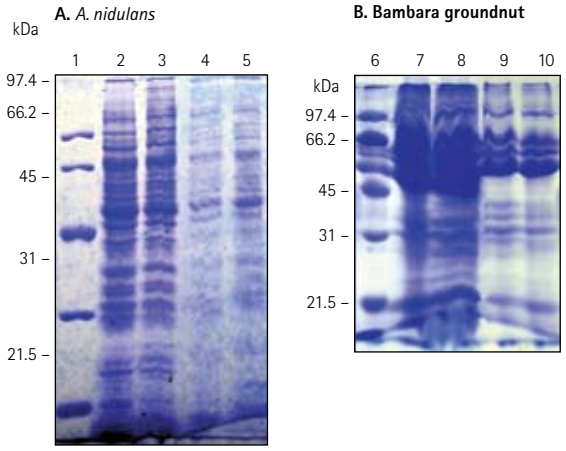
YeastBuster Protein Extraction Reagent extracted proteins with a similar efficiency over a wide molecular mass range. Starting with 50 mg tissue, the YeastBuster method extracted approximately 50% more protein (9.4 mg) than the denaturing method (6.3 mg).

Pulverized material (50 mg) was suspended in a 1.5 ml microcentrifuge tube by pipetting in 1 ml of extraction buffer [978 µl YeastBuster Protein Extraction Reagent, 10 µl THP, 1 µl Benzonase® Nuclease (25 U), and 11.4 µl 100 mM PMSF] and then agitated in a MiniMix microcentrifuge shaker (Thermo Life Sciences) at top speed for 20 minutes. Insoluble debris was removed by centrifugation at 16,000 × g at 4°C for 10 minutes. The supernatant containing the soluble fraction was transferred to another tube and retained. The pellet was resuspended in 1 ml denaturing buffer (see Figure 1 on p. 82). ▶▶

(† author for correspondence)

*Adapted from *inNovations* **19**, (2004).

For comparison, protein extracts were also prepared according to a described method (4) with modifications. In a microcentrifuge tube, 50 mg ground material was suspended in 1 ml 10% (w/v) TCA. Next, 10 µl cold acetone containing 0.07% (v/v) 2-mercaptoethanol was added, mixed, and left overnight at –20°C. After centrifugation at 16,000 × g at 4°C for 20 minutes, the supernatant was discarded and the pellet was washed with 900 µl cold acetone/mercaptoethanol mixture and dried under vacuum for 10 minutes. Proteins were resuspended in 1 ml denaturing buffer [40 mM Tris, 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 100 mM DTT], sonicated (six 10-second bursts with a 10-second cooling period between each burst) on ice, and then spun at 16,000 × g at 4°C for 20 minutes. The supernatant (soluble fraction) was transferred to a new tube and the remaining pellet resuspended in 500 µl denaturing buffer. A Bradford assay was used to determine the concentration of recovered protein.



Lane	Sample
1	Protein markers
2	5 µl denaturing extract
3	5 µl YeastBuster extract
4	10 µl pellet obtained with the denaturing method and resuspended in denaturing buffer
5	10 µl pellet obtained with YeastBuster Protein Extraction Reagent and resuspended in denaturing buffer
6	Protein markers
7	10 µl denaturing extract
8	10 µl YeastBuster extract
9	10 µl pellet obtained with the denaturing method and resuspended in denaturing buffer
10	10 µl pellet obtained with YeastBuster Protein Extraction Reagent and resuspended in denaturing buffer

Figure 1. Comparison of YeastBuster and denaturing extraction methods

Proteins extracted using the methods described in the text were analyzed by SDS-PAGE (10% gel) and Coomassie blue staining. Panel A: Protein extracts from *A. nidulans*. Panel B: Protein extracts from bambara groundnut.

Figure 1, panel A shows that YeastBuster™ Protein Extraction Reagent extracted proteins with a similar efficiency over a wide molecular mass range. Starting with 50 mg tissue, the YeastBuster method extracted approximately 50% more protein (9.4 mg) than the denaturing method (6.3 mg). Figure 1, panel B shows that the YeastBuster method was equally effective for release of proteins from seeds of bambara groundnut: both YeastBuster and denaturing extracts contained similar amounts of protein (6.7 mg and 7.1 mg, respectively). Most of the extractable protein was released (Figure 1, lanes 4, 5, 9, and 10). It should be noted that the same YeastBuster method described here yielded up to 15 mg protein from *S. cerevisiae* extract (data not shown).

YeastBuster Protein Extraction Reagent is capable of extracting proteins from *A. nidulans* and seeds of bambara groundnut with similar or even higher efficiencies than the denaturing method. Protein extracts can be used immediately or frozen as portions in liquid nitrogen and stored at –80°C for future use.

Summary

The YeastBuster non-denaturing method is rapid, simple, efficient, relatively inexpensive, flexible enough to be scaled up or down, and compatible with downstream applications. The results shown here suggest that the YeastBuster extraction method also could be used with a variety of filamentous fungi and plant tissues. ■

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Protocol for Protein Extraction, http://www.biotech.unl.edu/proteomics/mercaptoethanol.html

Product	Cat. No.	Page
YeastBuster™ Protein Extraction Reagent	71186	20

NEW Analysis of signal transduction pathways in human carcinoma cells following drug treatment*

Jörg von Hagen, Merck KGaA, Germany

Signal transduction pathways regulate fundamental biological processes and cellular responses to external signals. Aberrant pathway components (e.g., receptors, ligands, kinases) are responsible for, or contribute to, a wide variety of human diseases, including atherosclerosis, asthma, cancer, diabetes, and rheumatoid arthritis. As a result, understanding receptor signaling and transduction of the associated response from cell membrane to the nucleus provides important information for therapeutic drug design. To study signal transduction pathways, a convenient and reproducible method for preparing phosphorylated protein samples is a valuable tool.

Enrich cellular fractions for phosphoproteomes

Experiments in this report demonstrate use of the ProteoExtract® Phosphoproteome Profiler Kit in combination with the ProteoExtract Subcellular Proteome Extraction Kit (S-PEK) or the ProteoExtract Native Membrane Protein Extraction Kit (M-PEK) to enrich samples for phosphoproteins. Phosphoprotein samples are then used in several different downstream applications. The procedure (Figure 1) combines subcellular protein extraction followed by precipitation, a trypsin digest, and phosphopeptide capture with zirconium-based immobilized metal affinity chromatography (IMAC). Optimized to monitor signal transduction from the receptor to the nucleus, the method is ideal for measuring cellular response to drug treatment. Here phospho-enriched protein samples are analyzed to profile the cellular response to new drugs compared to standard drugs, to determine the mode of action for potential drugs, and to identify specific proteins in a signal transduction pathway.

MAPK-pathway inhibitor profiles

Excessive inflammation is considered a critical factor in many human diseases, including autoimmune disorders, neurodegenerative conditions, infection, cardiovascular diseases, and cancer (Modugno 2005). Identification of signaling pathways preferentially activated in inflammatory conditions has advanced drug discovery targeting these mechanisms. Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine protein kinases that regulate fundamental biological processes and cellular responses to external stress signals, including the synthesis of inflammation mediators. Clinical trials conducted with MAPK inhibitors have shown benefits in patients with inflammatory diseases, such as sepsis (Fijen 2001).

Important questions in drug discovery include: "How does the small molecule drug work?" and "What drug concentration provides the appropriate effect?" The following experiment demonstrates a method to answer these questions using the S-PEK and the Phosphoproteome Profiler Kit to determine the mode of action of an unknown inhibitor compared to previously described MAPK-pathway inhibitors. HCT116 human colon carcinoma cells were treated with a Raf inhibitor, a mitogen-activated ERK-activating kinase (MEK) inhibitor, an inhibitor of unknown function, or left untreated, and were processed using the ProteoExtract kits. Comparing nuclear fraction MS data of the cell extracts (Figure 2) shows that the unknown inhibitor (Inhibitor X) behaves like the MEK inhibitor, but has a mode of action different from the Raf inhibitor. Results were validated by Western blot analysis using phospho-specific antibodies (data not shown). ▶▶

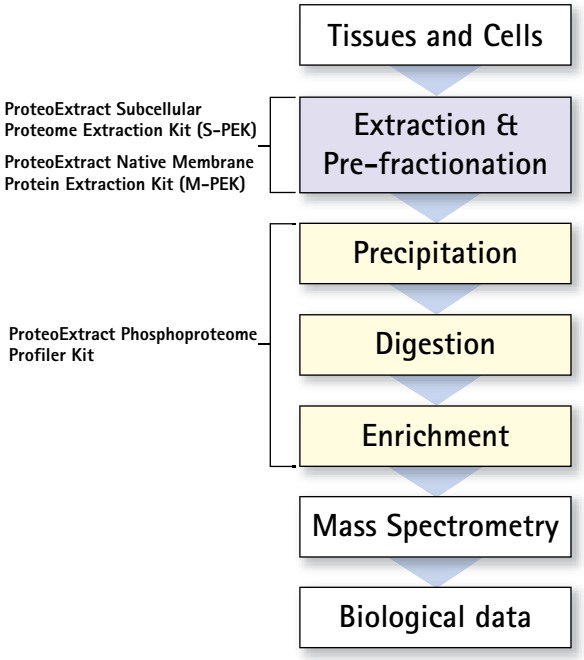

Figure 1. ProteoExtract® Phosphoproteome Profiler method

Cells are processed to give subcellular fractions using the ProteoExtract Subcellular Proteome Extraction Kit (S-PEK) or the ProteoExtract Native Membrane Protein Extraction Kit (M-PEK). The resulting fractions are digested with trypsin and enriched for phosphopeptides using the ProteoExtract Phosphoproteome Profiler Kit prior to mass spectometry (MS) analysis.

*Adapted from *inNovations* **23**, 16 (2006).

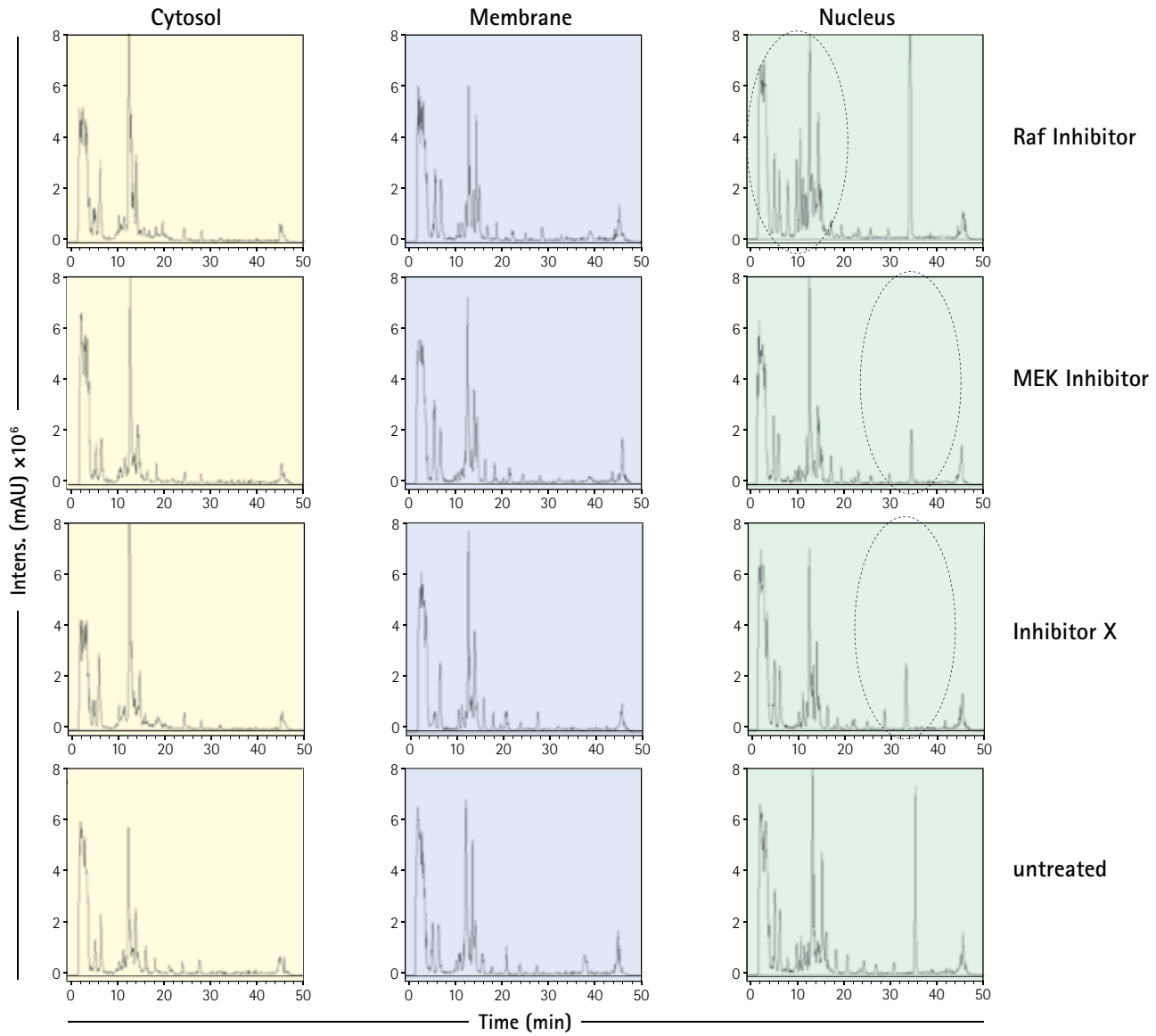

Figure 2. Analysis of a potential MAPK-pathway inhibitor compared to known Raf and MEK kinase inhibitors

HCT116 human colon carcinoma cells were seeded at 3 × 10^6 cells per 75 cm^2 flask in MEM Alpha. Cells were allowed to adhere for 16 h before incubating for 45 min with 10 µM Raf inhibitor (BAY439006, Bayer), MEK inhibitor (CL-1040, Pfizer), an inhibitor of unknown function (X), or left untreated. After treatment, cells were processed using the S-PEK procedure and each protein fraction (100 µg) was precipitated. Proteins were digested with trypsin and a portion (40 µl) was phosphopeptide enriched using the ProteoExtract® Phosphoproteome Profiler Kit. The crude cell digest and the phosphopeptide-enriched fraction were analyzed by electrospray ionization liquid chromatography mass spectrometry (ESI LC/MS) using a Bruker Daltonics Esquire. Results were analyzed with Matrix Science's Mascot® software and mass spectrometry database (MSDB) for proteins.

