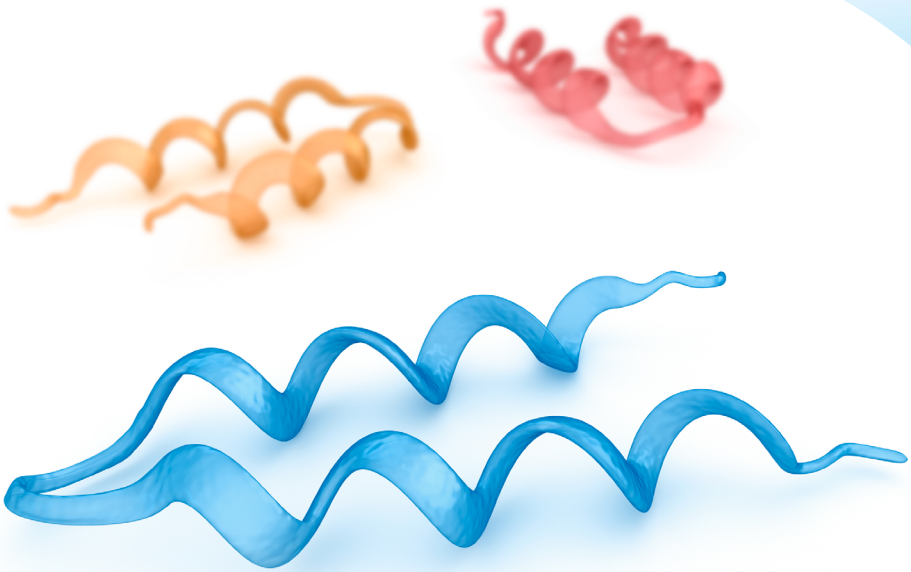
Protein purification and preparation.

High purity and recovery for better discovery.



Merck Millipore is a division of MERCK

Introduction

Today, researchers are challenged to create high quality protein samples for successful proteome analysis, often using cumbersome traditional sample preparation methods. With over 50 years of experience in developing protein sample preparation technologies, Merck Millipore is constantly innovating new tools to offer you rapid and efficient solutions that can be smoothly integrated into your workflow.

Why spend your time on arduous sample preparation protocols when you can focus your efforts on exciting experiments? With the right pure protein, in the buffer you need, at the concentration you want, your next discovery is only a step away. From protein isolation to purification, you can count on Merck Millipore to support your research.

To learn more, please visit: *www.merckmillipore.com/psp*

Key Features

Unmatched Flexibility

Isolate proteins from a diverse range of sample types with our flexible, broad range of kits.

Multiple downstream applications

Our kits enable you 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.

Scale-up compatibility

It's easy to scale up to high-throughput recombinant protein purification and solubility screening using our sample preparation reagents.

Protein Extraction

When purifying proteins for functional or structural 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 affect the target protein's integrity and activity, or otherwise expose it to degradative conditions. Merck Millipore's complete range of reagents and enzymes for cell lysis and protein extraction provide you with an array of options so that you can put together the perfect extraction protocol for your particular cells and protein.

Protein Extraction Reagents Application Guide

Products by Cell Type	Starting Material		Applications			Comments
	Total Culture	Cell Pellet	1D PAGE	2D PAGE / IEF	Activity Assay	
E. COLI						
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® Protein Extraction Reagent		•	•	•	•	Efficient protein extraction from *E. coli* under non-denaturing conditions.
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.
PopCulture® Reagent	•		•		•	Protein extraction from cells directly in the culture medium; no centrifugation required.
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 insect cells with retention of protein activity for assays and purification. Can use with monolayers or pellets derived from suspension cultures.
Insect PopCulture® Reagent	•		•		•	Lysis of insect cells directly in serum-free medium. Ideal for expression screening of many small samples.
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.
ProteoExtract® Kits		•	•	•*	•	Extract protein fractions from different parts of the cell. A range of kits offering maximum flexibility.
LYSIS AND EXTRACTION ENHANCEMENT						
Gram-negative bacteria (*E. coli*)						
rLysozyme™ Solution	•	•	•		•	Cleaves bond in peptidoglycan layer of *E. coli* cell wall.
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 chromatography, and reduced interference in assays.

1D PAGE—One-dimensional Polyacrylamide Gel Electrophoresis
2D PAGE—Two-dimensional Polyacrylamide Gel Electrophoresis
IEF—Isoelectric Focusing
* —Salt must be removed before IEF

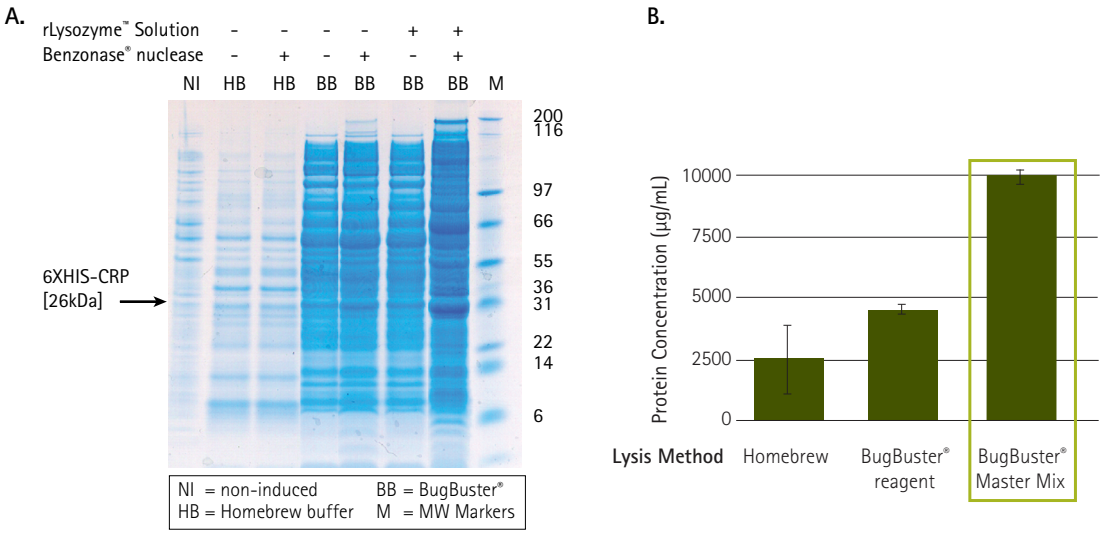
Protein Extraction with Cell Lysis Reagents ("Busters")

Featured Products

BugBuster® Protein Extraction Kits and Reagents

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

Gently disrupt the cell wall of *E. coli* and liberate soluble proteins with BugBuster® Kits and Reagents. BugBuster® reagent provides a simple, rapid, low-cost alternative to mechanical methods such as French press or sonication for releasing expressed target proteins in preparation for purification or other applications. The proprietary formulation uses a detergent mix to perforate cell walls without denaturing soluble protein. Simply harvest cells by centrifugation and suspend in BugBuster® reagent. Following a brief incubation, remove insoluble cell debris by centrifugation. The clarified extract is ready to be purified.



BugBuster® reagent is superior to "Homebrew" lysis buffer and BugBuster® reagent with both Benzonase® nuclease and rLysozyme™ solution yielded lysates with the most 6XHIS-CRP. (A) *E. coli* lysates (5 µL of 1 mL total lysate) from various lysis protocols were fractionated and analyzed by SDS-PAGE. A band corresponding to 6XHIS-CRP is prominently visualized in the BB +/+ lane. (B) Cleared cell lysates (2 µL of 1 mL total) were spotted on assay cards and quantified using the Direct Detect® spectrometer. In each case, bars represent the average of 3 independent samples.

*The Direct Detect® system is a novel quantitation platform from Merck Millipore (Catalogue No. DDHW00010-WW). Visit www.merckmillipore.com/directdetect for details.

How do I choose between BugBuster® Products?

Components of Bacterial Lysis Reagents

	BugBuster® Reagent	Buffer	Benzonase® Nuclease	rLysozyme™ Solution	Notes
BugBuster® Reagent	X	X			
BugBuster® 10X	X				Flexibility to customize dilution & buffer composition
BugBuster® Plus Benzonase® Nuclease	X	X	X		2 separate vials for greater flexibility
BugBuster® Plus Lysonase™ Kit	X	X	X	X	2 separate vials for greater flexibility
BugBuster® Master Mix	X	X	X	X	1 convenient reagent
PopCulture® Reagent	X	X			Buffer protects protein from the pH extremes produced in high density culture media, enabling extraction directly in medium.

We offer a family of protein extraction reagents for gentle, efficient, non-mechanical extraction of soluble proteins from bacteria, yeast, plant, mammalian, and insect cells.

CytoBuster™ reagent – Obtain protein extracts from mammalian and insect cells in their native state in 5 minutes.

NucBuster™ reagent – Extract nuclear proteins in less than 30 minutes with a simple 2 step protocol.

PhosphoSafe™ Extraction reagent – The PhosphoSafe™ Extraction Buffer is a detergent and Phosphatase inhibitor mixture optimized for fast, efficient extraction of soluble proteins from mammalian and insect cell preserving the phosphorylation state of your sample.

YeastBuster™ reagent – Extract proteins from yeast and plants without mechanical disruption and enzymatic lysis. The reagent has been tested with *Saccharomyces cerevisiae*, *Pichia pastoris*, *P. stipidis*, and *Schizosaccharomyces pombe* strains and with plant cells.

Insect PopCulture® reagent – Insect PopCulture® Reagent is a detergent-based lysis reagent specifically formulated for total insect cell culture (in suspension or adherent) extraction without the need for centrifugation.