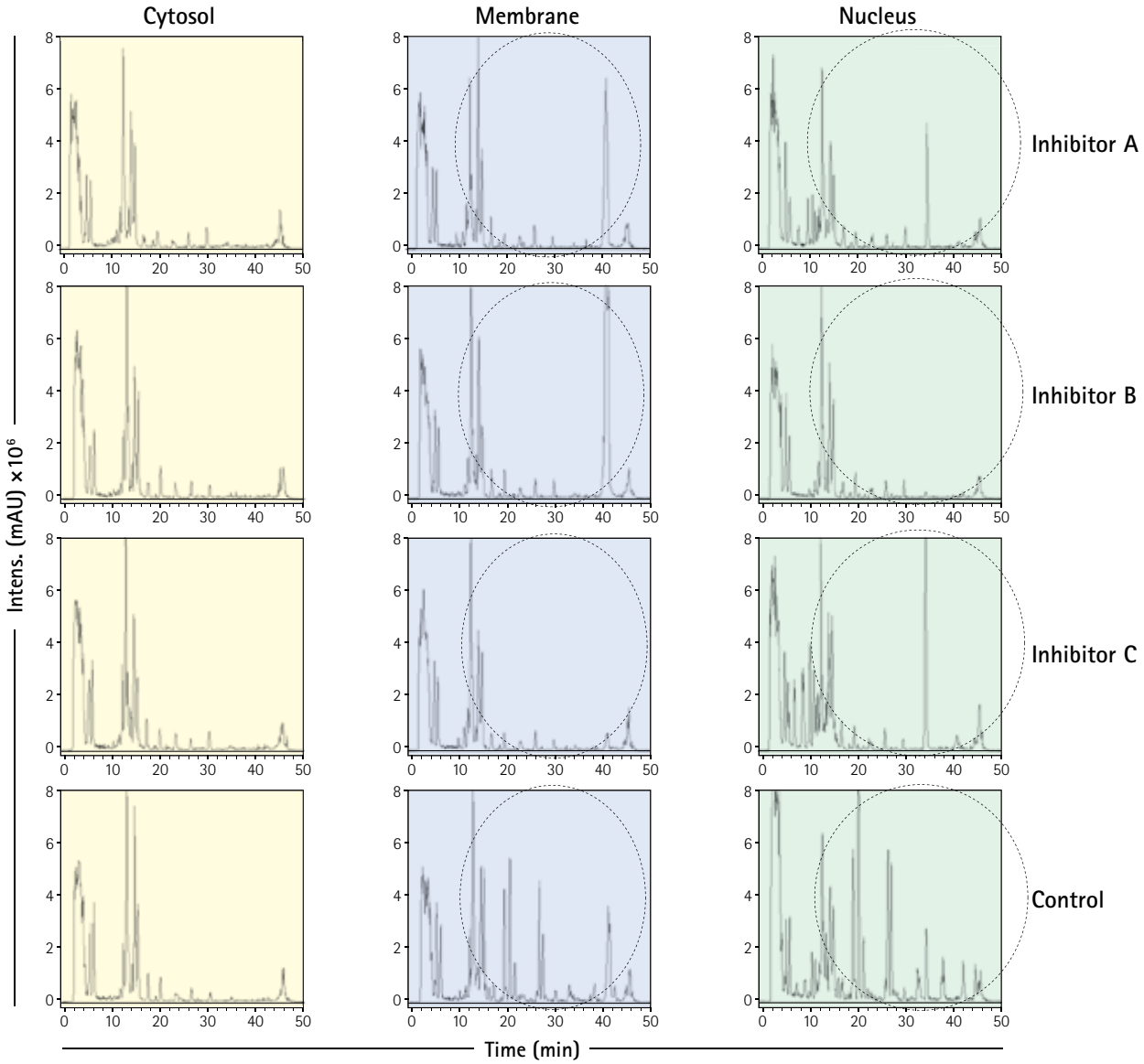

Figure 3. Pathway analysis of therapeutically relevant RTK-inhibitors

A431 human epidermal carcinoma cells were seeded at 3 × 10^6 cells per 75 cm^2 flask in RPMI 1640 with 10% FCS and 2 mM L-glutamine. Cells were stimulated with 50 ng/ml EGF for 10 min prior to RTK-inhibitor treatment (10-100 µM, depending upon inhibitor) for 45 min. Extracts of cellular protein fractions were prepared using S-PEK and 100 µg of each fraction was precipitated. Proteins were digested with trypsin and a portion (40 µl) was phosphopeptide enriched using the ProteoExtract® Phosphoproteome Profiler Kit. Phosphopeptide-enriched fractions were analyzed by ESI LC/MS.

RTK inhibitor mode of action

Epidermal growth factor (EGF), its cognate receptor (EGFR), and related family members are important in the growth of normal cells and malignant tumors, including: glioblastomas, astrocytomas, medulloblastomas, non-small cell lung carcinomas (NSCLC) and breast cancer (Harari 2004). Two EGFR inhibitors, Cetuximab and Gefitinib, have been approved for treatment of colorectal and lung cancer, respectively, in Switzerland and the United States (Snyder 2005, Chung 2005, Harari 2004). In the following experiment, human epidermal carcinoma cells (A431) were stimulated with EGF, followed by EGFR inhibition using different therapeutically relevant receptor tyrosine kinase (RTK) inhibitors. Figure 3 shows that all three RTK inhibitors repress signaling from the receptor (membrane fraction) to the nucleus. ▶▶

Results from the membrane fraction and the nuclear fraction for the different RTK inhibitors indicate that they inhibit by different modes of action.

IGF-1 stimulated protein phosphorylation

Insulin-like growth factor (IGF-1) signaling is highly implicated in cancer, with the IGF-1 receptor (IGF-1R) being the predominant significant factor. IGF-1R has a limited role in normal cell growth but is crucial for tumor transformation and survival of malignant cells, partially due to interactions with oncogenes. (Pollak 2004, Giovannucci 2000, Hankinson 1998, Baserga 1994). Targeting of IGF-1R is seen as a promising option for cancer therapy. Single chain antibodies and small molecules targeting IGF-1R dependent malignant growth are of particular interest.

This experiment uses the M-PEK and the Phosphoproteome Profiler Kit to show the results of IGF-1 stimulation on membrane fraction protein phosphorylation. MCF-7 human breast carcinoma cells were treated with IGF-1. Treated and untreated cells were processed using the ProteoExtract kits and analyzed by MS (Figure 4). Further analysis using Tandem MS (MS/MS) identified proteins known to be involved in IGF-1 signaling (data not shown). This technique can be used to identify relevant proteins and to obtain information about mechanisms involved in signal transduction pathways.

Summary

These experiments describe flexible, convenient, and reproducible methods, using ProteoExtract® kits, to prepare phosphorylated protein cellular fractions. Using this technique, researchers can confidently analyze phospho-enriched samples to profile the mechanism of unknown inhibitors, compare the efficiency of different inhibitors, determine dose-effects for potential therapeutic drugs, and, after MS/MS analysis, identify proteins involved in stimulated or inhibited signal transduction pathways. In addition to analyzing drug effects, the method can be used to measure the effect of overexpression (transient transfection of tissue culture cells) or of gene silencing with short interfering RNA (siRNA) on signal transduction pathways. ■

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Product	Cat. No.	Page
ProteoExtract® Phosphoproteome Profiler Kit	539181	44
ProteoExtract® Subcellular Proteome Extraction Kit	539790	28
ProteoExtract® Native Membrane Protein Extraction Kit	444810	30

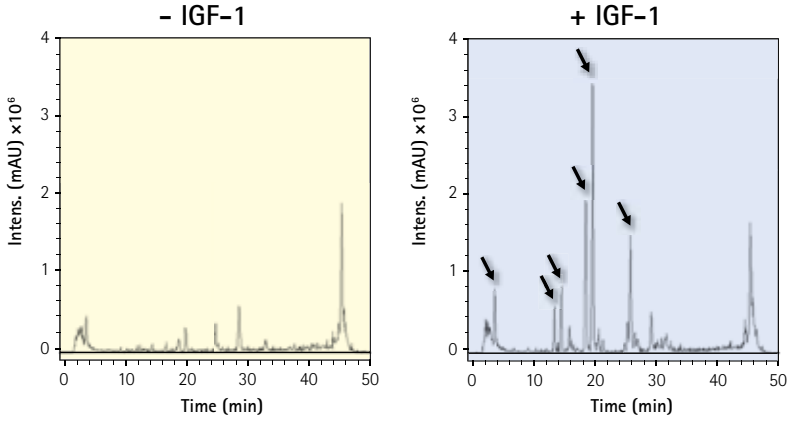

Figure 4. Phosphopeptide signal patterns of MCF-7 cells (membrane fraction) induced by IGF-1

MCF-7 human breast carcinoma cells were seeded at 3 × 10^6 cells per 75 cm^2 flask in RPMI 1640. Cells were serum-starved for 24 h and treated for 5 min with 100 ng/ml IGF-1. Membrane fractions were isolated from treated and untreated cells using the M-PEK. Each protein fraction (100 µg) was precipitated, digested with trypsin, and phosphopeptide enriched (40 µl) using the ProteoExtract Phosphoproteome Profiler Kit. Phosphopeptide-enriched fractions were analyzed by ESI LC/MS. Results were analyzed with Matrix Science's Mascot® software and MSDB protein database. Arrows indicate IGF-induced phosphorylation of specific peptides.

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Double your chance to discover less-abundant, low-solubility proteins on 2D electrophoresis gels*

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Introduction

Proteome analysis requires the handling of highly complex samples consisting of both a large number of analytes as well as large variances in the amount of the analytes. A typical eukaryotic cell contains approximately 70 pg of total protein, representing roughly 4000 unique proteins. In this mixture, the 250 most abundant protein species (mainly cellular housekeeping proteins) account for roughly 90% of the protein mass. These proteins are most easily visualized by standard methods and, without prefractionation, result in redundancy of collected MS-spectra during protein identification as often more than 30 MS spectra are collected per housekeeping protein.

Currently, two-dimensional electrophoresis (2DE) is the highest resolution technique for protein separation (Link 1999), capable of routinely separating 1000 proteins on a single gel. Despite representing approximately 50% of cellular proteins, low-abundance proteins are less frequently identified by 2DE of total proteomes (Gygi 2000) due to sample loading considerations. Furthermore, despite the high resolving power of 2DE, co-migration of proteins is a common problem (Gygi 2000), making differential protein analysis of complete proteomes by image analysis software unreliable, if at all possible. Hence, identification of less-abundant proteins and accurate protein expression profiling would profit significantly from robust and easy-to-use prefractionation schemes to reduce the protein complexity prior to analysis. This article describes the use of the recently developed ProteoExtract® Mammalian Partial Proteome Extraction Kit (P-PEK). The kit is designed for a serial sample preparation of complex protein mixtures using reagents with increasing solubilization power. Up to four sub-proteomes can be obtained with the provided reagents. Due to the reduction of protein complexity within each of the partial proteomes combined with better solubilization of proteins, the use of the ProteoExtract kit enables the production of 2D displays in which the full proteome is better characterized. Standardization of both protocol and reagents results in optimal resolution and reproducibility of 2DGE-protein patterns.

*Adapted from Calbiochem ProteoExtract Partial Mammalian Proteome Extraction Kit Application Note PE 0302.

Methods and Results

Sample preparation, analytical 2DGE, and image analysis

Human HepG2 and A-431 skin carcinoma tissue culture cells were grown in the appropriate media until 1×10^8–2×10^8 cells at 80% confluency were reached. To extract complete proteomes in one fraction, cell pellets were treated with the ProteoExtract Complete Mammalian Proteome Extraction Kit (C-PEK) (Cat. No. 539779), according to the manufacturer's instructions. For selective extraction of proteins based on differential solubility, cell pellets were prepared and proteins sequentially extracted with the ProteoExtract Partial Mammalian Proteome Extraction Kit (Cat. No. 539789) according to the manufacturer's instructions.

For analytical gels, 200 µg of protein was used for each 17-cm pH 4–7 immobilized pH gradient (IPG) strip (BioRad). SERVA ampholytes were added to 0.5% and the appropriate extraction reagent for dilution and rehydration (two for fraction 2 and three for fraction 3). In the case of partial proteome fraction 4, which contains SDS, proteins were precipitated using TCA/acetone (Yuan 2002) and resuspended in extraction buffer 3. IEF was performed in parallel for each extract in one tray for 50,000 Vh according to standard focusing conditions (Gorg 1999). Following SDS-PAGE using 13% gels, proteins were visualized by silver staining as described by Heukeshoven and Dernick (1988). With the ProteoExtract Complete Mammalian Proteome Extraction Kit, the proteins of a given sample are in one extract (Figure 1, panel A). Sequential extraction with the ProteoExtract Partial Mammalian Proteome Extraction Kit expands the proteome into four partial proteome fractions, each successive fraction containing proteins of increasing hydrophobicity (Figure 1, panel B, p. 60). Computer-assisted image analysis using PDQuest™ software v6.2.1 (Bio-Rad) indicated 891 protein spots in the complete extract and a total of 2545 spots in the four gels of the sequential extraction. By deducing the number of protein spots detected in more than one fraction, 2004 spots are found to be unique to their respective fraction (see also Table 1, p. 60). This means that after sequential extraction ▶▶

of human tissue culture cells with the ProteoExtract® Partial Mammalian Proteome Extraction Kit 1113 *additional* protein spots could be detected as compared to the complete extract. Since gels with partial proteomes of fractions 2–4 contain less complex 2D patterns, the corresponding image analysis is easier.