Ordering Information

Available from www.merck4biosciences.com

Application	Description	Catalogue No.
Bacteria	BugBuster® Protein Extraction Reagent	70584
	BugBuster® Master Mix	71456
	BugBuster® Plus Benzonase® Nuclease	70750
	BugBuster® Plus Lysonase™ Kit	71370
	BugBuster® 10X Protein Extraction Reagent	70921
	PopCulture® Reagent	71092
Mammalian	CytoBuster™ Protein Extraction Reagent	71009
	NucBuster™ Protein Extraction Reagent	71183
	PhosphoSafe™ Extraction Reagent	71296
Yeast	YeastBuster™ Protein Extraction Reagent	71186
Insect	Insect PopCulture® Reagent	71187

Cell Lysis & Nucleic Acid Removal Enhancers

Featured Products

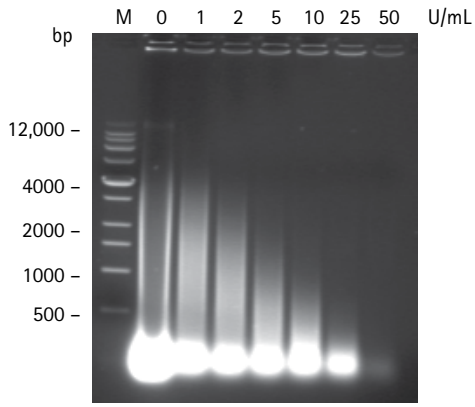
Benzonase® Nuclease

Effectively reduce viscosity and remove 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 has an exceptionally high specific activity. Benzonase® is an excellent choice for viscosity reduction to shorten processing time and increase yields of protein.

Benzonase® Advantages

- Compliant with FDA guidelines for nucleic acid contamination
- Functional between pH 6 and 10, from 0 °C to 42 °C for maximum versatility
- Active in the presence of ionic and non-ionic detergents, reducing agents, PMSF (1 mM), EDTA (1 mM) and urea.
- Available in ultrapure (> 99% by SDS-PAGE) and pure (> 90%) grades
- Available in standard concentration (25 U/µL) and high concentration (HC, 250 U/µL).



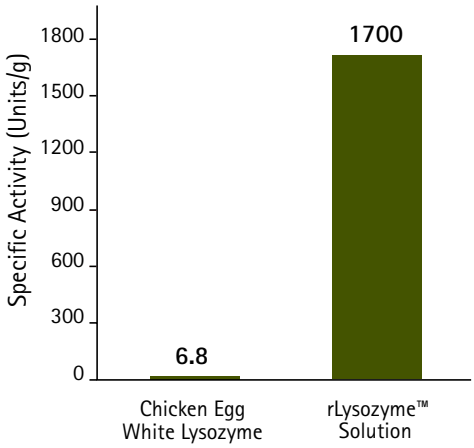
Nucleic acid digestion by Benzonase® Nuclease.
E. coli BL21(DE3) cells containing a pET construct were suspended in BugBuster® Reagent (5 mL/g wet weight). Identical volumes of the suspension were treated with the indicated amounts of Benzonase® Nuclease for 30 min at room temperature. Samples were clarified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

***E. coli* lysate without Benzonase® Nuclease.**
Gooey, viscous, difficult to handle.

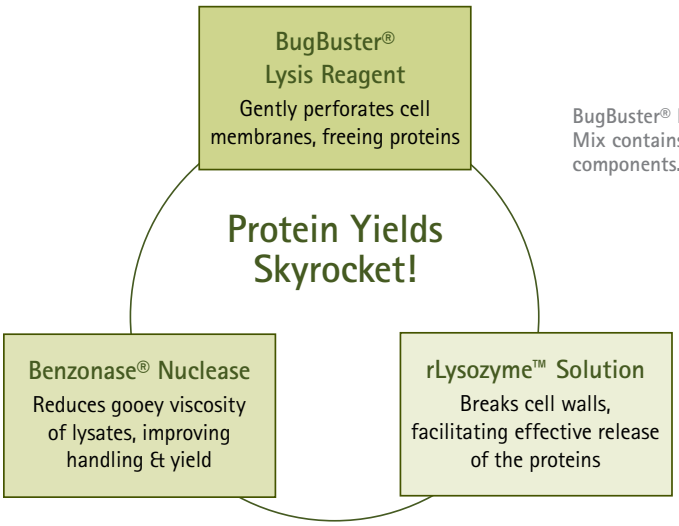
rLysozyme™ Solution

Degrade bacterial cell walls with stabilized recombinant lysozyme

rLysozyme™ Solution contains a highly purified and stabilized recombinant lysozyme that can be used for lysis of *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 traditional chicken egg white lysozyme. rLysozyme™ is optimally active at physiological pH. Very small amounts of rLysozyme™ enhance the efficiency of protein extraction with BugBuster® and PopCulture® Reagents. The product is supplied as a ready-to-use solution and is stable at –20 °C.



rLysozyme™ exhibits 250 times higher specific activity than chicken egg white activity when measured using a standard activity assay.



BugBuster® Master Mix contains all three components.

Ordering Information

Available from www.merck4biosciences.com

Description	Catalogue No.
Benzonase® Nuclease, Purity >90%	70746
Benzonase® Nuclease HC, Purity >90%	71205
Benzonase® Nuclease, Purity >99%	70664
Benzonase® Nuclease HC, Purity >99%	71206
rLysozyme™ Solution	71110
Chicken Egg White Lysozyme Solution	71412
Lysonase™ Bioprocessing Reagent	71230

Protein Extraction with ProteoExtract® Kits

Featured Products

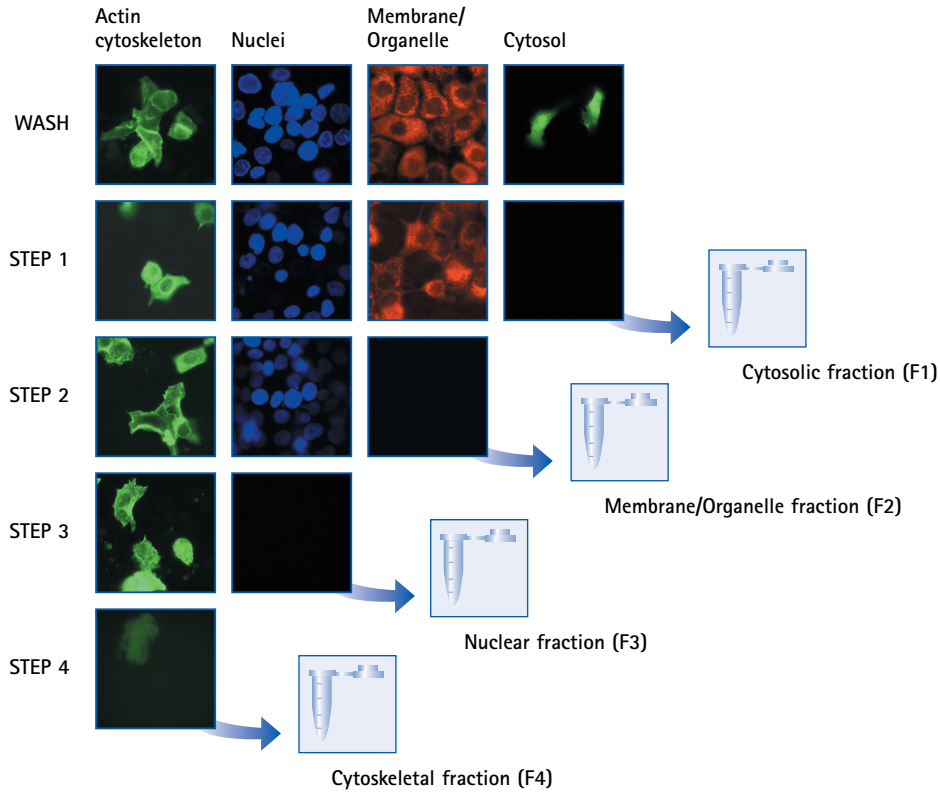
ProteoExtract® Subcellular Proteome Extraction Kit (S-PEK)

Reproducible extraction of subcellular proteomes from mammalian cells.

Based on different solubilities of certain subcellular compartments, the S-PEK uses proprietary chemistries to yield four subproteome fractions which are enriched in cytosolic, membrane/organelle, nuclear, and cytoskeletal proteins. In the case of adherent cells, the procedure is performed directly in the tissue culture dish without the need for cell removal. For suspension-grown cells, extraction starts with gentle sedimentation and washing of cells. Extraction from tissues requires isolation of viable cells before proceeding with the extraction protocol.

Applications of S-PEK:

- Subcellular redistribution assays to monitor protein translocation
- Enzyme activity assays including reporter gene assays and kinase assays
- SELDI (surface-enhanced laser desorption/ionization)-profiling
- Non-denaturing gel electrophoresis
- Assaying protein expression levels using fluorescent-labeled subcellular extracts in microarrays



Four distinct protein fractions separated using S-PEK. A431 cells were incubated with DAPI (nuclei), phallicidin (to stain actin) and MitoTracker® probes, extracted and monitored by fluorescence microscopy. These results show that the sequential extraction results in a stepwise degradation of the cell's structure yielding 4 subcellular fractions. In cases where a 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.

ProteoExtract® Native Membrane Protein Extraction Kit (M-PEK)

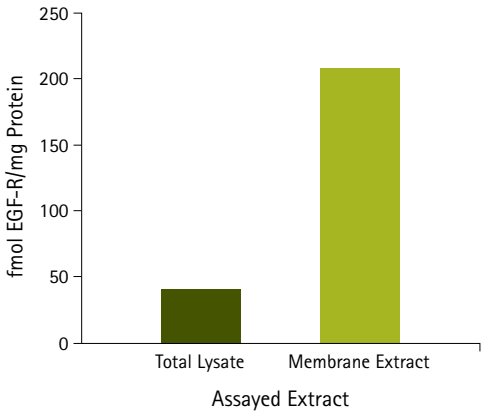
Isolation of native membrane proteins from mammalian cells and tissue.

Extract proteins associated with cellular membranes with M-PEK. The extremely mild extraction conditions yield a 3-5 fold enrichment of integral membrane and membrane-associated proteins. The simple, two-step procedure enables processing of multiple samples in parallel. Extraction from tissues requires isolation of viable cells before proceeding with the extraction protocol.

Applications for Extracted Membrane Proteins:

- Enzyme activity assays, including reporter gene assays and kinase assays
- Non-denaturing and denaturing gel electrophoresis, immunoblots and immunoassays
- Assaying post-translational modifications, such as phosphorylation
- SELDI-profiling of integral and membrane-associated proteins
- NHS ester labeling of membrane proteins

EGF-Receptor Enrichment



Greatly increased enrichment of EGF receptor using M-PEK compared to total cell lysate. HEK293 cells were extracted with buffered 1% Triton® X-100 surfactant to generate a total lysate or extracted with M-PEK to yield a membrane fraction. Equal volumes of these fractions were utilized to quantitate the concentration of EGF receptor in the samples using a EGF-R ELISA Kit. Protein concentrations were used to calculate the amount of EGF-R per mg protein in the total lysate and the membrane fraction. The measurements demonstrate a 4.5 fold enrichment of the EGF receptor in the M-PEK-extracted membrane fraction.