To readily visualize the differences in 2D patterns of the obtained partial proteomes, the gel images of fractions 1–3 were matched using different colormodes. Image 1 in Figure 1, panel C shows the comparison of protein pattern of fraction 1 containing the most soluble proteins from HepG2 cells (green) with fraction 2 containing proteins of intermediate solubility (red). The comparison of the protein pattern of fraction 2 containing proteins of intermediate solubility from HepG2 cells (red) with fraction 3 containing less soluble proteins (green) is depicted in image 2 in Figure 1, panel C. Note that the majority of protein spots appear to be unique for the respective fraction (also documented in Table 1). Despite successful prefractionation, some of the higher-abundance proteins appear in more than one fraction (yellow), thereby facilitating the matching during the image analysis process. For example, the white arrow points to a highly abundant protein spot detected in all three fractions, which was used as a landmark for matching the gel images. Protein quantitation reveals that approximately 60% of the total amount of extracted protein is obtained in fraction 1. This fraction contains the most soluble proteins, many of which are highly abundant. Detection of additional protein spots in the subsequent fractions 2 and 3 (Table 1) significantly increases the chance to discover and characterize less-abundant proteins. The supplementary SDS-soluble proteins in fraction 4, which can optionally be analyzed by 1DE rather than 2DE, would even enhance this but have not been considered in this analysis. ▶▶

Table 1. Sequential extraction of human tissue culture cells enables detection of additional protein spots

ProteoExtract kit used	Protein spots	Unique protein spots
Complete	891	**891**
Partial fraction 1	1024	91
Partial fraction 2	860	684
Partial fraction 3	661	407
Combined partial fraction 1–3	2545	**2004**

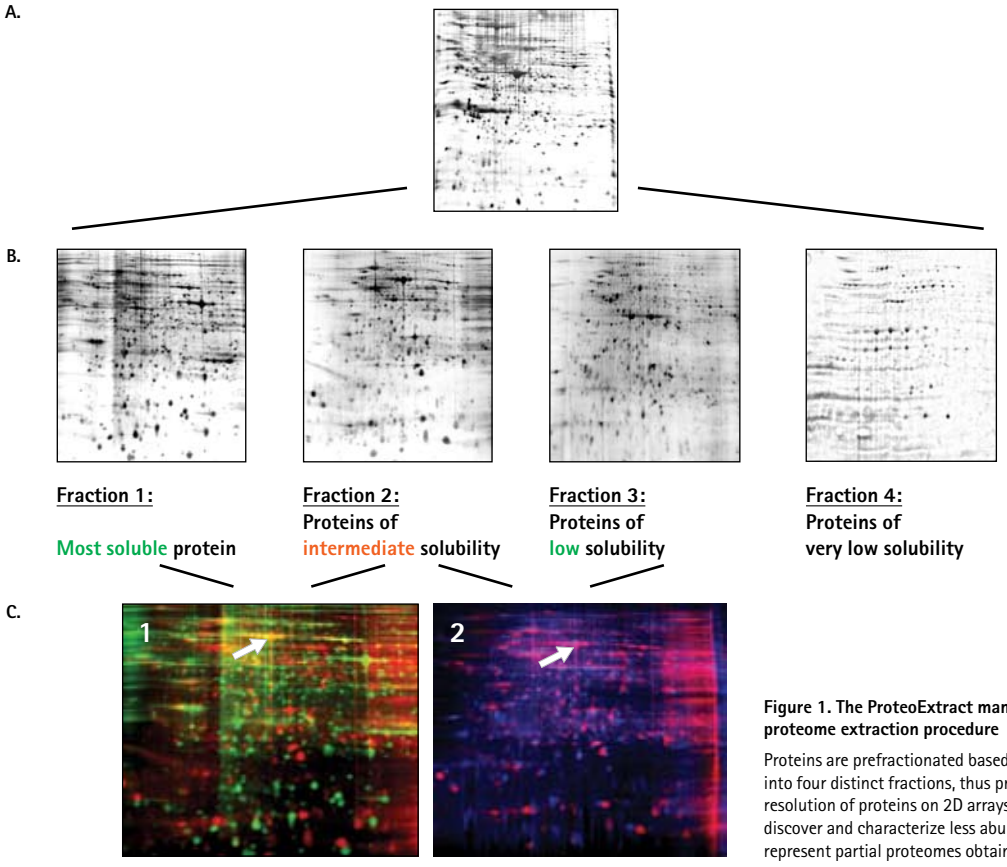

Figure 1. The ProteoExtract mammalian partial proteome extraction procedure

Proteins are prefractionated based on differential solubility into four distinct fractions, thus providing increased resolution of proteins on 2D arrays and a better chance to discover and characterize less abundant proteins. Images represent partial proteomes obtained from HepG2 cells.

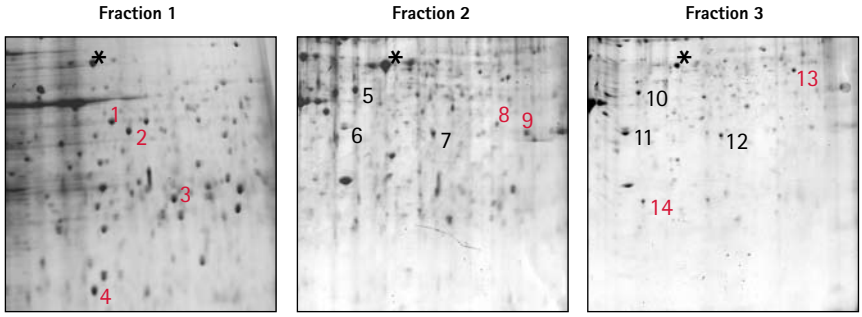

Figure 2. Two-dimentional electrophoresis protein arrays of fractions from A-431 cells following the ProteoExtract® mammalian partial proteome extraction procedure

Spots used as landmark (*) and for identification by nLC-MS/MS peptide mapping (numbers 1–14) are indicated and correspond to the proteins listed in Table 2.

Sample preparation, preparative 2DE, and identification by MS

To validate the findings of the computer-assisted image analysis by direct protein identification using mass spectrometry, preparative 2DE was performed. For preparative loading on IPG-strips, aliquots of fractions 1–3 from A-431 cells were precipitated using TCA/acetone (3) and pellets resuspended directly in a suitable volume of extraction reagent. Extraction Reagent 2 was used for fractions 1 and 2 and Extraction Reagent 3 for fraction 3. The IEF was performed with 1 mg protein per 17-cm pH 4–7 immobilized pH gradient strips for 50,000 Vh according to standard focusing conditions. Following SDS-PAGE using 13% gels, proteins were visualized by colloidal Coomassie blue staining (Patton 2002). The different partial proteomes of fractions 1–3 are shown in Figure 2. The predominant protein spot present in all three fractions (indicated by an asterisk) was used as a landmark during gel matching. This spot represents ER 60 protease as identified by MS (data not shown). Representative spots chosen for each fraction, were excised from the gel and the proteins were fragmented using trypsin according to standard protocols (Shevchenko 2002). Proteins were identified by peptide mapping using a capillary HPLC with 100 µm Chromolith® CapRod® monolithic silica capillary (at 3 µl/min flow rate), interfaced with a standard ESI ion source of an IonTrap Mass Spectrometer and the Mascot® search engine (Perkins 1999).

Protein identification by MS and MS/MS of the four spots picked from the gel array of fraction 1 (spots 1–4 in Figure 2) revealed readily soluble proteins of intermediate to high abundance (Table 2). Since the image analysis indicated that the divergence seemed to be smallest between the partial proteomes of fractions 2 and 3, three pairs of protein spots were chosen that appeared to be common between the two fractions. Spots 5 and 10, 6 and 11, and 7 and 12 (Figure 2) were either more prominent, less prominent, or equally prominent in fractions 2 and 3, respectively. MS and MS/MS analysis confirmed that the spots represent identical proteins of high abundance such as actin and ribosomal proteins (Table 2). Despite the fact that high-abundance proteins can be present in more than one fraction, protein identification of other spots, such as spots 8, 9, 13, and 14, revealed that in later fractions of the sequential extraction, membrane-associated and less-soluble proteins predominate. For example, annexin I could be identified in fraction 2 but not in fraction 3 and vice versa for NADPH reductase.

The reagents and the protocol of the extraction kit have been optimized to solubilize proteins in distinct partial proteomes, however the degree of redundancy between the different protein arrays obtained will depend on the type of cells or tissue and their condition. Therefore, in some cases it might be advantageous to perform the extraction in only three, rather than four, sequential steps, using Extraction Reagent 1, followed by either Extraction Reagent 2 or 3, and finally Extraction Reagent 4. ▶▶

Conclusions

The sequential proteome extraction procedure results in distinct and reproducible partial proteomes with reduced complexity, thereby facilitating an easier computer-assisted image analysis. The discovery of low-abundance, low-solubility proteins is considerably enhanced by 2DGE arrays that lack the most soluble and highly abundant proteins. ■

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Table 2. MS and MS/MS analysis

Fraction	Spot	Assignment	Mascot Mowse score	Sequence coverage (in %) MS	MS/MS
1	1	alpha enolase	410	66, 8	27, 6
	2	pyruvate kinase	476	43, 3	20, 5
	3	triosephosphate isomerase	578	86, 7	45,2
	4	statmin	173	59, 7	26, 8
2	5	ubiquinol-cytochrome c reductase core protein I	307	34, 8	17, 3
	6	actin	228	49, 7	19, 0
	7	ribosomal protein p0, acidic	424	56, 5	28, 7
	8	mitochondrial ribosomal protein S22	180	48, 1	13, 3
	9	annexin I	258	47, 8	20, 4
3	10	ubiquinol-cytochrome c reductase core protein I	268	34, 8	11, 7
	11	actin	209	46, 2	19, 6
	12	ribosomal protein p0, acidic	280	44, 4	19, 2
	13	RuvB like 1	234	31, 1	14, 7
	14	NADPH ubiquinone reductase	211	38, 4	19, 4

Product	Cat. No.	Page
ProteoExtract® Complete Proteome Extraction Kit	539779	26
ProteoExtract® Partial Proteome Extraction Kit	539789	27
ProteoExtract® Subcellular Proteome Extraction Kit	539790	28

Convenient and versatile subcellular extraction procedure that facilitates classical protein expression profiling and functional protein analysis*

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Introduction

One of the major challenges in functional proteomics is the separation of complex protein mixtures to allow detection of low-abundance proteins and provide for reliable quantitative and qualitative analysis of proteins impacted by environmental parameters (Banks 2000, Westbrook 2001). Prerequisites for the success of such analyses are standardized and reproducible operating procedures for sample preparation prior to protein separation by one- and two-dimensional gel electrophoresis and/or liquid chromatography (Rabilloud 2000, Krapfenbauer 2001). Due to the complexity of total proteomes, especially of eukaryotic proteomes, and the divergence of protein properties, it is often beneficial to prepare standardized partial proteomes of a given organism to maximize the coverage of the proteome and to increase the chance to visualize low-abundance proteins and make them accessible for subsequent analysis (Krapfenbauer 2001).

Besides expression profiling, activity assays (Adam 2002) or protein interaction studies (e.g., in a microarray format) (Zhu 2001), are extremely informative for elucidating protein function. Such analyses often require that the proteins of interest remain in a nondenatured state. Another valuable and complementary data-set for clarifying protein function is the subcellular localization of proteins (Huber 2003) and the detection of redistribution events within a cell. Changes in the topology of proteins, i.e., spatial rearrangements to another subcellular compartment, are important cellular events and crucial for biological processes such as signal transduction or apoptosis.

Therefore, it is evident that there is an increasing need for standardized but versatile sample preparation methods, which yield the total proteome fractionated into nondenatured subproteomes of decreased complexity and that simultaneously provide topological information. Selective purification of cellular organelles, based on popular cell biological techniques, has been utilized previously for proteome analysis (Jung 2000, Pasquali 2999, Fountoulakis 2001) and has meanwhile led to numerous efforts concerning organelle proteomics (Scianimanico 1997, Bell 2001, Taylor 2000, Fountoulakis 2003). Although this approach can be extremely powerful, examples of which have been described recently (Gagnon 2002, Desjardins 2003), isolation of organelles with high purity is mainly realized by timely and costly techniques such as differential centrifugation and/or affinity enrichment, thereby mostly neglecting the remaining cellular components and the changes therein. Furthermore, the homogenization techniques employed require usually relatively large amounts of starting material and are generally more efficient with tissues than tissue culture cells. Hence, we have systematically investigated scalable biochemical extraction methods, applicable to both adherent as well as suspension-grown mammalian tissue culture cells, that enable simple fractionation of the total proteome into distinct subcellular fractions. Since both membrane proteins and nuclear proteins are of particular pharmacologic interest, a suitable extraction method is desirable that selectively harvests these proteins (which are frequently present in minuscule quantities) into distinct subcellular fractions, separated from high-abundance proteins present in cytosol or cytoskeleton.

Previously, an elegant sample preparation procedure based on differential detergent extraction has been described (Ramsby 1994), however, we have found that the selectivity of extraction, especially for nuclear proteins, was insufficient for our purposes [own unpublished observation and (Chiang 2000)]. As a result, we have studied alternative approaches resulting in an optimized sequential extraction procedure that ensures sufficient selectivity in a convenient and rapid format. The validated procedure has recently become commercially available (Calbiochem ProteoExtract® Subcellular Proteome Extraction Kit). We assessed the selectivity of the developed procedure ▶▶

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Abbreviations: A-431, epidermoid carcinoma cell line; **DAPI**, 4´,6-diamidino-2-phenylindole; **GFP**, green fluorescent protein; **MAPK**, mitogen activated protein kinase; **NFκB**, nuclear factor kappa B; **Saos-2**, osteosarcoma cell line; **TNFα**, tumor necrosis factor alpha

*Adapted from (2004) *Proteomics* **4**, 1397–1405 with permission.

by several independent but complementary methods: morphological analysis, one- and two-dimensional electrophoresis (1DE, 2DE) protein analysis, immunoblot, ELISA, and enzyme activity profiling. To demonstrate the versatility of the extraction method, spatial changes of two important signaling molecules were monitored: mitogen activated protein kinase (MAPK) migrates upon phosphorylation from the cytosol to the nucleus and nuclear factor kappa B (NFκB) relocates from the cytosol to the nucleus upon stimulation with tumor necrosis factor alpha (TNFα).

Materials and methods

Materials

Laboratory chemicals were obtained in extra pure grade from Merck (Darmstadt, Germany), as well as Coomassie Brillant Blue R250, Benzonase® Nuclease, nonfat dry milk, and BSA fraction V. ProteoExtract® Subcellular Proteome Extraction Kit, Protease Inhibitor Cocktail Set III (Cat. No. 539134), Calpain-1 ELISA Kit (Cat. No. QIA118), and Calpain Activity Assay Kit, Fluorogenic (Cat. No. QIA120) are from EMD Biosciences. Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum was from Gibco (Grand Island, NY, USA). ER-Tracker™; MitoTracker®; 4′,6-diamidino-2-phenylindole (DAP); and phallicidin were from Molecular Probes (Eugene, OR, USA). DC protein assay, IPG strips 4–7, carrier ampholyte 3–10, Criterion precast gels, MultiProtean IEF cell, PDQuest™ software, PVDF membrane, and SYPRO® Ruby Protein Stain were from Bio-Rad (Munich, Germany). Primary antibodies that were used in this study were obtained against the following antigens listed from the supplier indicated in parentheses: calpain, actin, NFκB (Calbiochem), heat shock protein 70 (HSP 70), cadherins, cytochrome P450 reductase, c-Fos, cytokeratins, vimentin (Sigma, Steinheim, Germany), c-Jun (BD Transduction Laboratories, Lexington, KY, USA), histone-1 (Santa Cruz Biotechnology, Heidelberg, Germany), p44/42 MAP kinase, and Phospho-p44/42 MAP Kinase (Cell Signaling Technology, Beverly, MA, USA). Conjugated horseradish peroxidase secondary antibody, ECL Western blotting detection reagent and Hyperfilm® were from Amersham Biosciences (Freiburg, Germany). GeneTools® software was from Syngene (Cambridge, UK).

Cell culture and stimulation

An osteosarcoma cell line, Saos-2 (ATCC HTB-85), and an epidermoid carcinoma cell line, A-431 (ATCC CRL-1555) were grown to form subconfluent (80%) layers at 37°C in DMEM supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2. To study NFκB translocation, A-431 cells were stimulated with 0.2 mg/ml TNFα for 0, 5, and 15 minutes, essentially as described previously (Butcher 2001). Following stimulation, cells were put on ice and extracted to generate the four subcellular proteomes.

Subcellular fractionation and protein extraction

The extraction procedure was performed as described in the protocol included with the ProteoExtract® Subcellular Proteome Extraction Kit. Additives such as protease inhibitor cocktail and an unspecific nuclease, Benzonase® Nuclease were included. Adherent-growing cells (such as A-431 cells or Saos-2) allowed the procedure to be performed directly in cell culture flask or coverslips without detachment of the monolayer. In the case of suspension culture cells, tissue pieces, frozen cell pellet, or when adherent cells were detached during extraction, the resulting suspension was transferred into a microcentrifuge tube and pelleted by centrifugation. The supernatants (e.g., cytosolic fraction) were stored at –70°C for later use. A flow chart describing the subcellular fractionation method is depicted in Figure 1.

Fluorescence microscopy

Prior to extraction, live cells were prestained with ER-Tracker™, MitoTracker®, DAPI, or phallicidin as fluorescent markers for endoplasmic reticulum (ER), mitochondria, nuclei, and filamentous actin cytoskeleton, respectively. For use as an indicator for cytosolic extraction, cells were transfected with a plasmid encoding green fluorescent protein (GFP). The treated cells were washed and extracted, and subcellular structures of cells or cell remnants that remained attached during extraction were documented by fluorescence microscopy prior to and after each extraction step. Exposure times for the digital imaging were kept identical throughout the extraction procedure for the individual fluorescent labels and GFP, so that subcellular disintegration, i.e., extraction, leads to a decreased signal strength. In cases where loss of signal was observed following the extraction, phase contrast images were recorded of the identical field to prove that cells or cell remnants were still present. ▶▶

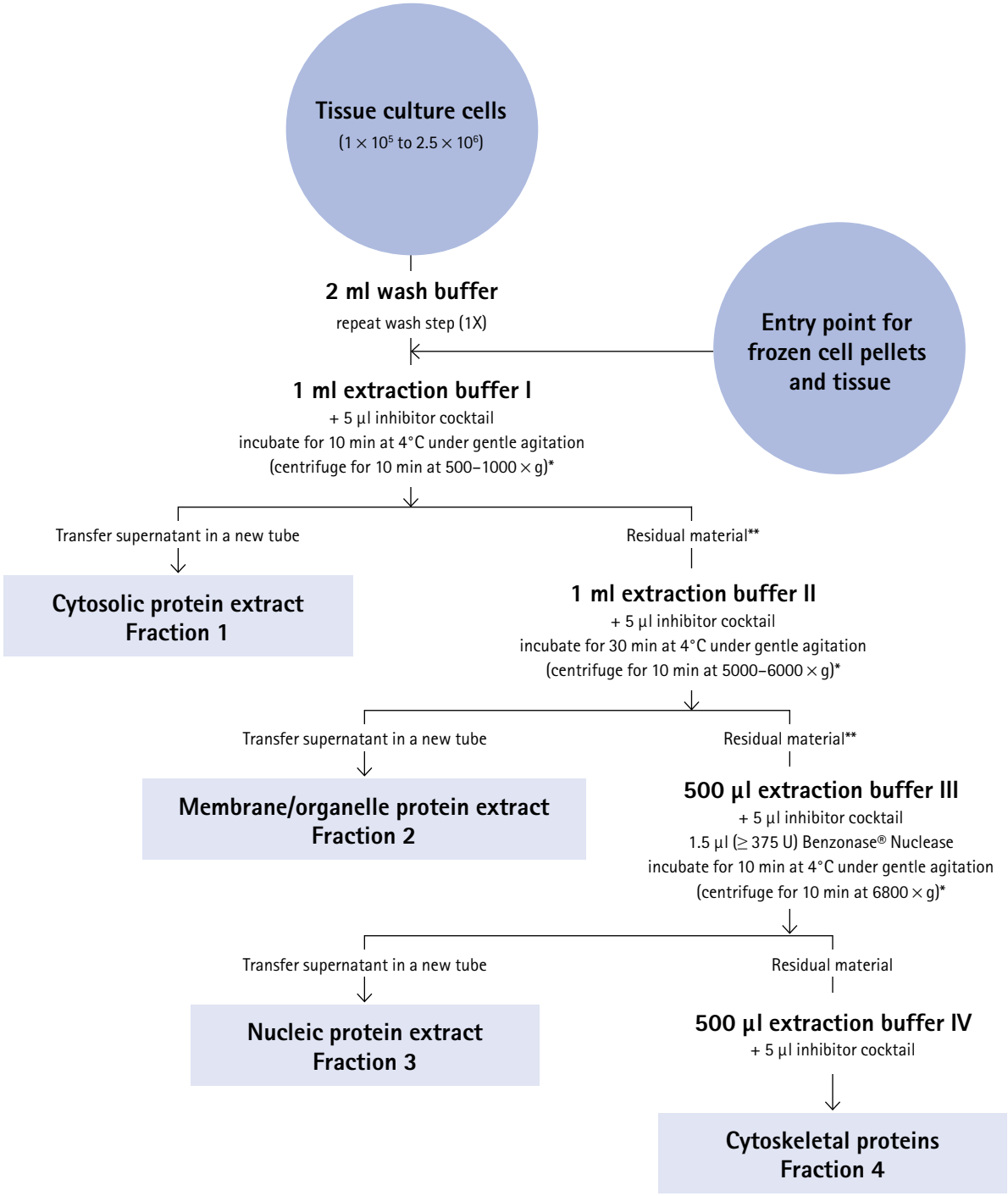

Figure 1. Schematic representation of the subcellular extraction procedure, depicting the individual steps of the sequential biochemical fractionation

The scheme provides the buffer volumes used for approximately 1 × 10⁶ cells. The asterisk (*) indicates that for adherent cells, centrifugation is not required as long as cells or cell remnants remain attached to the growth support.

Gel electrophoresis and gel analysis

In the case of conventional 1DE, volume equivalents of the four subcellular fractions obtained were separated by SDS-PAGE (10% gel) (Laemmli 1970) and stained by Coomassie Blue R-250 (Wong 2000). For 2DE, 200 mg of each subcellular extract was precipitated using TCA/acetone, as previously described (Yuan 2002), and resuspended in 7 M urea, 2 M thiourea, 4% CHAPS, 40 mM DTT, and 0.2% carrier ampholytes pH 3–10. IEF was performed in parallel on 11-cm IPG strips with linear pH gradient from 4–7 in a MultiProtean IEF cell for 38,000 Vh according to standard focusing conditions (Gorg 1999). Following second dimension run with Criterion precast gels (10% resolving, 4% stacking gel with a gel size of 13.3 × 8.7 cm) as previously described (Vidarte 2001), gels were fixed, stained with SYPRO® Ruby, and scanned (Seliger 2003). Comparative analysis of the four subcellular proteomes by 2DE was performed with PDQuest™ software (v6.2.1) following matching using 29 landmarks.

Immunological investigations

For Western blot analysis, proteins were transferred to PVDF membranes after SDS-PAGE. Blots were blocked overnight with 5% nonfat dry milk in PBS and membranes were probed, washed, and developed according to the manufacturer's instructions. Films were scanned by densitometry analysis using GeneTools® Software, essentially as described elsewhere (Tanaka 2002). To be able to detect c-Fos reliably, the protein was immunoprecipitated prior to SDS-PAGE. In addition, calpain was quantified using a commercial ELISA kit (formerly available from Calbiochem) according to the manufacturer's instruction.

Protein determination and enzyme activity analysis

Protein concentrations of extracts were determined by a modified Lowry method (DC protein assay) using BSA as a standard according to the manufacturer's instruction. Calpain activity was measured with a commercial kit according to the manufacture's instruction. Alkaline phosphatase activity was determined as previously described (Broquet 2003). RNAse activity was measured in a proprietary versatile RNAse assay utilized in-house for quality control of molecular biology reagents. Briefly, following incubation with a subcellular sample under investigation, remaining fluorescence is measured after affinity depletion of a biotin-tagged fluorescent probe that carries consensus sequences for numerous RNAses between both the labels. All enzyme activities were measured as the change in signal strength per minute to make sure that measurements were taken within the linear range of the assay used.

Results and discussion

Morphological analysis of the subcellular extraction procedure

For the sequential extraction of cell content, the developed procedure takes advantage of the differential solubility of certain subcellular compartments in special reagent mixtures. The four extraction buffers utilized have been optimized to provide reproducible protein isolation from distinct subcellular compartments. Defined surfactants maintained in select buffers of appropriate ionic and osmotic composition enable selective extraction. A flow chart describing the method to prepare the subcellular extracts is depicted in Figure 1.

The success of such a procedure depends on the preservation of the integrity of the subcellular structures before and during the extraction, which was investigated using adherent-growing A-431 cells. This allows the procedure to be performed directly on chambered coverslips without the need for cell removal. Hence, early destruction of the cellular structure by enzymatic or mechanical detachment of cells from the growth support is prevented. In the case of strongly attached adherent cells, the cells or the parts of the cells that remain upon sequential extraction stay attached to the surface during the extraction procedure, thereby facilitating image analysis. Cells treated with fluorescent dyes that selectively label chosen subcellular compartments were washed and extracted, and subcellular structures of cells or cell remnants that remained attached during extraction were visualized by fluorescence and phase contrast microscopy prior to and after each extraction step (Figure 2). In cases where loss of signal was observed following the extraction, phase contrast images were recorded of the identical field ▶▶

to prove that cells or cell remnants were still present (Figure 2). These results show that the sequential extraction results in a stepwise disintegration of the cell's structure, yielding four subcellular fractions. Upon treatment with the first extraction buffer, cells shrink as a result of the release of the cytosolic content but remain intact in their overall structure (Figure 2, step 1). After the second extraction step, due to the solubilization of membranes and membrane organelles, only isolated nuclei and the cytoskeleton remain intact (Figure 2, step 2). The treatment of the residual material with the third extraction buffer destroys the structure of the nucleus (Figure 2, step 3), thus probably solubilizing the nuclear proteins. Finally, the cytoskeleton components are liberated during the fourth and final extraction (Figure 2, step 4).

Protein pattern analysis of the subcellular extracts

The selectivity of the procedure was further investigated by 1DE and 2DE protein profiling. A typical result of the 1DE and 2DE protein profiling with subcellular extracts generated from Saos-2 cells is depicted in Figure 3, panel A, and Figure 4, respectively. Comparative analysis of the four subcellular proteomes by 2DE, which is depicted in complementary colors for the cytosolic (red), membrane/organelle (green), nuclear (dark blue), and cytoskeletal (gray green) fractions in Figure 4, revealed that more than 35% of the total protein spots visualized (45% in the given example) are detected in only a single subcellular fraction (see also Table 1). Examples of such protein spots are SSP 6103, 7206, 6611, and 7010 in Figure 4. Spots present in all four fractions appear light blue in color. More detailed quantitative analysis revealed that for more than half (62% in the given example) of the visualized proteins, at least 80% is detected in a single subcellular fraction. The protein overlap between neighboring fractions is ≤ 25%, resulting in protein patterns of membrane/organelle and nuclear fractions that are clearly distinct from the protein patterns of the cytosolic and the cytoskeletal fractions. ▶▶

Table 1. Computer-assisted image analysis indicated a total of 1107 unique spots in matched gels of the sequential extracts. By deducing the number of protein spots detected in more than one subcellular fraction, 499 spots (i.e., 45% of the total of 1107 spots detected) are solely found in a single fraction. See the "Protein pattern analysis of the subcellular extracts" section and Figure 4, page 71, for further details.