Ordering Information

Available from www.merck4biosciences.com

Application	Description	Catalogue No.
Organelle Fractionation	ProteoExtract® Subcellular Protein Extraction Kit	539790
	ProteoExtract® Complete Mammalian Protein Extraction Kit	539779
	ProteoExtract® Cytosol/Mitochondria Fractionation Kit	QIA88
	ProteoExtract® Native Cytoskeleton Enrichment Kit	17-10210
	ProteoExtract® Cytoskeleton Enrichment & Isolation Kit	17-10195
Membrane Proteins	ProteoExtract® Native Membrane Protein Extraction Kit	444810
	ProteoExtract® Transmembrane Protein Extraction Kit	71772
Mass Spec Peptide Enrichment	ProteoExtract® All-in-One Trypsin Digestion Kit	650212
	ProteoExtract® Glycopeptide Enrichment Kit	72103
	ProteoExtract® Phosphopeptide Enrichment TiO_2 Kit	539722
Albumin & IgG Depletion	ProteoExtract® Albumin Removal Kit	122640
	ProteoExtract® Albumin/IgG Removal Kit	122642

Protein Extraction with Inhibitors

Featured Products

Protease Inhibitor Cocktails

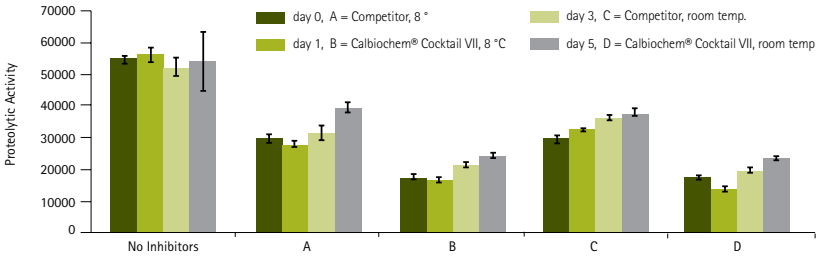
Prevent protein degradation by proteases during extraction and purification

Ensure the integrity of purified proteins by using protease inhibitor cocktails and highly specific protease inhibitors. During protein expression and isolation, endogenous proteases rapidly begin to degrade protein samples, reducing the quality and quantity of protein samples required for characterization and analysis. By using the right combination of protease inhibitors, you can protect your purified protein preparations from common proteases including serine proteases, metalloproteases, cysteine proteases, aminopeptidases, and aspartic proteases.

Protease Inhibitor Advantages:

- Convenient—Flexible protocol and ready-to-use formulations
- Consistent—High quality ensures reproducibility and excellent inhibition over a wide range of protease classes
- Flexible—Comprehensive selection of specific cocktail formulations designed to inhibit proteolytic activity from most tissue or cell type extracts, including mammalian, bacterial, yeast, fungal, and plant cells
- Application-Specific—Available without EDTA for purification schemes involving metal ion chelation
- Chromatography or analysis using 2-D gel electrophoresis. New protease inhibitor cocktail formulations include recombinant aprotinin for applications that require the use of animal-free reagents

Calbiochem® Protease Inhibitors offer greater efficiency and stability



Stability of Protease Inhibitor Dilutions in BugBuster® Lysis Reagent. Protease inhibitors were diluted to the prescribed working concentration (Competitor or Calbiochem® Cocktail VII, Catalogue No. 539138). The ability of the inhibitors to inhibit the proteolytic activity of PRONASE® reagent (Catalogue No. 537088) was measured by using the Universal HT Protease Assay on days zero, one (24 h post dilution), three (72 h) and five (120 h). The Universal HT Protease Assay quantifies protease activity using a fluorescein thiocarbamoyl-casein derivative (FTCcasein). Proteolytic activity liberates FTC-labeled peptides, which results in enhanced fluorescence (Ex.max: 495 nm; Em.max: 525 nm). Addition of the protease inhibitor cocktails inhibits the proteolytic activity of the PRONASE® reagent (Catalogue No. 537088), resulting in reduced fluorescence. On day 1, for samples incubated at 8 °C, the competitor tablet inhibited the proteolytic activity by 50% and the Calbiochem® Cocktail VII inhibited the proteolytic activity by 70%. On day 5, for samples incubated at 8 °C, the competitor tablet caused a 29% decrease in proteolytic activity in comparison to the Calbiochem® cocktail VII, which caused a 57% decrease in proteolytic activity. The data show that the efficiency of the cocktail remained higher than the competitor's tablet in this study.

Featured Protease Inhibitor Cocktails

Protease Inhibitor Cocktail Set III, EDTA-Free (Cat. No. 539134)

This popular cocktail is widely cited in publications, and has been used in multiple applications, such as Western blot, immunoprecipitation, kinase assay and ubuiquitination assay. This cocktail is recommended for use with mammalian cell and tissue extracts and is also suitable for bacterial cell extracts for metal chelation chromatography. It contains six protease inhibitors (in 1 mL DMSO) with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases. Each vial contains the concentrations of inhibitors shown in the table below. One mL is sufficient for about 20 g tissue.

Visit www.merck4biosciences.com/inhibitors for a complete listing of our inhibitor cocktails.

Phosphatase Inhibitor Cocktails

Prevent protein dephosphorylation for cell signaling studies

It is critical to preserve the phosphorylation state of proteins of interest during their extraction from cell and tissue lysates. To effect cell signaling, target proteins are phosphorylated by protein kinases that transfer a phosphate group to specific sites, typically at serine, threonine, or tyrosine residues. These phosphate groups can be removed by protein phosphatases, restoring the protein to its original dephosphorylated state. Using phosphatase inhibitors help reveal the signaling status inside a cell at a specified timepoint. Merck Millipore offers four different Phosphatase Inhibitor cocktails and a PhosphoSafe™ Extraction Reagent that help protect phosphoproteins from different families of phosphatases.

Featured Phosphatase Inhibitor Cocktail

Phosphatase Inhibitor Cocktail Set II (Cat. No. 524625)

This cocktail of five phosphatase inhibitors for the inhibition of acid and alkaline phosphatases as well as protein tyrosine phosphatases (PTPs) is widely cited and has been used, for example, in studies of EGFR signaling, apoptosis pathways and inflammation. Suitable for use with tissue and cell extracts, including extracts containing detergents. Each vial contains 1 mL aqueous solution of the phosphatase inhibitor cocktail. The concentrations of the individual inhibitors are shown in the table below. Note: 1 set = 5 x 1 mL.

Ordering Information

Available from www.merck4biosciences.com

Description	Recommended Application	Catalogue No.
Protease Inhibitor Cocktail Set I	General Use	539131
Protease Inhibitor Cocktail Set II	Bacterial cell extracts (except those intended for metal chelation chromatography)	539132
Protease Inhibitor Cocktail Set III, EDTA-Free	Mammalian cells and tissue extracts purified using metal chelation chromatography; samples to be analyzed by 2-D gel electrophoresis	539134
Protease Inhibitor Cocktail Set IV	Fungal and yeast cell extracts	539136
Protease Inhibitor Cocktail Set V, EDTA-Free	Mammalian cells and tissue extracts purified using metal chelation chromatography; samples to be analyzed by 2-D gel electrophoresis	539137
Protease Inhibitor Cocktail Set VI	Plant cell extracts	539133
Protease Inhibitor Cocktail Set VII	Proteins containing His•Tag® sequences	539138
Serine Protease Inhibitor Cocktail	Broad range serine protease inhibition	565000
Phosphatase Inhibitor Cocktail Set I	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A	524624
Phosphatase Inhibitor Cocktail Set II	Protection against acid and alkaline phosphatases and Protein Tyrosine Phosphatases (PTPs)	524625
Phosphatase Inhibitor Cocktail Set III	Protection against acid, alkaline and Ser/Thr phosphatases and Protein Tyrosine Phosphatases (PTPs)	524627
Phosphatase Inhibitor Cocktail Set IV	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A	524628
PhosphoSafe™ Extraction Reagent	Protection against Ser/Thr phosphatases and Protein Tyrosine Phosphatases (PTPs)	71296

Visit www.merck4biosciences.com/inhibitors for a complete listing of our inhibitor cocktails.

Protein Purification

Affinity purification is based on the specific interaction of a target molecule with an immobilized ligand. Merck Millipore offers a wide range of tools for protein purification, including affinity magnetic beads, affinity agarose resins, Amicon® Pro purification system and protease cleavage enzymes.

- PureProteome™ magnetic beads are ideal for small volume affinity purification assays, such as immunoprecipitation and serum depletion or enrichment.
- Affinity agarose portfolio for larger volume applications, such as antibody purification and recombinant protein purification.
- Amicon® Pro purification system is ideal for small volume affinity purification assays followed by buffer exchange and/or concentration.
- Protease cleavage enzymes available in restriction grade or in kits for cleaving fusion proteins.