Subcellular fraction	Total spots	Unique spots
Cytosolic	572	200
Membrane/organelle	484	111
Nuclear	393	99
Cytoskeleton	272	89
Combined master gel	1107	499

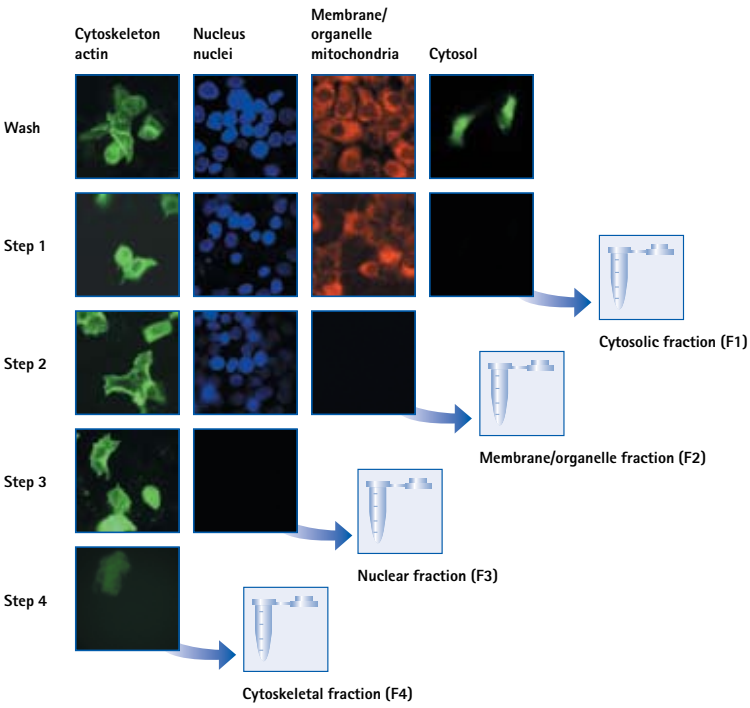

Figure 2. Visualization of subcellular extraction procedure selectivity using morphological analysis of adherently growing A-431 cells

By stepwise disintegration of the cells' structure upon sequential extraction, the developed method selectivity extracts cellular proteins into four fractions: cytosolic (F1), membrane and organelle-localized (F2), soluble and DNA-associated nuclear (F3), and cytoskeletal (F4) proteins. Recombinant GFP was used as cytosolic marker, MitoTracker® and ER-Tracker™ as membrane stains, DAPI to visualize nuclei, and phallicidin to visualize filamentous actin skeleton.

Thus the subcellular prefractionation can increase the chance to visualize low-abundance proteins in membranes and organelles and nuclei since these are efficiently separated from high-abundance proteins commonly found in the cytosol and cytoskeleton that might otherwise hamper analysis. Intriguingly, although some proteins are extracted in multiple subsequent fractions (e.g., SSP 2101, Figure 4), the majority (on average 70% in example) of the proteins detected in more than one subcellular fraction do not overlap with adjacent fraction(s); see for example SSP 1503, 2603, and 1101 in Figure 4. As many of these are also located near landmarks that were set, it is unlikely that this results from misalignment during matching. Therefore, this probably reflects a functional relevance rather than resulting from inefficient extraction or mismatching.

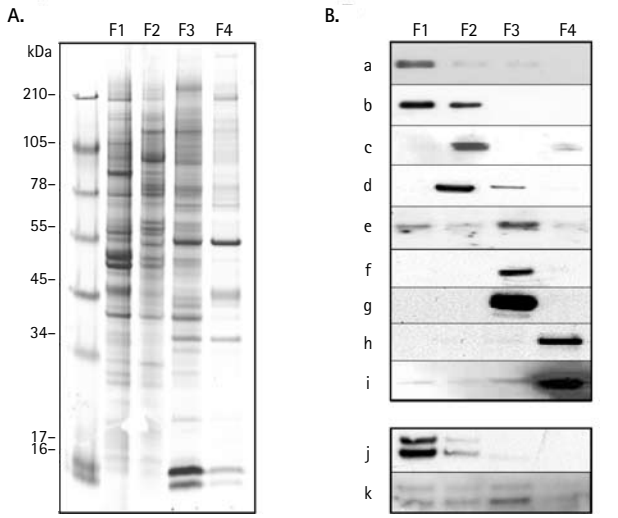

Figure 3. Selectivity of the subcellular extraction procedure is demonstrated by 1DE protein profiling and by monitoring marker protein distribution of obtained subcellular fractions

Visualization of total protein by SDS-PAGE and Coomassie staining revealed clearly distinct protein profiles of the individual fractions obtained (panel A). Fractionated proteins were also subjected to immunoblot analysis, revealing a distinct topological distribution of the analyzed marker proteins to individual subcellular fractions. Marker proteins of the cytosol (a, calpain; b, HSP 70) were mainly detected in fraction 1, membrane and organelle markers (c, cadherins; d, cytochrome P450 reductase) were predominantly in fraction 2, transcription factors as well as DNA-associated proteins (e, c-Fos; f, c-Jun; g, histone 1) were in fraction 3, and cytokeratins and vimentin representing cytoskeletal markers (h and i, respectively) were found in fraction 4. To study changes in protein topology upon post-translational modification, mapping of total p44/42 MAPK (j) and phosphorylated p42/42 MAPK (k) revealed that the phosphorylated protein form is mainly present in the nucleus, i.e., fraction 3, even though p44/42 MAPK as such is found by and large in the cytosol, i.e., fraction 1.

Immunological analysis of the subcellular extracts

The efficiency and selectivity of the subcellular extraction procedure was further investigated by immunological analysis using both Western blot and ELISA. Following SDS-PAGE, proteins were electroblotted to membranes and probed with antibodies against calpain and HSP 70 as cytosolic markers (a and b in Figure 3, panel B), cadherins and cytochrome P450 reductase as membrane markers (c and d), c-Fos, c-Jun, and histone-1 as soluble and DNA-associated nuclear markers (e, f, and g), and cytokeratins and vimentin as cytoskeletal markers (h and i). Immunoblotting revealed that the majority of marker proteins were primarily (0.85%) detected in distinct subcellular fractions and that the distribution of markers expected to be localized within the same subcellular context was virtually identical. Please note that HSP 70 is known to be present in both the cytoplasm as well as associated with cell membranes (Broquet 2003), reflected by detection in both the cytoplasmic and membrane/organelle fraction after subcellular extraction. In the case of actin, however, immunodetection revealed that the protein was not only present in the cytoskeletal fraction, but also in the cytosolic and to some extent in the membrane/organelle and nuclear fractions as well (data not shown). The cytosolic fraction most likely corresponds to soluble actin monomers, which are not detected by the phallicidin stain during morphological analysis. Independently, calpain was quantified using an ELISA, providing highly comparable results to the data collected by immunoblotting (data not shown). Taken together, the immunological data are consistent with the morphological findings and demonstrate the efficiency and selectivity of the subcellular extraction procedure at the protein level. Thus the developed procedure readily fractionates proteins according to their subcellular localization, yielding four subproteomes enriched in (a) cytosolic, (b) membrane/organelle-localized, (c) soluble and DNA-associated nuclear, and (d) cytoskeletal proteins. In addition to the analysis for the above-mentioned marker proteins, the subcellular distribution of p44/42 mitogen-activated protein kinase (MAPK) was investigated by immunoblotting as described, using commercial antibodies detecting total (phosphorylation-state independent) p44/42 MAPK (j in Figure 3, panel B), as well as antibodies that detect endogenous levels of the kinase only when it is phosphorylated (k in Figure 3, panel B). MAPKs are known to mediate signaling from the cell membrane to the nucleus upon phosphorylation at conserved threonine and tyrosine residues within their activation loops ▶▶

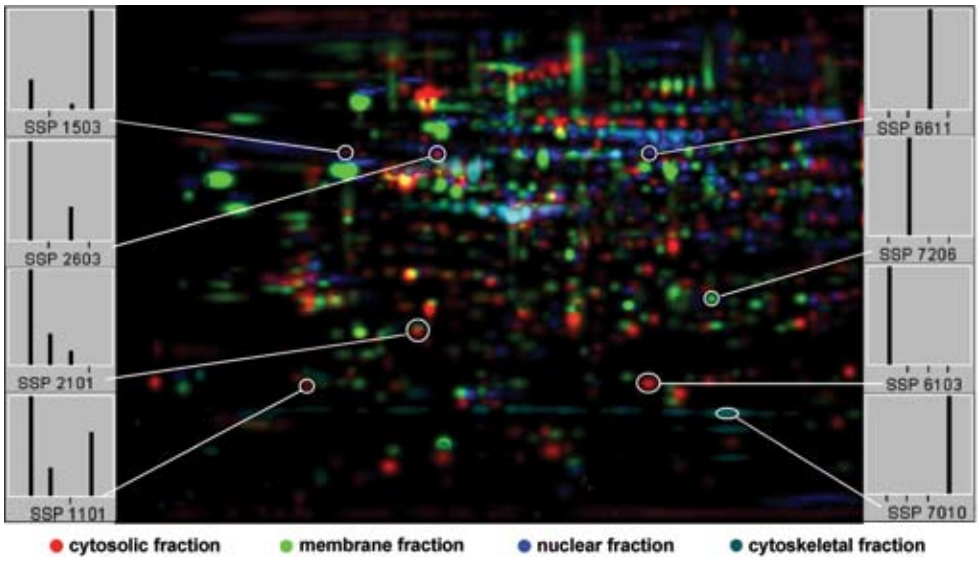

Figure 4. Separation and staining of subcellular proteomes by 2DE reveals clearly distinct protein profiles in the collected fractions.

Two hundred micrograms from each sequentially extracted subcellular fraction was separated on linear 4–7 IPG strips and 10% PAGE gels and visualized by staining with SYPRO® Ruby. Gel images were matched using PDQuest™ software and artificially colored to represent individual subfractions: red for the cytosolic, green for the membrane/organellar, dark blue for the nuclear, and gray green for the cytoskeletal subcellular fraction. See Table 1, page 69, for further details.

(Marshall 1995, Toledano-Katchalski 2003). Quantification by densitometric analysis revealed that although 0.80% of total p44/42 MAPK was detected in the cytosolic fraction under steady-state conditions, about 50% of the phospho-p44/42 MAPK was present in the nucleus (j and k in Figure 3, panel B, respectively). These results indicate that the subcellular extraction procedure facilitates studies that monitor changes in protein topology induced by transient post-translational modification, such as after phosphorylation.

Enzymatic analysis of the subcellular extracts

During the optimization phase of the extraction method, we found that extracts from Chinese hamster ovary (CHO)-K1 cells expressing *Renilla* luciferase maintained luciferase activity (data not shown), despite this enzyme being sensitive to denaturation. Encouraged by these findings, we set out to test chosen endogenous enzyme activities after subcellular fractionation of Saos-2 cells. As a cytosolic marker, calpain activity was measured and alkaline phosphatase activity was used as membrane marker. Finally, as a surrogate enzyme marker for both membrane-associated (Egesten 1997) and nuclear fractions (Frank 1998), RNAse activity was measured. Subcellular fractions were thus assayed and the measured relative enzymatic activities graphically displayed (Figure 5). The activity profiling independently confirms the selective separation of cellular components according to their subcellular localization. Moreover, it also demonstrates that

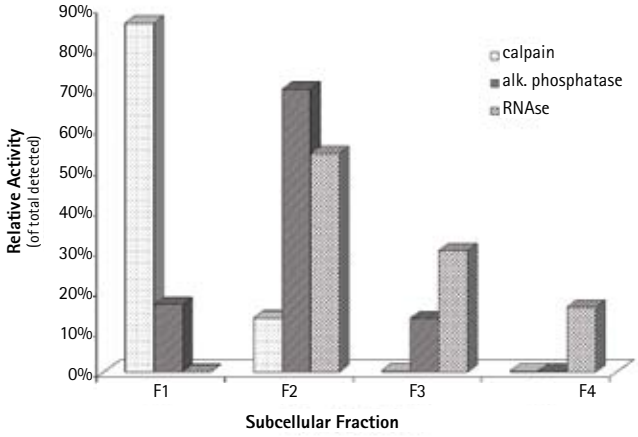

Figure 5. Documentation of enzyme activities in the obtained subcellular fractions

Respective fractions were assayed for three endogenous enzymatic markers: calpain activity (localized to cytoplasm), alkaline phosphatase activity (membrane-associated), and RNAse activity (membrane- and nuclear-associated). The activity profiling independently confirms selective separation of cellular components according to their subcellular localization and demonstrates that the assayed components are extracted in an active and thus functional state.

the assayed components are extracted in an active and thus functional state.

Utilization of the subcellular extraction procedure to monitor redistribution of regulatory proteins

The developed subcellular proteome extraction procedure should be suitable to investigate changes in subcellular localization of regulatory proteins impacted by ▶▶

experimental or disease parameters. A strong indication for this were the encouraging results with p44/42 MAPK (Figure 3, panel B, j and k). To demonstrate this application we chose the well-described translocation of NFκB from the cytosol to the nucleus upon stimulation of cells with TNFα (Mejdoubi 1999, Butcher 2001). NFκB translocation, studied in A-431 cells stimulated for different times with TNFα, was detected and quantified using densitometric analysis of immunoblots. Time course analysis demonstrates a measurable translocation of NFκB from the cytoplasm to the nucleus in as little as 5 minutes with a stronger response observed at 15 minutes (Figure 6) while the control protein calpain did not translocate between fractions upon TNFα-stimulation of cells. Therefore, the subcellular extraction method allows the assessment of spatial rearrangements of various proteins.

The subcellular extraction procedure has successfully been used with a variety of adherent and nonadherent tumor cells. The procedure is scalable and has routinely been used with 4×10^5 to 2×10^7 cells. Although the relative protein amounts obtained vary slightly from cell type to cell type, the overall reproducibility is satisfying with CV < 10%. Lastly and importantly for the investigation of clinical and pharmacological relevant samples, the protocol has been applied successfully with frozen cell pellets and with dissected colon carcinoma tissue that had been fragmented into cell clusters.

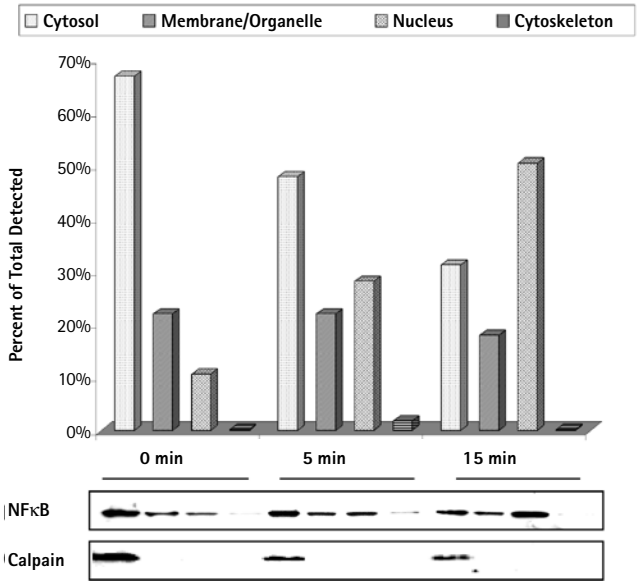

Figure 6. Feasibility demonstration of exploiting the subcellular extraction procedure to detect and measure redistribution of proteins affected by disease or experimental factors

NFκB redistribution was recorded in a time course analysis in A-431 cells that were stimulated with TNFα by quantification of immunoblots generated with the subcellular fractions and anti-NFκB antibodies. The time course analysis demonstrates a measurable translocation of NFκB from the cytoplasm to the nucleus within 5 min with a stronger response observed at 15 min upon cell stimulation, whereas the control protein calpain does not change its topology.

Concluding remarks

Although the purity of the four subcellular fractions resulting from sequential extraction might not be as high as with dedicated purification techniques for the isolation of singular organelles, the procedure has been shown to be very selective and provides for enriched membrane/ organellar or nuclear proteins in solution, separated for the most part from cytosolic and cytoskeletal proteins. The procedure is applicable to a wide variety of mammalian cellular sources, is scalable, fast, simple, and robust. The developed method has a broad application range and facilitates protein expression profiling and functional analysis of a vast array of proteins, including mode of action studies. Finally, the sequential extraction procedure can also be advantageous for protein-interaction studies (e.g., with immobilized baits), as it extracts proteins in a functional state and it decreases the risk of false positives when probing with the relevant subcellular fraction. ▶▶

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Product	Cat. No.	Page
ProteoExtract® Subcellular Proteome Extraction Kit	539790	28

Preserve phosphorylation state and kinase activity in cell extracts*

Scott Hayes, Yuping Ambuel, David Bartnicki, and Michael Batenjany — EMD Biosciences/Novagen

Here we highlight the advantages of combining the PhosphoSafe™ Extraction Reagent with both the ProteoEnrich™ ATP-Binders™ Kit (Bartnicki 2004), which extracts phosphorylated proteins, and the Calbiochem K-LISA™ PTK Screening Kit, which measures protein tyrosine kinase (PTK) activity. We prepared extracts from rat L6 myoblasts with PhosphoSafe Extraction Reagent and CytoBuster™ Protein Extraction Reagent under identical conditions (Trim 2001). The PhosphoSafe Buffer is based on the same formulation as CytoBuster Reagent, but includes four phosphatase inhibitors (sodium fluoride, sodium vanadate, β-glycerophosphate, and sodium pyrophosphate) to help preserve phosphorylation throughout extraction and processing. Western blot analysis assessed by an antibody to phosphorylated myosin light chain 2 (anti-phospho-MLC2; Thr18/Ser19) demonstrated specific recognition of the phosphorylated MLC2 with both reagents (Figure 1). The signal intensity increased dramatically in samples prepared with the PhosphoSafe Buffer, indicating that a greater number of the phosphorylated residues remained intact.

To test whether PhosphoSafe Extraction Reagent could maintain the phosphorylation state of target proteins through multiple processing steps, we prepared cell extracts from A-431 human cell lines using PhosphoSafe Buffer and processed the extracts with the ProteoEnrich ATP-Binders Kit. The ATP-Binders protocol involves buffer exchanges, multiple wash steps, and an overnight dialysis at 4°C. We analyzed the ATP-binding protein–enriched fraction for Src, a known ATP-binding protein, by Western blotting (Figure 2). The blot revealed multiple bands, indicating that the PhosphoSafe Extraction Reagent enabled retention of multiply phosphorylated forms of Src. ▶▶

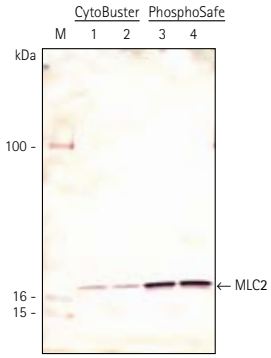

Figure 1. Detection of phosphorylated MLC2

Monolayers of subconfluent L6 myoblasts were extracted with CytoBuster Reagent or PhosphoSafe Buffer for 10 min at room temperature. Extracts were centrifuged and assayed for protein concentration using BCA. Duplicates of each extract (10 µg) were separated by SDS-PAGE (4–20% gradient gel). After transfer to nitrocellulose, protein phosphorylation state was probed with anti-phospho-MLC2 primary antibody. Goat Anti-Rabbit IgG AP Conjugate was added and detected by staining with the AP Detection Reagent Kit.

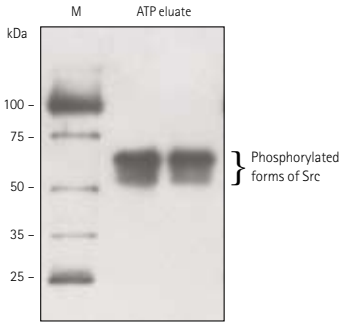

Figure 2. Detection of phosphorylated Src

Cell extract from the A-431 cell line was prepared by the standard PhosphoSafe Extraction Reagent protocol and dialyzed to remove endogenous ATP. Dialyzed extract was processed according to the standard ProteoEnrich ATP-Binders Kit protocol (Trim 2001). Duplicate extract samples (5 µg) were separated by SDS-PAGE (4–20% gradient gel). After transfer to nitrocellulose, phosphorylated Src was detected with a 1:500 dilution of primary antibody for rabbit anti-human Src, followed by an Anti-Rabbit IgG, H & L Chain Specific (Goat) Peroxidase Conjugate. Detection was by chemiluminescence using SuperSignal® HRP Substrate.

*Adapted from *inNovations* **20**, 14, (2004).

To measure PTK activity of different human cell line extracts (HeLa, MCF-7, HEK 293, and A-431) prepared with PhosphoSafe™ Extraction Reagent, we used the K-LISA™ PTK Screening Kit (Calbiochem Cat. No. 539701). The kit uses an ELISA-based, colorimetric assay and plate format that includes a multiplex array of three synthetic polypeptide PTK substrates composed of glutamic acid, alanine, and tyrosine (E_4Y, EAY, and EY) to differentiate substrate specificity. Using the kit, we quantified PTK activity in less than two hours. Each of the kinase-containing extract and peptide substrate combinations produced activity levels significantly higher than the corresponding background (Figure 3).

Based on these data, we conclude that extracts prepared with PhosphoSafe Extraction Reagent offer significant advantages over preparations lacking phosphatase inhibitors. Specifically, the inhibitors in this formulation preserve a protein's phosphorylation state and maintain enzymatic activity. Extracts prepared with this buffer are compatible with Western blot, kinase activity, protein interaction, and other analysis methods. ■

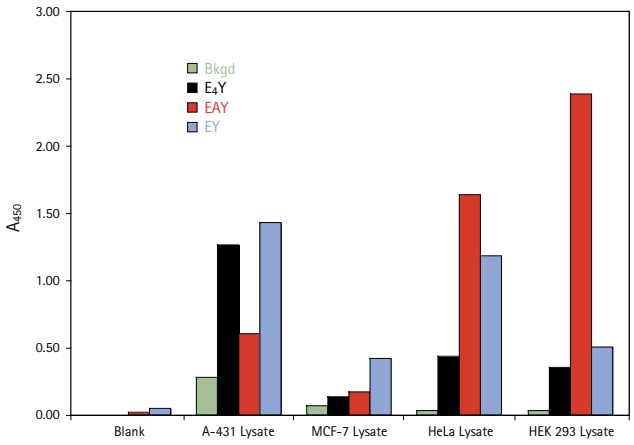

Figure 3. Detection of tyrosine kinase activity

Crude lysates from A-431, MCF-7, HeLa, and HEK 293 cells prepared with PhosphoSafe buffer were diluted in kinase reaction buffer and tested for enzymatic activity in an ELISA format using a synthetic set of tyrosine kinase substrates, E_4Y, EAY, and EY, as provided in the K-LISA PTK Screening Kit (Calbiochem Cat. No. 539701). Substrate phosphorylation was detected using Anti-Phosphotyrosine (PY20) (Mouse) Peroxidase Conjugate (Calbiochem Cat. No. 525320).

References:

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Product	Cat. No.	Page
CytoBuster™ Protein Extraction Reagent	71009	24
PhosphoSafe™ Extraction Reagent	71296	25
ProteoEnrich™ ATP-Binders™ Kit	71438	38

Automated solubility screening of recombinant proteins in a 96-well format*

Anthony Grabski, Don Drott, and Mark Mehler—EMD Biosciences/Novagen

We describe here a novel HT–compatible method for protein solubility screening. The procedure incorporates a nonfouling filtration plate capable of retaining insoluble inclusion bodies while allowing soluble proteins to be collected for rapid quantification and analysis.

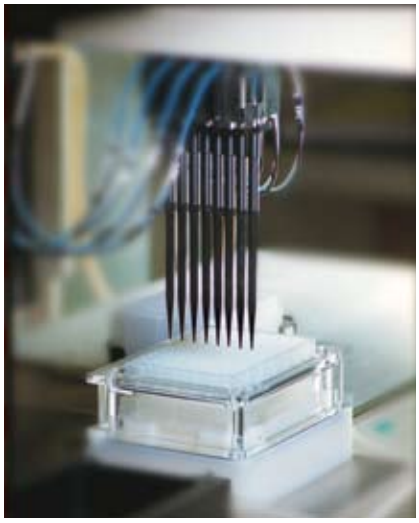


Figure 1. MultiPROBE® II HT EX workstation set up for automated solubility screening

Table 1. RoboPop™ solubility screening protocol

1. Culture *E. coli* cells in liquid medium under conditions for target protein production.
2. Add 0.1 culture volume premixed PopCulture® Reagent + Lysonase™ Bioprocessing Reagent to each culture, mix, and incubate 10 min at room temperature.
3. Place the 96-well Collection Plate and Filter Plate into the vacuum manifold.
4. Transfer 200 µl extract from each culture to the 96-well Filter Plate.
5. Apply vacuum*, collecting the flow-through containing soluble proteins in the Collection Plate.
6. Remove the Collection Plate containing the soluble fraction and replace it with a new Collection Plate.
7. Add 200 µl 4% SDS denaturing solution to each well of the Filter Plate and incubate 10 min at room temperature. This step solubilizes the inclusion body fraction.
8. Apply vacuum*, collecting the solubilized proteins in a Collection Plate.
9. Quantify target proteins present in soluble and insoluble fractions (step 5 and step 9, respectively). His•Tag® fusion proteins may be detected by Western blotting or ELISA using the His•Tag Monoclonal Antibody or His•Tag Antibody Plates, respectively. S•Tag™ fusion proteins may be quantified directly using the FRETWorks™ S•Tag Assay Kit (Novagen Cat. No. 70724-3). Activity of native proteins may be assayed directly from the soluble fraction. SDS-PAGE followed by staining may also be used for highly expressed proteins.

* Samples may be collected by substituting centrifugation of the Filter Plate over the Collection Plate at 2000 × g for 5 min.

Bacterial expression systems are frequently employed to produce large quantities of heterologous protein for structural and functional analysis irrespective of the source, sequence, or abundance of the protein in its natural host. Unfortunately, the ease and efficiency of bacterial expression systems for recombinant protein production do not always correlate with high yield of soluble, correctly folded, active protein. Instead, insoluble inclusion bodies are formed due to rapid, high expression of the protein, inadequate or low concentrations of chaperone helper proteins, complexities of folding, and limited solubility of folded domains (Mukhopadhyay 1997). The yield of soluble, correctly folded protein can often be increased by optimizing the primary sequence of the target protein, the genetic background of the host strain, and growth conditions, such as temperature and induction methods (Wigley 2001).

Conventional methods for screening the effectiveness of these solubility optimization experiments are tedious and inefficient. These methods typically involve cell harvest by centrifugation, mechanical disruption by sonication or French press, and separation of soluble proteins from insoluble proteins, debris and residual intact cells by a second centrifugation step. The soluble supernatant fraction is subsequently analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot, and/or activity assay to detect and quantify the presence and quality of the target protein.

Parallel processing of hundreds of samples for high-throughput (HT) proteomics research requires biological, chemical, and engineering solutions to eliminate tasks that are difficult to automate, such as sonication and centrifugation, while minimizing multiple processing steps. We have developed specialized lysis reagents that eliminate the need for mechanical disruption of cells. These reagents include BugBuster®, YeastBuster™, and CytoBuster™ protein extraction reagents, for *E. coli*, yeast, and insect or mammalian cells, respectively. The effectiveness of BugBuster Reagent and the need to simplify the purification process ▶▶

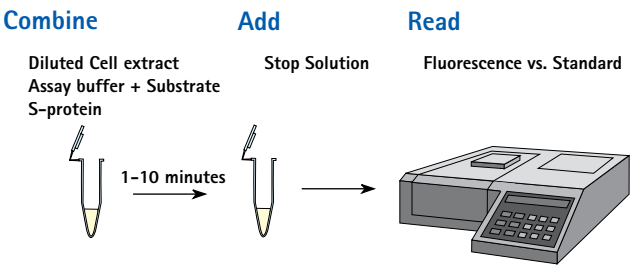
*Adapted from *inNovations* **16**, 11–13, (2003).

for automation led us to the development of PopCulture® Reagent. PopCulture Reagent is a concentrated mixture of specialized detergents that when combined with rLysozyme™ Solution and Benzonase® Nuclease enables extraction and purification of recombinant proteins from *E. coli* directly from the culture media without cell harvest, mechanical disruption, or extract clarification. The combined activities of rLysozyme Solution and Benzonase Nuclease are now available as Lysonase™ Bioprocessing Reagent. Lysonase Reagent significantly increases protein extraction efficiency and reduces sample viscosity, thereby facilitating downstream processing and robotic pipetting. PopCulture, rLysozyme, Benzonase, affinity resins, and plasticware are conveniently formatted into the RoboPop™ Protein Purification Kits for automated protein purification from *E. coli* or insect cells.

We have now expanded the applications of PopCulture and Lysonase Reagent for use in parallel or automated expression-level solubility screening. We describe here a novel high throughput–compatible method for protein solubility screening.

The procedure incorporates a nonfouling filtration plate capable of retaining insoluble inclusion bodies while allowing soluble proteins to be collected for rapid quantification and analysis. Insoluble proteins retained by the filtration plate are solubilized with 4% SDS denaturing solution, collected, and quantified separately. The protocol has been automated on the MultiPROBE® II from PerkinElmer Life Sciences (Figure 1). The general protocol we used for automation is described in Table 1.