Agarose Portfolio

Application	Magnetic	Agarose	Amicon® Pro System
IP & Antibody Purification	Protein A Protein G Kappa Ig Binder Lambda Ig Binder	Protein A Protein G Protein G/Protein A	✔
Recombinant Tag Purification	His•Tag® purification	His•Tag® purification GST•Tag™ purification S•Tag™ purification Strep •Tag® II purification T7•Tag™ purification	✔
Protease Cleavage	Thrombin Factor Xa Enterokinase HRV 3C Protease		
Biotinylated Molecule Purification	Streptavidin	Streptavidin	✔
Depletion/Enrichment	Albumin Albumin/IgG Depletion Kit Human Albumin/Ig Depletion Kit	ProteoExtract® Albumin Kit ProteoExtract® Albumin/IgG Kit	✔
Custom Labeled	NHS FlexiBind Carboxy FlexiBind		✔

Affinity Purification with PureProteome™ Magnetic Beads

PureProteome™ Protein A & G Beads

Fast and easy immunoprecipitation

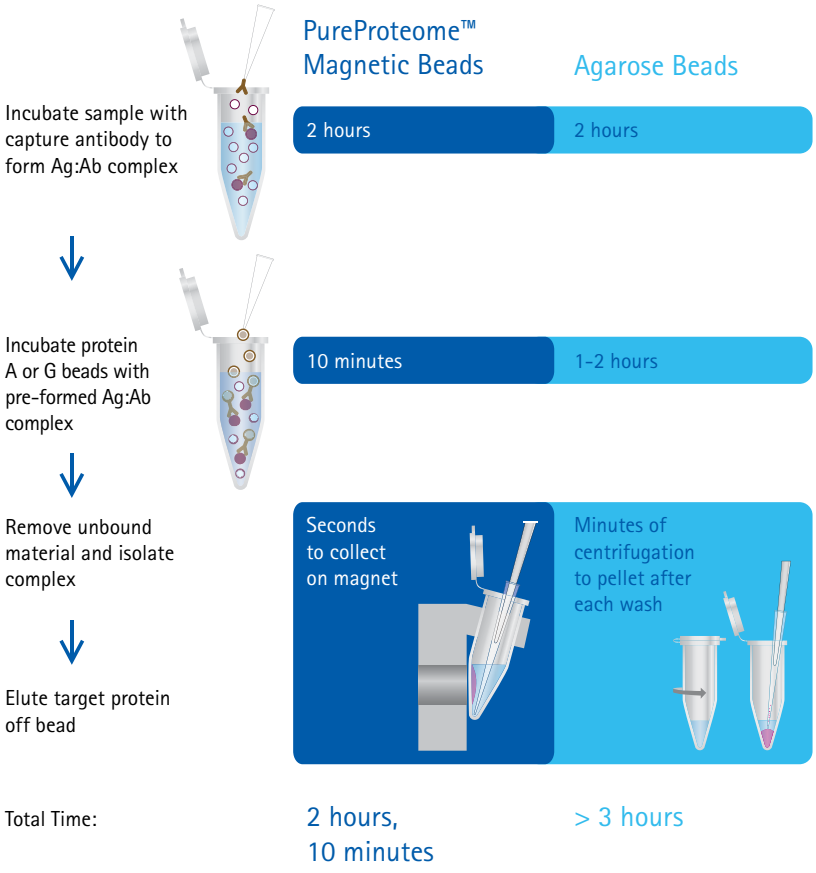
Traditional methods require hours of incubation time and minutes of harsh centrifugation to isolate sample. In contrast, PureProteome™ magnetic beads enhance binding equilibrium, enabling faster, gentler processing. The beads are easily resuspended for fast mixing and efficient interaction between the beads and protein.

PureProteome™ Protein A/G Mix Beads

Bind all mammalian immunoglobulin G (IgGs) efficiently using PureProteome™ Protein A/G mix magnetic beads, which provide a 50:50 blend of Protein A and Protein G.

Advantages of PureProteome™ Immunoprecipitation:

- Be efficient with high capacity beads: increased surface area allows for significantly greater binding capacity than non-Merck Millipore beads
- Achieve high purity: low non-specific binding of other proteins
- Save time with fast sample processing: enhanced binding equilibrium decreases incubation times by > 50%

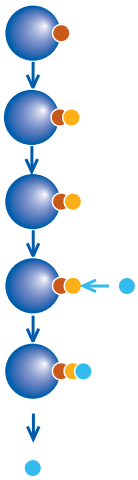


High speed immunoprecipitation with magnetic beads compared to agarose. In parallel indirect immunoprecipitations, PureProteome™ magnetic beads offered a 50% reduction in incubation time while yielding results equivalent to agarose beads.

PureProteome™ NHS & Carboxy FlexiBind beads

Customize your beads quickly & easily

Tailor your beads to match your application. Studying protein-protein interactions? Immobilizing enzymes, nucleic acids or small molecules? PureProteome™ NHS and Carboxy FlexiBind magnetic beads offer you flexibility in binding your target ligand. To customization your bead, the only requirement is that your target ligand has a free amine group.



- **Flexibility:** Choose from a range of sizes and chemistries to fit your application
- **Speed:** Get results faster
- **Cost Savings:** Less sample and reagent waste

PureProteome™ NHS FlexiBind Magnetic Beads (perfect for the first time user)

- **Fast:** Customize your own bead in <60 min
- **Easy to Use:** Kit contains everything you need: beads, all buffers and Amicon® Ultra centrifugal filters for eliminating unreacted species
- **Robust:** Little experience or optimization required

PureProteome™ Carboxy FlexiBind Magnetic Beads (for the experienced user)

- **Flexible:** Choice of 0.3 µm, 1 µm or 2.5 µm COOH magnetic beads
- **Automation-Compatible:** Smaller beads have higher buoyancy properties while retaining strong magnetic capability
- **Economical:** Aggressive pricing

PureProteome™ Kappa & Lambda Ig Binder beads

Immunoprecipitate all Human Antibodies (including IgA, IgD, IgE and IgM)

PureProteome™ Kappa Magnetic Beads bind to the kappa light chain constant region on human immunoglobulins with high specificity, and the Lambda Magnetic Beads bind to the lambda light chain constant region on human immunoglobulins with high specificity. These novel magnetic beads are capable of capturing all immunoglobulin subtypes (IgG, IgA, IgD, IgE, and IgM) and provide a rapid, scalable, and reproducible means to capture human antibody or antibody fragments containing kappa or lambda light chains – including Fab and F(ab')2.

Depletion of all human immunoglobulins can be performed by mixing PureProteome™ Kappa and Lambda Magnetic beads.

Relative Affinity

Antibodies	Protein A/G Mix	Protein A	Protein G	Kappa Ig Binder	Lambda Ig Binder	Kappa/Lambda mix*
Rabbit IgG	●	●	●			
Mouse IgM	●	●				
Mouse IgG_3	●	●	●			
Mouse IgG_{2b}	●	●	●			
Mouse IgG_{2a}	●	●	●			
Mouse IgG_1	●	●	●			
Human IgM	●	●		●	●	●
Human IgE	●	●		●	●	●
Human IgD	●	●		●	●	●
Human IgA	●	●		●	●	●
Human IgG_4	●	●	●	●	●	●
Human IgG_3	●		●	●	●	●
Human IgG_2	●	●	●	●	●	●
Human IgG_1	●	●	●	●	●	●
Rat IgM	●	●				
Rat IgG_{2c}	●	●	●			
Rat IgG_{2b}	●	●	●			
Rat IgG_{2a}	●	●	●			
Rat IgG_1	●	●	●			
Rat IgG	●	●	●			

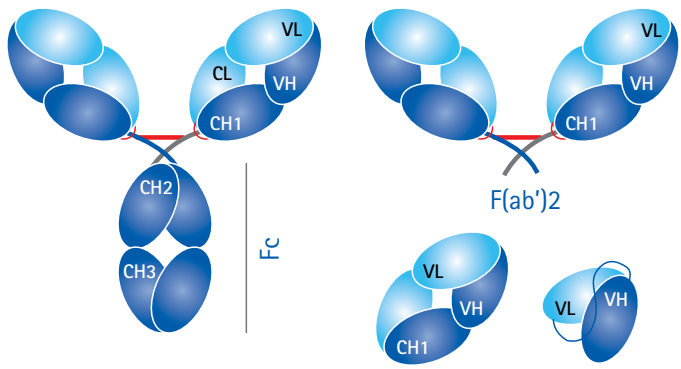
Fragments	A/G Mix	Protein A	Protein G	Kappa Ig Binder	Lambda Ig Binder	Kappa/Lambda mix*
Human					●	●
Human				●		●
Human Fc	●	●	●			
Human scFv	●	●		●	●	●
Human F(ab')2	●	●	●	●	●	●
Human Fab	●	●	●	●	●	●

Key code for relative affinity of protein A and G; PureProteome™ Kappa and Lambda magnetic beads for respective antibodies:

- ● Strong affinity
- ● Moderate/slight affinity
- ● Requires evaluation

** PureProteome™ Kappa/Lambda mix is not a catalog item.*
Simply procure the Kappa and Lambda beads individually and mix at a 1:1 ratio.

PureProteome™ Kappa or Lambda light chain ligands bind to the constant region of the antibody light chain, so PureProteome™ Kappa or Lambda ligands will not bind scFv.



Ordering Information

Available from www.merckmillipore.com/psp

Application	Description	Catalogue No.
IP, antibody purification, Fab purification	PureProteome™ Protein A Magnetic Beads	LSKMAGA10
	PureProteome™ Protein G Magnetic Beads	LSKMAGG10
	PureProteome™ Protein A/G Mix Magnetic Beads	LSKMAGAG10
	PureProteome™ Kappa Ig-Binder Magnetic Beads*	LSKMAGKP02
	PureProteome™ Lambda Ig-Binder Magnetic Beads*	LSKMAGLM02
Biotinylated molecule purification	PureProteome™ Streptavidin Magnetic Beads	LSKMAGT10
His•Tag® tagged protein purification	PureProteome™ Nickel Magnetic Beads	LSKMAGH10
Custom labelled (flexibility to bind ligand of choice)	PureProteome™ NHS FlexiBind Magnetic Beads	LSKMAGN04
	PureProteome™ Carboxy FlexiBind Magnetic Beads**	LSKMAG1CBX10
Depletion/Enrichment	PureProteome™ Albumin Magnetic Beads	LSKMAGL10
	PureProteome™ Albumin/IgG Depletion Kit	LSKMAGD12
	PureProteome™ Human Albumin/Immunoglobulin Depletion Kit*	LSKMAGHDKIT
Magnetic Stands	PureProteome™ Magnetic Stand, 8-well	LSKMAGS08
	PureProteome™ Magnetic Stand, 15 mL	LSKMAGS15

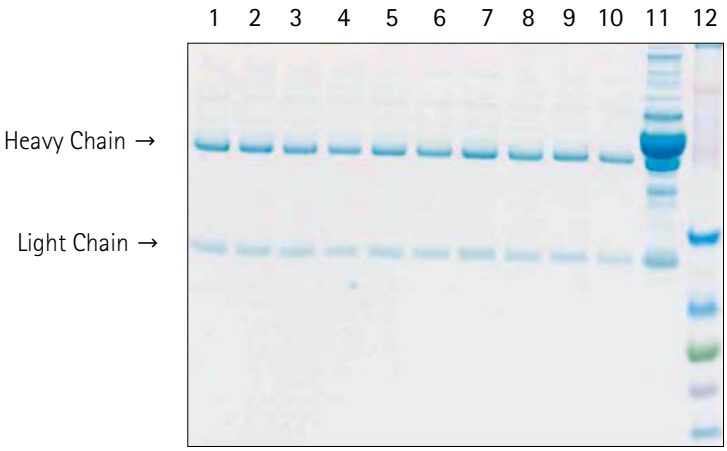
** Human only.*
*** Available in 0.3, 1.0 and 2.5 µM.*

Agarose Based Affinity Purification

Agarose resins are the preferred approach for large purifications and a convenient option when up-scaling will be needed. We offer a complete portfolio of agarose resins and kits for antibody purification and immunoprecipitation, purification of tagged proteins

Antibody Purification & Immunoprecipitation

Protein A and Protein G are proteins of microbial origin that bind specifically to mammalian immunoglobulins. When coupled to agarose, they provide an efficient tool for purification and immunoprecipitation of antibodies. Immunoglobulins of various species interact differently with the two proteins. **A combination of Protein A and Protein G agarose** is a good choice to have the characteristics of each in one reagent.