Figure 2. FRETWorks™ S•Tag Assay



FRETWorks S•Tag Assay

Substrate	FRET ArUAA
Endpoint	fluorescence (520 nm)
Elapsed time	< 10 min
Detection limit	< 1 fmol

Solubility screening of fusion proteins from *E. coli* total culture extracts

We used four different plasmid recombinants for expression-level solubility screening, as listed in Table 2 . All of these fusion proteins can be purified by immobilized metal chelation chromatography using His•Bind® affinity resins and detected using either the His•Tag® Monoclonal Antibody or the FRETWorks™ S•Tag™ Assay (Figure 2). Figure 3 shows the results of a solubility screening experiment. The data demonstrate the effectiveness of the RoboPop Solubility Screening Kit for fractionation and quantification of the expressed soluble and insoluble target protein from each construct. The SDS-PAGE analysis (Figure 3, panel A) shows that under the expression conditions tested, GUS was entirely insoluble, β-gal was approximately 50% soluble, NusA was approximately 90% soluble, and GST was approximately 75% soluble. Although the fractions obtained with the kit may be analyzed by conventional SDS-PAGE to estimate the degree of soluble target protein expression, sensitivity and throughput are low and quantification is difficult by this method. A highly sensitive, robot-friendly protocol has been developed to quantify S•Tag fusion proteins in crude extracts using the homogeneous FRETWorks S•Tag Assay (3). This assay is based on the affinity of the 15–amino acid S•Tag peptide in the fusion proteins for the 104–amino acid S-protein in the assay buffer. The S•Tag–S-protein interaction reconstitutes ribonuclease activity, cleaving the FRET substrate and producing a fluorescent signal as the quencher is released from the fluorescent molecule. Results of the FRETWorks assay for quantification of GUS, β-gal, NusA and GST (Figure 3, panel B) correlated well with the SDS-PAGE analysis of the same samples. The RoboPop Solubility Screening Kit may also be used without the aid of a robotic platform by employing simple multichannel pipetting for the robotic liquid handling steps, and vacuum filtration may be replaced by centrifugation in the robotic or manual protocol. ▶▶

Table 2. Vector constructs used for analysis in Figure 3

Vector/construct	Fusion protein – "name"	Expected size
pET-41b(+)	GST•Tag™/His•Tag/S•Tag – "GST"	35.6 kDa
pET-30b(+)/β-gal	His•Tag/S•Tag/β-gal – "β-gal"	121 kDa
pET-43.1b(+)	Nus•Tag™/His•Tag/S•Tag/HSV•Tag® – "NusA"	66.4 kDa
pTriEx™-4/GUS	His•Tag/S•Tag/GUS/HSV•Tag – "GUS"	73.5 kDa

Summary

The RoboPop™ Solubility Screening Kit increases the efficiency of solubility optimization by streamlining tedious cell lysis, extract fractionation, and sample analysis procedures. The screening kit provides a robot-friendly alternative to conventional labor-intensive, expression-level protein solubility screening through PopCulture® cell lysis and HT 96-well filtration-based fractionation. We have combined the chemistry of PopCulture, the biological activities of Lysonase™ Reagent, and the engineering of an innovative Filtration Plate to eliminate common bioprocessing bottlenecks. Together, the RoboPop Solubility Screening Kit and RoboPop purification kits allow rapid identification and selection of ideal host-vector combinations, expression conditions, and purification parameters toward production of proteins for structural or functional analysis.

References

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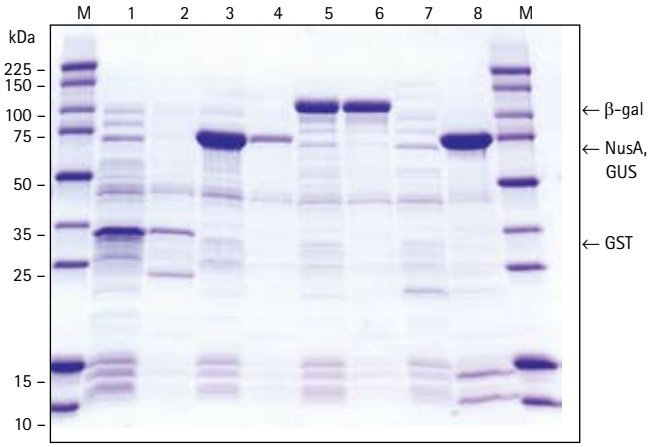
Mukhopadhyay, A. 1997. *Adv. Biochem. Eng. Biotechnol.* **56,** 61.
Wigley, W.C., et al. 2001. *Nature Biotechnol.* **19,** 131.

See the following pages for related product details and ordering information:

Product	Cat. No.	Page
Lysonase™ Bioprocessing Reagent	71230	18
PopCulture® Reagent	71092	47
RoboPop™ Solubility Screening Kit	71255	48

A. SDS-PAGE analysis of soluble and insoluble fractions



Lane	Sample
M	Perfect Protein™ Markers, 10–225 kDa
1	GST, soluble
2	GST, insoluble
3	NusA, soluble
4	NusA, insoluble
5	β-gal, soluble
6	β-gal, insoluble
7	GUS, soluble
8	GUS, insoluble

B. FRETWorks S•Tag Assay of soluble and insoluble fractions

Construct	Fraction	pmol/ml	µg/ml	% in fraction
GST	soluble	116	209	65.1
	insoluble	62.2	112	34.9
NusA	soluble	33.2	121	74.7
	insoluble	11.2	41	25.3
β-gal	soluble	33.7	202	44.7
	insoluble	41.7	250	55.3
GUS	soluble	1.0	3	0.7
	insoluble	140	477	99.3

Figure 3. Solubility screening using four different vector constructs

Cultures of *E. coli* strain BL21(DE3) containing the vector constructs described in Table 2 were grown at 37°C to an OD_{600} = 1.5 and target protein expression was induced by adding IPTG to a final concentration of 1 mM. Following incubation for approximately 3 h at 30°C (final OD_{600} = 5–6), the cultures were dispensed (1 ml/well) into sequential rows of 2-ml 96-well plates, and 100 µl PopCulture Reagent containing 2 µl Lysonase Reagent was added to each well. The procedure described in Table 1 (page 77) was used to generate soluble and insoluble protein fractions. Panel A shows SDS-PAGE analysis (Coomassie blue staining) of the indicated samples (15 µl extract). Panel B shows the results of the FRETWorks S•Tag Assay performed with the same fractions. For the assay, samples were serially diluted 1:25 to 1:2500, and the dilutions analyzed according to the standard protocol (20 µl diluted sample was used per assay). The S•Tag fusion proteins were quantified with a standard curve based on known amounts of S•Tag Standard.

NEW Convenient optimization of protein refolding conditions with iFOLD™ Protein Refolding System 1*

Peter Leland, James Rane, and Anthony Grabski — EMD Biosciences, Novagen

Introduction

Proteomic studies often require large quantities of very pure and correctly folded protein. *E. coli* expression systems are commonly used to produce these recombinant proteins because these systems are generally simple and fast, and have the potential to express significant quantities of target protein. However, overexpressing recombinant proteins in *E. coli* can result in the formation of inclusion bodies, which is usually perceived as a problem.

Inclusion bodies actually have some unique and beneficial attributes: they are highly enriched for the target protein, resistant to proteolysis, and easily separated from other host cell components. After purification, inclusion bodies can be solubilized in a buffer containing a chaotrope and a reducing agent. However, defining the conditions necessary to convert an unfolded inactive protein that is soluble only in the presence of a chaotrope, to a correctly folded active protein that is soluble in a physiological buffer, can be both arduous and time consuming. Typically, an efficient refolding buffer can be identified only after several rounds of empirical testing.

The iFOLD™ Protein Refolding System 1 is designed to simplify traditional refolding screening methods. The system provides a comprehensive set of refolding buffers, arrayed in a 96-well plate (Figure 1, p. 106). Optimal refolding conditions for a target protein are determined by simultaneously evaluating 92 different buffers. In addition to the pre-dispensed refolding plate, the iFOLD Protein Refolding System 1 includes the reagents necessary for preparing and denaturing inclusion bodies, following optimized procedures.

iFOLD™ Protein Refolding System 1

In part, the iFOLD System 1 is based on an artificial chaperone system (Rozema 1995) using N-lauroylsarcosine (LS), which is a chaotropic anionic detergent, and methyl-β-D-cyclodextrin, which acts as a detergent sponge. LS coats hydrophobic segments of the target protein, solublizing inclusion bodies (Frankel 1991). The cyclodextrin, present in some the refolding buffers, strips the LS from the target protein, allowing the protein to refold. For some proteins, low concentrations of LS promote, rather than inhibit, successful refolding, so not all wells contain cyclodextrin. The remaining components and pH range used for the iFOLD Protein Refolding Plate (Table 1, p. 106) were determined by fractional factorial and rational design after an extensive review of successful refolding experiments reported in literature and in the REFOLD database (http://refold.med.monash.edu.au) (Vincentelli 2004, Willis 2005, Chow 2005). The single 96-well plate, containing 92 refolding buffers and 4 wells for user-provided controls, is convenient for bench-top use and is compatible with high-throughput robotic systems.

Isolating inclusion bodies

E. coli cells are lysed by sonication or an alternative mechanical cell disruption method. Lysonase™ Bioprocessing Reagent, a combination of rLysozyme™ Solution and Benzonase® Nuclease, is included to improve protein extraction from the lysed cells. Inclusion bodies are harvested by low speed centrifugation and purified by a series of detergent and buffer washes.

λ Protein Phosphatase

Using the iFOLD System 1, we identified multiple conditions that supported successful refolding of λ PP. Results indicate an absolute dependence on reducing equivalents for efficient refolding (Table 2, p. 109); 12 of the 13 wells resulting in the greatest catalytic activity contained the reducing agent TCEP and the remaining well contained glutathione buffer (which has a net negative reduction potential). The dependence on a reducing environment is consistent with a report by Voegtli, et al. (2000), who found it was necessary to block a surface-exposed cysteine to prevent aggregation and precipitation of λ PP during crystallization trials. Wells with the greatest amount of aggregation (Figures 3A, B) had the lowest catalytic activity (Figures 3C, D). Reducing the refolding temperature from 22°C to 10°C did not change the pattern of aggregation or successful refolding (compare Figure 3A to 3B and Figure 3C to 3D), but did tend to increase the amount of aggregation and lower the yield of active λ PP.

trx–GFP fusion

Several buffer conditions supported successful refolding of trx-GFP. Every protein refolding solution resulting in relative fluorescence intensity >35,000 contained cyclodextrin (Table 2, p. 109). Since cyclodextrin sequesters LS, residual LS is apparently detrimental to refolding trx-GFP. Refolding reaction temperature had an effect similar to that observed for λ PP; the pattern of successful refolding did not change, but the fluorescence intensity of samples refolded at 10°C was consistently lower than for samples refolded at 22°C (Figure 4, p. 108). ▶▶

*Adapted from *inNovations* 23, 3 (2006).

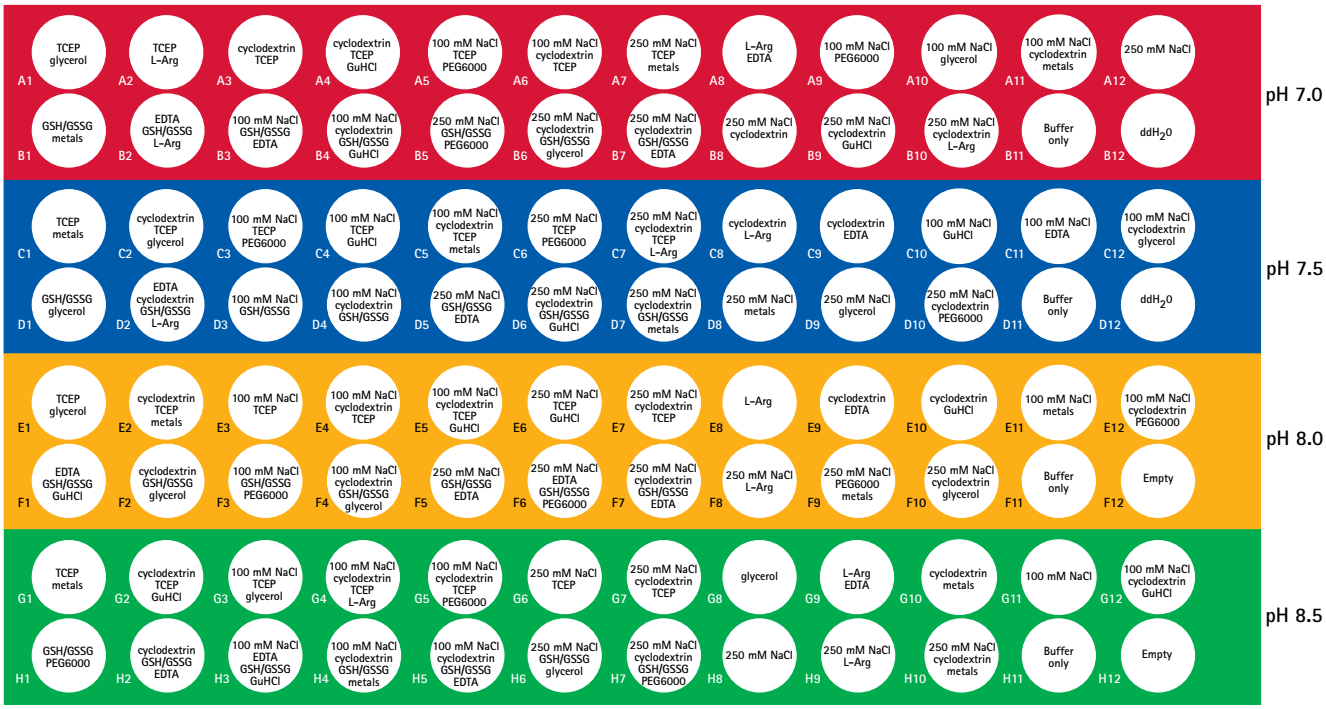
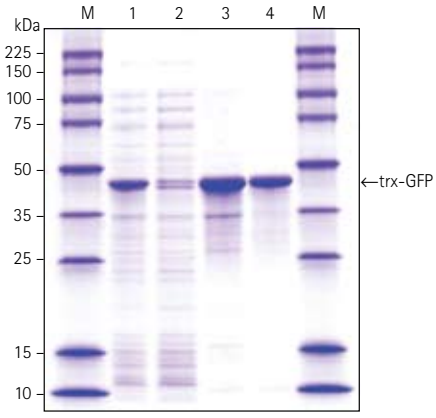


Figure 1. Buffer matrix for the iFOLD™ Protein Refolding Plate 1

Key: **buffer:** Tris-HCl; **cyclodextrin:** methyl-β-D-cyclodextrin; **EDTA:** ethylenediaminetetraacetic acid; **GSH/GSSG:** reduced glutathione/oxidized glutathione; **GuHCl:** guanidine hydrochloride; **L-Arg:** L-arginine; **metals:** $CaCl_2/MgCl_2$; **PEG:** polyethylene glycol average M.W. 6000, **TCEP:** Tris(2-carboxyethyl)phosphine. See Table 1 for concentrations.