Montage® Antibody Purification Kits

From the initial clarification stage to the final antibody concentration step. High capacity pre-packed spin columns: no tedious chromatographic steps, no expensive hardware. Purify 10–20 mg in less than 60 minutes.

Human IgG Purifications/10X reuse with Human Serum.
Human IgG was purified 10 consecutive times from normal serum using the regenerated Montage® spin column with PROSEP®-G media. An average of 12.96 mg of Human IgG was purified over 10 cycles with a CV of 7.3%.

Ordering Information

Description	Size	Catalogue No.
Protein A Agarose	1.5 mL	IP02-1.5ML
	10 mL	16-125
Protein A Agarose Fast Flow	10 mL	16-156
Protein G Agarose	1.5 mL	IP04-1.5ML
	10 mL	16-266
Protein A + Protein G Agarose	1.5 mL	IP05-1.5ML
	10 mL	IP10-10ML

Description	Size	Catalogue No.
Montage® Antibody Purification Kit with PROSEP®-A media	20 purifications	LSK2ABA20
Montage® Antibody Purification Kit with PROSEP®-G media	20 purifications	LSK2ABG20

His•Tag® Purification

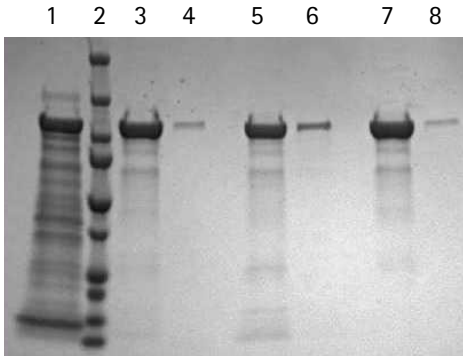
Purification is based on the affinity between the neighboring histidines of the His•Tag® sequence and an immobilized metal ion (usually Ni^{2+} or Co^{2+}). The metal is held by chelation with reactive groups covalently attached to a solid support. The most commonly used chelators include nitriloacetic acid (NTA) and iminodiacetic acid (IDA).

NTA has an additional chelation site that minimizes leaching of the metal during the purification and has a broad chemical compatibility including reducing agents like 2ME.

Ni-NTA His•Bind® Resin is always an optimal choice and has a binding capacity over 10 mg of His-Tagged fusion protein per mL resin.

The agarose matrix on the **Ni-NTA His•Bind® Superflow™ Resin** has a higher level of crosslinking for higher bead rigidity making it compatible with FPLC.

Our **IDA His•Bind®** resins are offered uncharged to allow flexibility of choice in the metal ion (Nickel, Cobalt, Zinc, Iron, Copper, etc.). IDA supports can be recycled many times with no loss in performance.



←Target protein

Ni-NTA His•Bind® performance vs. equivalent competitor resins Vector pET-28b (+) was used to express a His-Tag fusion protein of 119KDa in E. coli BL21 (DE3) cells, induced culture was processed with BugBuster® Master Mix, and protein extract was divided evenly to proceed to the His-Tag purification using Ni-NTA His•Bind®, Ni-NTA Competitor Q and Ni-NTA Competitor G resins. Ni-NTA His•Bind® resins show higher binding capacity and a better purification.

Lane	Sample
1	Crude Extract
2	Markers
3	Ni-NTA Competitor Q Elution
4	Ni-NTA Competitor Q Strip
5	Ni-NTA Competitor G Elution
6	Ni-NTA Competitor G Strip
7	Ni-NTA His•Bind® Elution
8	Ni-NTA His•Bind® Strip

Ordering Information

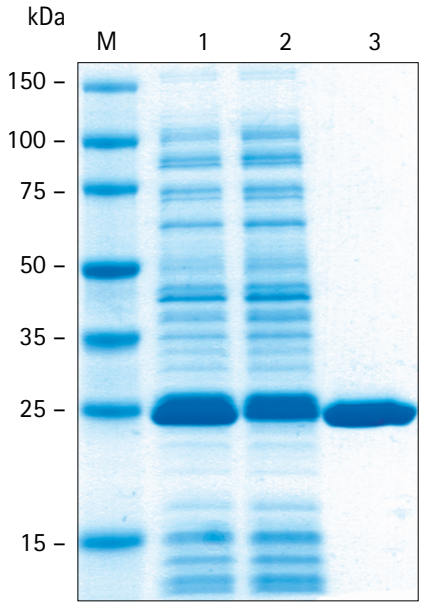
Available from www.merckmillipore.com/psp

Application	Description	Catalogue No.
Ni-NTA His•Bind® Resin		
Small to medium scale Gravity flow column Recommended for eukaryotic extracts	Ni-NTA His•Bind® Resin	70666
	BugBuster® Ni-NTA His•Bind® Purification Kit	70751
	Ni-NTA Buffer Kit	70899
Ni-NTA His•Bind® Superflow™ Resin		
Small to production scale FPLC or gravity flow column	Ni-NTA His•Bind® Superflow™ Resin	70691
	Ni-NTA Buffer Kit	70899
Uncharged IDA His•Bind® Resin		
Uncharged (metal flexibility) Reusability Small to medium scale Gravity flow column or batch mode	IDA His•Bind® Resin	69670
	His•Bind® Buffer Kit	69755
	His•Bind® Purification Kit	70239
	BugBuster® His•Bind® Purification Kit	70793

Affinity Purification with Recombinant Fusion Tags

GST•Tag™ Purification

The GST fusion system is based on the widely recognized affinity of glutathione-S-transferase (GST) fusion proteins for immobilized glutathione. Our GST Resin utilizes an 11-atom spacer arm to covalently attach reduced glutathione to the solid support via a sulfide linkage. The resin can be reused several times without loss of capacity and the high degree of substitution of glutathione ensures a high binding capacity.



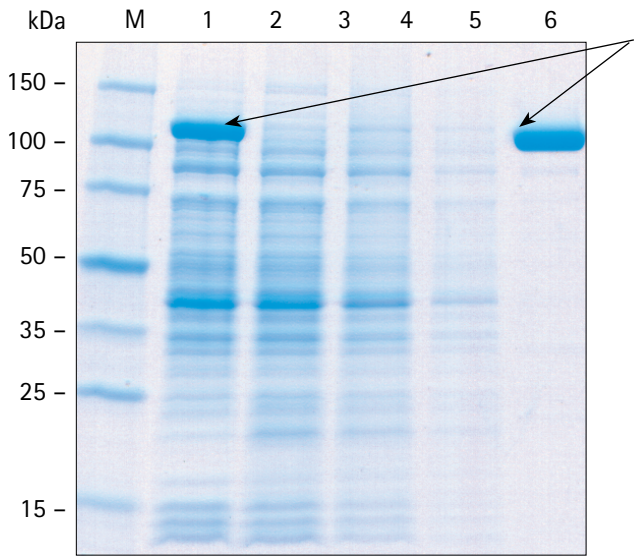
Lane	Sample
M	PerfectProtein™ markers 15-150 kDa
1	BugBuster® extract
2	Flow-through
3	Eluate

GST•Bind™ purification. A crude extract containing unfused GST was applied to a 2 mL GST•Bind™ Resin column. Total protein yield after purification was 8 mg/mL resin.

S•Tag™ Purification

Featured Product

The S•Tag™ fusion protein is a short 15-aa sequence that specifically binds with high affinity the 104-aa S-Protein (Kd=10^{-9} M, 1000 times stronger that the interaction between Nickel and His•Tag® fusion protein). Fusion proteins can be easily purified by cleavage with site specific proteases or in acidic buffers.



Lane	Sample
M	PerfectProtein™ markers 15-150 kDa
1	Crude extract
2	Flow-through
3	Wash 1
4	Wash 2
5	Eluate + Biotinylated Thrombin
6	Eluate after Biotinylated Thrombin removal

S•Tag™ affinity purification
S•Tag™ β-gal expressed from a pET construct was purified from a crude soluble fraction using S-protein Agarose under native conditions. Elution of the target protein from the agarose was performed by digestion with Biotinylated Thrombin, which was subsequently removed with Streptavidin Agarose. The fractions are indicated.

Strep•Tag® II Purification

The Strep•Tag® fusion protein II is an 8 aminoacid sequence that binds to the biotin pocket of Streptavidin with 100 times higher binding capacity.

T7•Tag® Purification

Purification is antibody-based. Covalently coupled to agarose beads, the T7•Tag® monoclonal antibody captures the T7•Tag® – a sequence of 11aminoacid.

Streptavidin Agarose

Cross-linked agarose is covalently coupled with pure streptavidin under controlled conditions. The stable linkage to the resin minimizes leaching of the streptavidin while maintaining full binding activity. The matrix is suitable for use in column and batch formats for any application that requires high biotin binding capacity and low non-specific binding and is ideal for affinity purification of biotinylated proteins or pull down experiments of biotinylated DNA/RNA probes. The resin has no detectable protease, DNAse, or RNAse.