Lane	Sample
M	Perfect Protein™ Markers 10-225 kDa
1	Total cellular protein
2	Soluble fraction
3	Insoluble fraction
4	Purified inclusion body fraction
M	Perfect Protein Markers 10-225 kDa

Figure 2. SDS-PAGE of trx-GFP inclusion body isolation using the iFOLD Protein Refolding System 1

4-20% gradient gel, Coomassie blue staining.

Table 1. Components of the iFOLD Protein Refolding Plate 1

Final concentrations are listed for the 500-µl reaction, after target protein addition.

Buffer	50 mM Tris-HCl*	pH 7.0, 7.5, 8.0, or 8.5
Ionic strength	NaCl	100 or 250 mM
Detergent trap	methyl-β-D-Cyclodextrin	12.5 mM
Redox agents	TCEP GSH/GSSG	1 mM 3.8/1.2 mM
Additive	Glycerol PEG 6000 L-Arg Guanidine-HCl $CaCl_2/MgCl_2$ EDTA	20% (v/v) 0.1% (w/v) 0.5 M 0.5 M 1 mM each 1 mM

* Tris buffer pH is measured at room temperature (22˚C)

HRV 3C Protease

The iFOLD™ System 1 revealed multiple successful refolding buffers for HRV 3C protease, as determined by a protease activity assay measuring cleavage of a peptide substrate (Figure 5A, p. 108). In contrast to trx-GFP, residual LS was essential for refolding HRV 3C protease; none of the 14 wells with the highest enzymatic activity contained cyclodextrin. Each of the 14 wells did contain either 100 or 250 mM NaCl (Table 2, p. 109). Decreasing the temperature from 22°C to 10°C had only a modest effect on the outcome of the refolding reactions, slightly increasing aggregation and reducing enzymatic activity (data not shown). HRV 3C protease refolding reactions with low, moderate, or high catalytic activity against the peptide substrate were assayed for their ability to cleave a 53-kDa HRV 3C cleavage control protein (CCP; Figure 5B). Overall, the results from the CCP assay correlate well with those from the peptide-based assay.

Scale-up

After the iFOLD System 1 screen, trx-GFP from 1.7 g inclusion bodies was successfully refolded using well E7 buffer and was purified to 140 mg of fluorescent protein. Details of this experiment can be accessed at www.novagen.com/ifold_gfp. ▶▶

Figure 3A

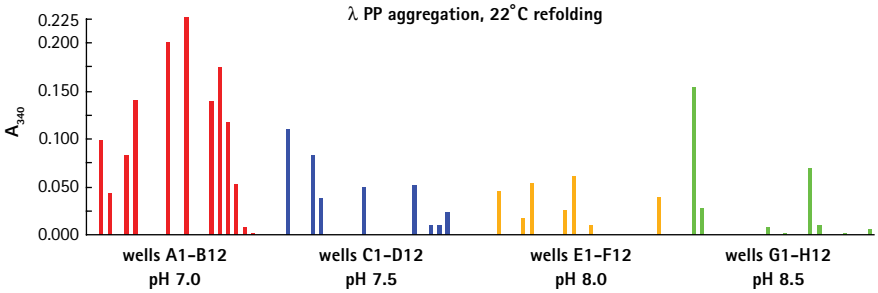


Figure 3B

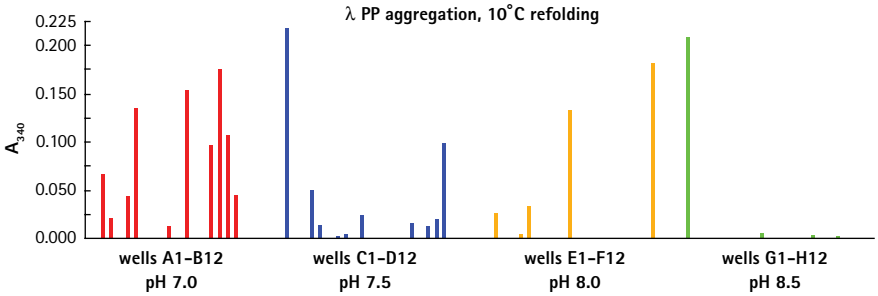


Figure 3C

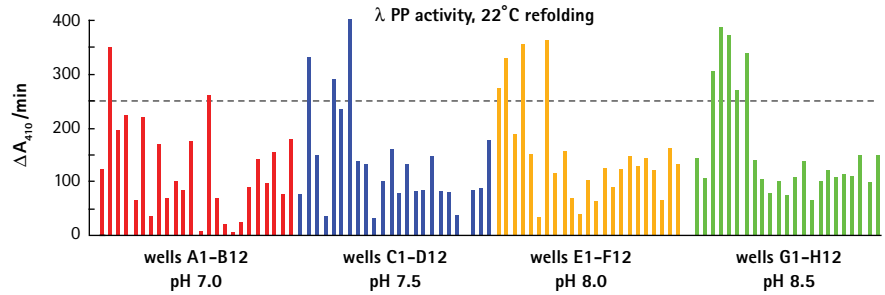


Figure 3D

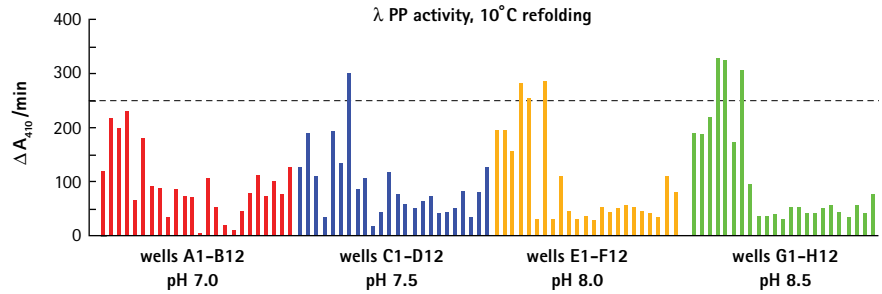


Figure 3. Refolding data for λ protein phosphatase

Aggregation of λ PP refolded at 22°C (A) and 10°C (B) was determined by the A_{340} value. Enzymatic activity of λ PP refolded at 22°C (C) and 10°C (D) was determined by measuring cleavage of 4-nitrophenyl phosphate (ΔA_{410}/min, Zhuo 1993).

Figure 4A

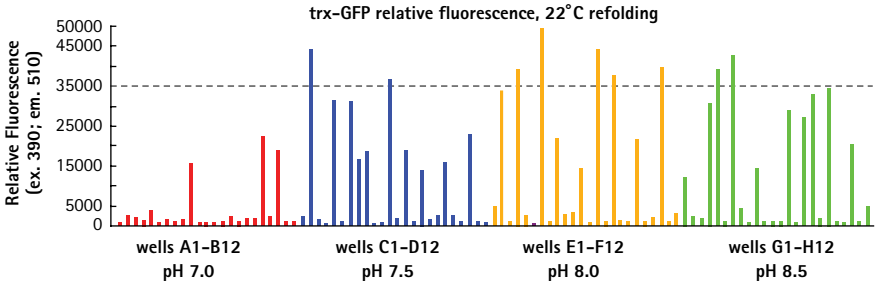


Figure 4B

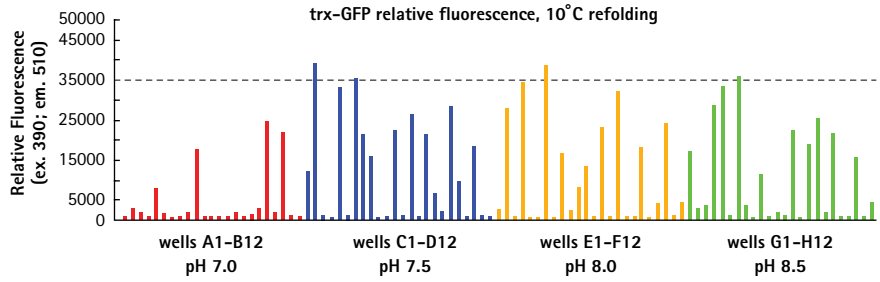


Figure 4. Refolding data for trx-GFP

Samples from 22°C (A) and 10°C (B) refolding experiments were diluted 1:4 with 50 mM Tris-HCl, pH 8.0, and the relative fluorescence intensity was recorded (excitation 390 nm; emission 510 nm).

Figure 5A

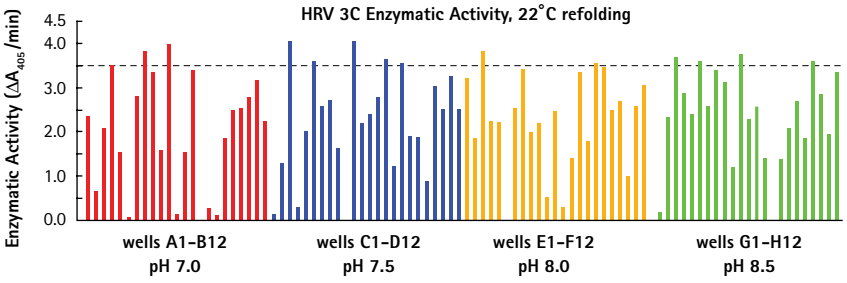
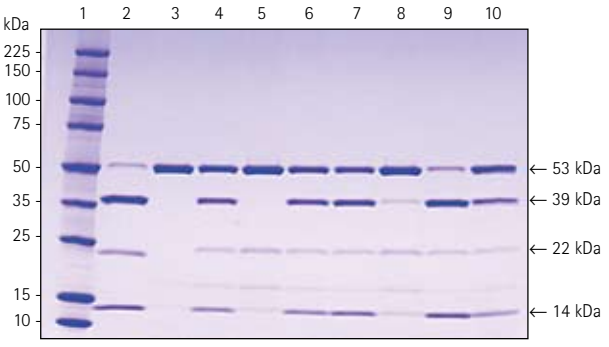


Figure 5B



Lane	Sample
1	Perfect Protein™ Markers, 10-225 kDa
2	Native HRV 3C protease (positive control)
3	No protease (negative control)
4	iFOLD well A9
5	iFOLD well B5
6	iFOLD well C6
7	iFOLD well D4
8	iFOLD well E6
9	iFOLD well F10
10	iFOLD well G10

Figure 5. Refolding data for HRV 3C protease

A. Enzymatic activity of HRV 3C protease (22 kDa) refolded at 22°C was determined by measuring ΔA_{405}/min, indicating cleavage of a *p*-nitroanilide-labeled peptide substrate (Wang 1997)

B. Selected samples with high, moderate, or low catalytic activity against the peptide substrate were tested for cleavage activity against a 53-kDa cleavage control protein (CCP). CCP cleavage reactions were incubated at 4°C for 30 min and analyzed by SDS-PAGE (4-20% gradient gel) and Coomassie blue staining. Samples with high activity against the peptide substrate (Lanes 4, 6, and 9) cleaved the CCP (39 and 14 kDa fragments) to a similar extent as the HRV 3C positive control (Lane 2). Refolding samples with moderate (Lanes 7 and 10) or low (Lanes 5 and 8) activity in the peptide assay had correspondingly less activity against the CCP.

Table 2. Trends in optimal refolding buffers for λ PP, trx-GFP, and HRV 3C protease based on data from refolding screens performed at 22˚C

Each protein was correctly refolded, as determined by activity or fluorescence assays, in a number of different buffers (λ PP, 13 wells; trx-GFP, 10 wells; HRV 3C, 14 wells). Components in the wells that successfully refolded the proteins were tabulated to determine which components enhanced refolding and which might inhibit refolding. This table shows the number of wells, of the total number of successful refolding wells, that contained a specific pH or component. Trends are highlighted in red. For λ PP, 13 of 13 successful refolding buffers contained reducing agents. For trx-GFP, 10 of 10 successful refolding buffers contained cyclodextrin to sequester the N-laurylsarcosine. For HRV 3C, 14 of 14 successful refolding buffers contained NaCl and no cyclodextrin.

Refolding Additive	Test Protein		
	λ PP[1]	trx-GFP[2]	HRV 3C protease[3]
pH 7.0	2/13	0/10	3/14
pH 7.5	4/13	2/10	5/14
pH 8.0	4/13	5/10	2/14
pH 8.5	5/13	3/10	4/14
cyclodextrin	9/13	**10/10**	**0/14**
100 mM NaCl	4/13	4/10	**8/14**
250 mM NaCl	4/13	3/10	**6/14**
TCEP	**12/13**	5/10	6/14
GSH/GSSG	**1/13**	2/10	3/14
glycerol	2/13	5/10	1/14
PEG 6000	1/13	2/10	4/14
L-Arg	4/13	0/10	0/14
Guanidine-HCl	0/13	0/10	0/14
$CaCl_2$ + $MgCl_2$	2/13	0/10	0/14
EDTA	0/13	0/10	3/14

[1] Refolding wells with a value of ΔA_{410}/min greater than 250. (Zhuo 1993)
[2] Refolding wells with an RFU value greater than 35,000.
[3] Refolding wells with a value of ΔA_{405}/min greater than 3.5. (Wang 1997)

Product	Cat. No.	Page
iFOLD™ Protein Refolding System 1	71552-3	67

Conclusion

Results from refolding three proteins using the iFOLD™ Protein Refolding System 1 indicate that each protein required a distinct set of components for refolding (Table 2). A single screen with the iFOLD System 1 simultaneously identifies the distinct factors important for refolding a specific protein, so that an optimal buffer can be prepared for large scale refolding reactions. The iFOLD procedure used to purify and denature inclusion bodies is also scaleable. ■

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Zhuo, S., et al. 1993. *J. Biol. Chem.* **268**, 17754.

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- Nuclease HC, Purity > 99% 54

BugBuster®
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- Plus Lysonase™ Kit 17
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