Ordering Information

Available from www.merckmillipore.com/psp

Description	Catalogue No.
GST•Tag™ Purification	
GST•Bind™ Resin	70541
GST•Bind™ Buffer Kit	70534
BugBuster® GST•Bind™ Purification Kit	70794
S-Tag Purification	
S-protein Agarose	69704
S•Tag™ Thrombin Purification Kit	69232
S•Tag™ rEK Purification Kit	69065
Strep•Tag® II Purification	
Strep-Tactin® Superflow Agarose	71592
Strep-Tactin® Buffer Kit	71613
Strep-Tactin® SpinPrep Kit	71608
D-Desthiobiotin	71610
T7•Tag® Purification	
T7•Tag® Affinity Purification Kit	69025
T7•Tag® Antibody Agarose	69026

Description	Size	Catalogue No.
Streptavidin Agarose	5 mL	69023-3
	10 mL	16-126

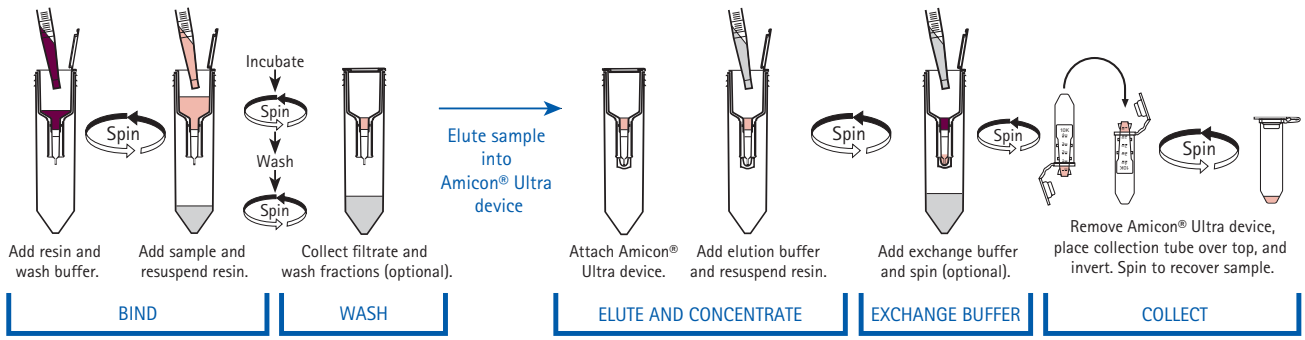
Amicon® Pro Purification System

Choose the direct route. Purify with a pro.

Traditional protein purification is a long process with many steps and multiple devices, often resulting in protein degradation and loss. Avoid the risks associated with sample transfer and reduce hands-on time when you bind, wash, elute and/or concentrate your protein in the all-in-one Amicon® Pro purification system. No matter what your workflow, the Amicon® Pro system delivers consistent, accurate sample preparation, resulting in more reliable recovery, uncompromised purity and easier data generation.

Because of the extremely flexible, modular design of the Amicon® Pro system, you can configure the perfect device for your protein preparation.

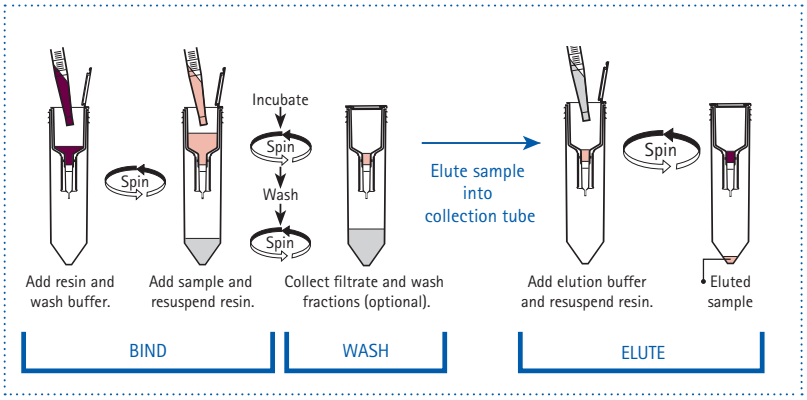
Amicon® Pro Application		Components	Protocol Steps	Resin Guideline	Benefits[3]
PURIFICATION	With buffer exchange and/or concentration	Exchange device + Amicon® Ultra filter	• Bind • Clear and Wash • Elute/Concentrate +/- Buffer Exchange	≤200 µL packed resin[1]	• Speed • No sample transfer - No loss • Improved yield



Direct, no-transfer protein workflow for affinity purification, concentration and buffer exchange using the Amicon® Pro purification system. If using ≤ 200 µL packed resin, you can attach the Amicon® Ultra 0.5 mL device for simultaneous concentration during the elution step and optional buffer exchange. Invert the Amicon® Ultra 0.5 mL device and spin to collect your final sample.

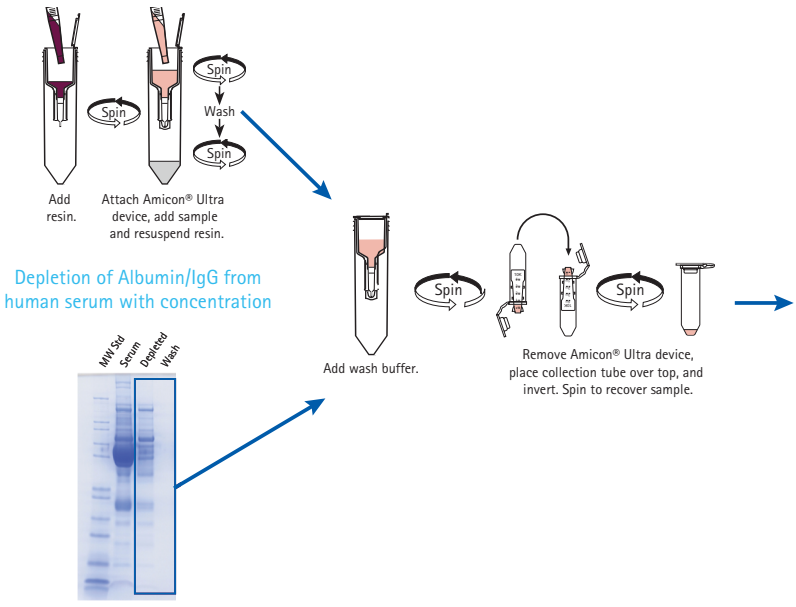
Protein Purification only

Amicon® Pro Application	Components	Protocol Steps	Resin Guideline	Benefits[3]
Purification only	Exchange device	• Bind • Clear and Wash • Elute	≤1000 µL packed resin[2]	• Range of sample volumes can be processed • Single elution -no fractions



Direct, no-transfer workflow for affinity purification. For larger scale protein purification (using > 200 µL packed resin, for example), you can take advantage of the Amicon® Pro system's efficient bind-wash-elute workflow to minimize your hands-on time.

Amicon® Pro Application	Components	Protocol Steps	Resin Guideline	Benefits[3]
DEPLETION OR ENRICHMENT	Exchange device + Amicon® Ultra filter	• Bind • Deplete/Concentrate +/- Wash/Concentrate +/- Buffer Exchange	≤200 µL packed resin[1]	• Speed • No sample transfer - No loss



One-step depletion enriches your protein sample for more informative proteomics data. After binding your sample to a depletion resin (i.e., Anti-albumin), attach the Amicon® Ultra filter prior to centrifugal passage of the unbound fraction for simultaneous sample depletion with concentration in one step. Invert the Amicon® Ultra 0.5 mL device and spin to collect your final sample. Your sample is ready for proteomic analysis.

Ordering Information

To choose the appropriate Amicon® Pro device, determine the molecular weight cut-off (MWCO) of your protein of interest and your desired affinity purification scheme. For convenience and ease of use, the Amicon® Pro purification kits contain devices, reagents and buffers optimized for twelve reactions. These kits are ideal for affinity purification of tagged recombinant proteins, antibody purification and depletion.

Amicon® Pro Purification Kits 12/pk Includes reagent kit (resin & buffers)	Reagent Kit Only	MWCO				
		3,000	10,000	30,000	50,000	100,000
Amicon® Pro Affinity Concentration Kit Ni-NTA	ACR5000NT	ACK5003NT	ACK5010NT	ACK5030NT	ACK5050NT	ACK5100NT
Amicon® Pro Affinity Concentration Kit Protein A	ACR5000PA	ACK5003PA	ACK5010PA	ACK5030PA	ACK5050PA	ACK5100PA
Amicon® Pro Affinity Concentration Kit Protein G	ACR5000PG	ACK5003PG	ACK5010PG	ACK5030PG	ACK5050PG	ACK5100PG
Amicon® Pro Affinity Concentration Kit GST	ACR5000GS	ACK5003GS	ACK5010GS	ACK5030GS	ACK5050GS	ACK5100GS

Amicon® Pro purification system – No Reagents Included	MWCO				
	3,000	10,000	30,000	50,000	100,000
Amicon® Pro Purification System Trial Pack 2/pk	ACS500302	ACS501002	ACS503002	ACS505002	ACS510002
Amicon® Pro Purification System 12/pk	ACS500312	ACS501012	ACS503012	ACS505012	ACS510012
Amicon® Pro Purification System 24/pk	ACS500324	ACS501024	ACS503024	ACS505024	ACS510024

Amicon® Pro purification system – Jump from lysate to concentrated, pure protein in a single device.
To view a video and learn more, please visit: www.merckmillipore.com/AmiconPro

Protein Purification with Protease Cleavage Enzymes

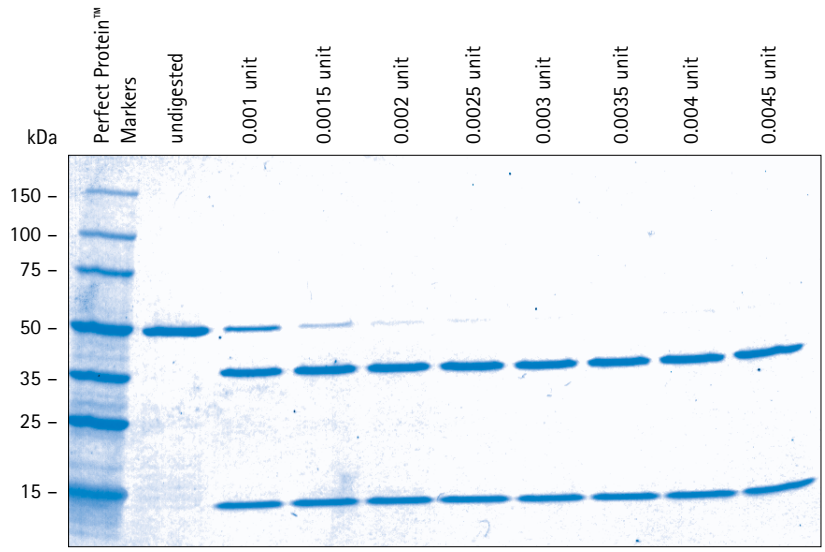
Featured Products

Restriction & Biotinylated Grade Thrombin

Highly efficient, specific cleavage of fusion proteins

Restriction Grade Thrombin is qualified to specifically cleave target proteins containing the recognition sequence LeuValProArg ↓ GlySer. The preparation is functionally tested for activity with fusion proteins and is free of detectable contaminating proteases. Thrombin is supplied with 10X Thrombin Cleavage Buffer and a Cleavage Control Protein.

Biotinylated Thrombin is identical in activity to Restriction Grade Thrombin, but has covalently attached biotin for easy removal of the enzyme from cleavage reactions using immobilized streptavidin. Our Thrombin Cleavage Capture Kit not only includes biotinylated thrombin and immobilized streptavidin but also all required buffers and filters for complete, convenient recovery of cleaved protein.



Biotinylated Thrombin cleavage. The indicated amounts of Biotinylated Thrombin were used to cleave 2 µg of Cleavage Control Protein in an overnight digestion. Samples were analyzed by SDS-PAGE (4–20% gradient gel) followed by staining with Coomassie blue. The 0.0045-unit lane represents a 2.25-fold over-digestion.

HRV 3C Protease

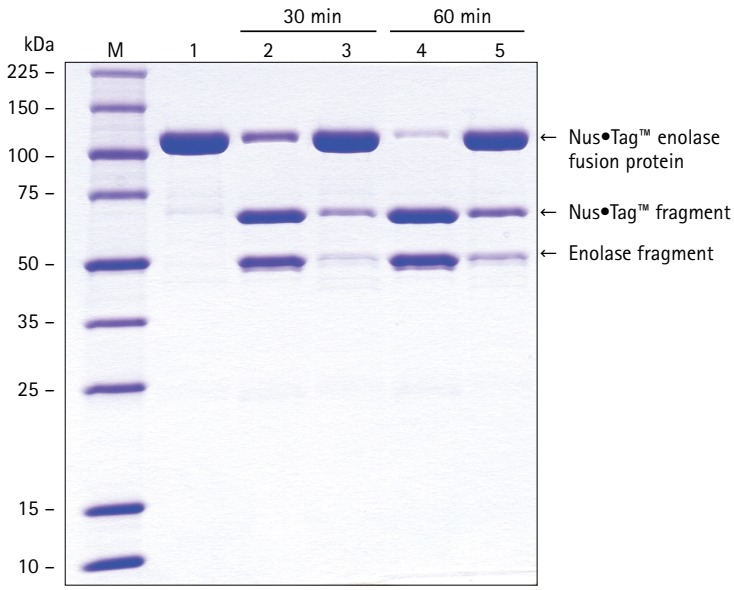
Highly efficient, specific cleavage of fusion proteins

Recombinant type 14 3C protease from human rhinovirus (HRV 3C) is a highly purified, recombinant 6XHis-tagged enzyme, which recognizes the cleavage site LeuGluValLeuPheGln ↓ GlyPro.

The small, 22-kDa size of the protease, with optimal activity at 4 °C, high specificity, and His-tag fusion make HRV 3C protease an ideal choice for rapid removal of fusion tags.

Lane	Sample
M	PerfectProtein Markers, 10-225 kDa
1	3 µg purified Nus•Tag™ enolase fusion protein
2	3 µg Nus•Tag™ enolase fusion protein with 30-min HRV3C protease reaction
3	3 µg Nus•Tag™ enolase fusion protein with 30-min competitor's protease reaction
4	3 µg Nus•Tag™ enolase fusion protein with 60-min HRV3C protease reaction
5	3 µg Nus•Tag™ enolase fusion protein with 60-min competitor's protease reaction

HRV 3C Protease cleaves fusion proteins more efficiently compared to cleavage with a competitor's protease. Using a 1:100 (w/w) ratio of protease:target protein, 500 µg of purified Nus•Tag™ enolase fusion protein was incubated in parallel 500 µL reactions at 4°C. The reactions was quenched by adding equal volume 4X SDS Sample Buffer and then immediately placing the samples into a water bath at 75 °C for 5 min.



Ordering Information

Available from www.merck4biosciences.com

Description	Catalogue No.
Restriction-Grade Thrombin	69671
Biotinylated Thrombin	69672
Thrombin Cleavage Capture Kit	69022
Restriction Grade Factor Xa	69036
Factor Xa Cleavage Capture Kit	69037
Recominant Enterokinase	69066
Enterokinase Cleavage Capture Kit	69067
HRV 3C Protease	71493
Tag·off™ High Activity rEK	71537
Tag·off™ rEK Cleavage Capture Kit	71540

Protein Buffer Optimization and Sample Concentration

When downstream quality matters, make sure your upstream tools are the best. The last steps of preparing a protein sample for downstream analyses, such as activity assays or structural studies, involve ensuring that the protein is in its native, soluble form, dissolved in the buffer of choice, and at an appropriate concentration. With Merck Millipore's tools for protein buffer optimization and sample concentration, obtain publication-quality data from every last microgram of protein.

Protein Buffer Exchange, Sample Desalting, and Dialysis

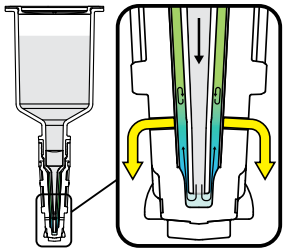
Each protein preparation is unique. Give it the special treatment it deserves with a perfectly designed device for dialyzing and buffer exchange. Select between fast and gentle diafiltration using the Amicon® Pro System or dialysis using D-Tube™ Dialyzers.

Fast: single spin
Gentle: unique design provides continuous diafiltration
Less Buffer: only 1.5 mL buffer required

Sample Needs	Amicon® Pro System	Amicon® Ultra Filter	D-Tube™ Dialyzer
Faster optimization	~20 minutes	<1 hour	5 hours
Sensitive samples which may precipitate at higher concentrations	+	-	+
Post-dialysis concentration	+	+	-
Limited amounts of exchange solvent	+	+	-
Temperature sensitive	Minimal effect of cold temperature on speed	Minimal effect of cold temperature on speed	Cold temperature reduces speed

Choose the direct route. Desalt with a single spin. Amicon® Pro Purification System

Maximize protein activity with gentle, single-spin diafiltration.
Buffer exchange using dialysis or diafiltration is often required to make a protein sample compatible with specific downstream analyses. But dialysis is time-consuming and multi-step diafiltration risks loss of activity and can require subsequent concentration. The Amicon® Pro device offers ground-breaking, gentle, single-spin diafiltration for simultaneous buffer exchange with concentration.



The uniquely designed interface between the exchange tube tip and the Amicon® Ultra device enables greater than 99% buffer exchange in a single spin. Buffer exchange, as shown in this diagram, was measured by the replacement of a low-molecular weight dye (yellow) with clear buffer (black arrows); while a high-molecular weight dye (bright blue) was retained inside the Amicon® Ultra device.

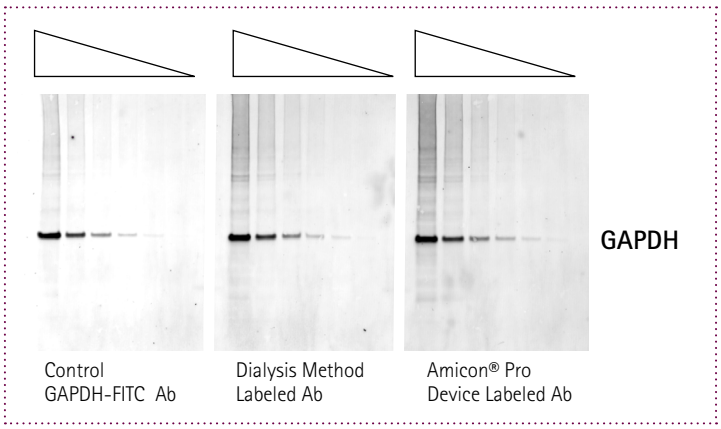
The gentleness of dialysis with the efficiency of diafiltration.

	Dialysis cassette + concentrator	0.5 mL diafiltration device (3 spin)	Amicon® Pro purification system
Process time	16 hours	50 min.	20 min.
Recovery	51%	> 95%	> 95%
Specific activity (signal/µg GST-LLP)	0.195	0.17	0.199

Gentler buffer exchange = greater activity. Eluted Samples of GST-lambda protein phosphatase (LPP) buffer exchanged and concentrated using Amicon® Pro device showed greater specific activity and percentage recovery than when prepared with a dialysis cassette (plus concentrator device) or 0.5 mL diafiltration spin column.

One hour antibody labeling.

The unique design of the exchange tip enables single spin diafiltration.



Generate FITC-labeled antibody in one hour. What's faster than labeling antibodies using other purification methods, and more economical than purchasing prelabeled antibodies? Using Amicon® Pro purification systems for antibody labeling.

Step	Dialysis-based buffer exchange pre/post labeling	Amicon® Pro purification system
Buffer exchange	Overnight	15 min
FITC labeling	3 h	30 min
Free FITC removal and buffer exchange	Overnight	15 min
Total time	3 days	1 h
Antibody recovery	39%	72%

Ordering Information

To choose the appropriate Amicon® Pro device, determine the molecular weight cut-off (MWCO) of your protein of interest and your desired affinity purification scheme.

Amicon® Pro purification system – No Reagents Included	MWCO				
	3,000	10,000	30,000	50,000	100,000
Amicon® Pro Purification System Trial Pack 2/pk	ACS500302	ACS501002	ACS503002	ACS505002	ACS510002
Amicon® Pro Purification System 12/pk	ACS500312	ACS501012	ACS503012	ACS505012	ACS510012
Amicon® Pro Purification System 24/pk	ACS500324	ACS501024	ACS503024	ACS505024	ACS510024

Amicon® Pro purification system – the gentleness of dialysis at the speed of diafiltration.
To view a video and learn more, please visit: www.merckmillipore.com/AmiconPro

Featured Products

D-Tube™ Dialyzers

Fast and easy dialysis

Gently dialyze intractable or sensitive samples and prevent them from precipitation or over-concentration. Providing maximum efficiency, D-Tubes™ dialyzers are designed with a double membrane to spread the sample over a large surface area enabling complete dialysis in just two to five hours.

D-Tube™ Dialyzer Advantages:

> 89% Sample Recovery

- Low binding membrane and housing enhance sample recovery

Reliable and Easy to Use

- Secure design prevents sample loss due to leaks—no knots or clamps to loosen and leak
- Easy to open and close with a screw cap
- Rigid frame permits smooth sample withdrawal of submilliliter volumes—removing every last drop is easy

Convenient Sample Loading

- No need to use a syringe to load or remove samples. Simply load your sample with standard pipette tip
- Floating racks fit most standard beakers to hold devices in exchange buffer
- D-Tubes™ dialyzers can also be used to electroelute samples from agarose or acrylamide

Ordering Information

Available from www.merckmillipore.com/psp

		Product	D-Tube™ Mini	D-Tube™ Midi	D-Tube™ Maxi	D-Tube™ Mega	D-Tube™ Mega
Proteins/DNA/RNA/ Oligonucleotides	Nominal Molecular Weight Cutoff	Maximum initial sample volume	10 to 250 µL	50 to 800 µL	100 µL to 3 mL	3 to 10 mL	10 to 15 mL
MW	NMWCO	Qty/pk					
MW < 7 k	3,500	10		71506-3	71508-3	71739-3	71742-3
		50				71739-4	71742-4
7 < MW < 24 k	7,000	10	71504-3	71507-3	71509-3	71740-3	71743-3
		50				71740-4	71743-4
		1 plate of 96	71712-3				
24 k < MW	13,000	10	71505-3		71510-3		
		50					
		1 plate of 96	71713-3				
	Floating Rack	Product (Qty/pk)	Mini (10)	Midi (10)	Maxi (10)	Mega (10)	Mega (10)
			71512-3	71513-3	71514-3	71748-3	71748-3

Fast and Easy Diafiltration With Amicon® Ultra Centrifugal Filters

Change buffers by gradually adding new solvent during simultaneous ultrafiltration

Because some macromolecules can lose activity or proper structure upon extreme changes of buffer conditions, use diafiltration, which involves removing microsolutes by adding solvent to the sample being filtered at the same time that ultrafiltration is being applied.

Advantages of Amicon® Ultra diafiltration:

- Fast – buffer exchange in as few as two spins
- Efficient – requires minimal volume of exchange buffer, easily contained in reservoir
- Easy to use – simply load your sample with standard pipette tip
- Enables simultaneous concentrating and desalting

For product selection consult the Amicon® Ultra selection chart on pages 32–34.

Centrifugal Concentration Devices

Featured Products

Amicon® Ultra Centrifugal Filters
Fast and easy protein concentration

Amicon® Ultra Centrifugal filters provide fast sample processing and promote high sample recoveries, even in dilute samples, through ultrafiltration. The unique features of the Amicon® Ultra centrifugal filters give you the fastest, most efficient concentration for sensitive downstream applications.

Amicon® Ultra Centrifugal Filter Advantages:

Maximize Concentration with Highest Protein Recovery

True Engineered Dead Stop

- Avoids spinning to dryness
- Provides a predictable concentration factor
- No need to calibrate for several samples to run in parallel

Reverse Spin Recovery

- Reverse spin devices enable you to maximize protein recovery, especially with small dilute samples, without introducing pipetting errors
- Low binding membrane and polypropylene housing for >90% sample recovery

Fast and Efficient Concentration Without Compromise

Ultracel® Low-binding Membranes

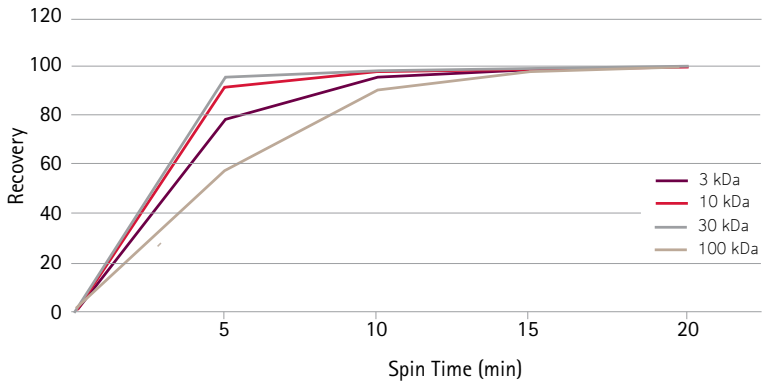
- Vertical membrane design aligns with filtrate rather than perpendicular for less clogging, less waste and faster filtration
- Ultra-fast sample processing achieving concentration in as little as 10 minutes
- 25- to 80-fold concentration in a single step

Broad Chemical Compatibility

- Heat-sealed membrane eliminates adhesives and downstream extractables
- Large spectrum of compatibility
- Compatible with pH 1 to 9

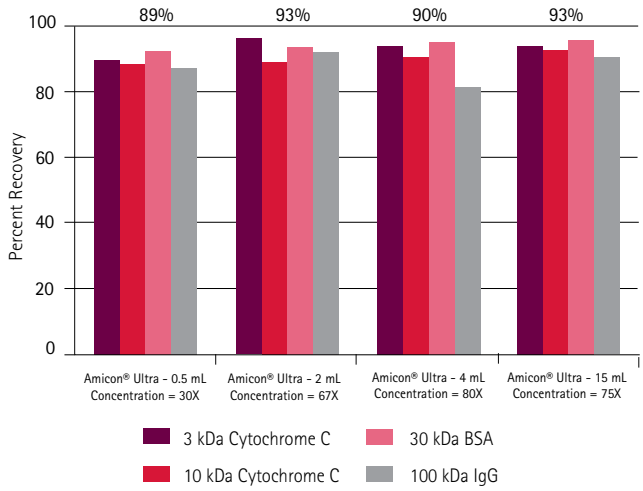
Reliable Samples

- Spin precious samples with confidence in one robust, sleek unit that prevents leakage



Amicon® Ultra 4 mL Filters—Fast Spin Times with Excellent Recovery

Average spin time for Amicon® Ultra-4 mL Filters:
Four different proteins (3 kDa Cytochrome C, 10 kDa Cytochrome C, 30 kDa BSA, and 100 kDa IgG) were tested on the Amicon® Ultra-4mL Filters for percent recovery and spin time. The data show that greater than 95% of all protein was recovered in 15 minutes or less.



Consistently high recovery of diverse proteins with Amicon® Ultra filters

Concentration and percent recovery using Amicon® Ultra Filters: 4 different devices (Amicon® Ultra-0.5 mL, Amicon® Ultra-2 mL, Amicon® Ultra-4 mL, Amicon® Ultra-15 mL), were tested with four different proteins (3 kDa Cytochrome C, 10 kDa Cytochrome C, 30 kDa BSA and 100 kDa IgG) to determine percent recovery and concentration factor.

To select an Amicon® Ultra Centrifugal Filter, identify the starting volume, molecular weight of protein or nucleic acid being concentrated, final volume and concentration factor.

Then consult the product selection chart below to choose the Amicon® Ultra filter with the right molecular weight cutoff (MWCO).

	Amicon® Ultra-0.5	Amicon® Ultra-2	Amicon® Ultra-4	Amicon® Ultra-15
Starting Volume	< 0.5 mL	< 2 mL	< 4 mL	< 15 mL
Proteins				
MOLECULAR WEIGHT (MW) 6 < MW < 20 k	3,000	3,000	3,000	3,000
20 < MW < 60 k	10,000	10,000	10,000	10,000
60 < MW < 100 k	30,000	30,000	30,000	30,000
100 < MW < 200 k	50,000	50,000	50,000	50,000
200 k < MW	100,000	100,000	100,000	100,000
Single-Stranded and Double-Stranded Nucleic Acids				
LENGTH 137-1159 bp	30,000	30,000	30,000	30,000
Nanoparticles				
PARTICLE DIAMETER (DIA) 1.5 < dia < 3 nm	3,000	3,000	3,000	3,000
3 < dia < 5 nm	10,000	10,000	10,000	10,000
5 < dia < 7 nm	30,000	30,000	30,000	30,000
7 < dia < 10 nm	50,000	50,000	50,000	50,000
10 nm < dia	100,000	100,000	100,000	100,000

MWCO: Molecular Weight Cut Off

10,000 MWCO Amicon® Ultra-4 and -15 filters are both CE marked for *in vitro* diagnostic use.

Once you've chosen the right Amicon® Ultra filter for your needs, choose your rotor, G force and spinning time for concentrating your molecule.

Designed as standard 1.5 mL, 15 mL conical or 50 mL conical tubes, Amicon® Ultra filters fit all stardard rotor types.

CHOOSE A ROTOR AND G FORCE

	Amicon® Ultra-0.5	Amicon® Ultra-2	Amicon® Ultra-4	Amicon® Ultra-15
Starting Volume	< 0.5 mL	< 2 mL	< 4 mL	< 15 mL
Final Volume	15–20 µL	15–70 µL	50 µL	200 µL
Design of the Device	Standard 1.5 mL	Standard 15 mL	Standard 15 mL	Standard 50 mL
Fixed-Angle (35 °) Rotor	14,000 g 1,000 g reverse spin	7,500 g 1,000 g reverse spin	5,000 g for 100,000 7,500 g for all other MWCO	5,000 g
Swinging Bucket Rotor	N/A	4,000 g 1,000 g reverse spin	4,000 g	4,000 g

CONCENTRATION FACTOR

Final Volume	15–20 µL with reverse spin	15–70 µL with reverse spin	50 µL	200 µL
Concentration Factor	X25–X30	X14–X67	X80	X75

ADJUST SPINNING TIME

For Proteins and Nanoparticles

3,000	30 min.	60 min.	40 min.	40 min.
10,000	15 min.	40 min.	15 min.	20 min.
30,000	10 min.	20 min.	10 min.	20 min.
50,000	10 min.	15 min.	10 min.	15 min.
100,000	10 min.	30 min.	10 min.	15 min.

Single-Stranded and Double-Stranded Nucleic Acids

30,000	10 min.	15 min., fixed angle 40 min., swinging rotor	10 min., 5,000 g, fixed angle	10 min., 5,000 g, fixed angle

Visit **www.merckmillipore.com/psp** to check both chemical compatibility and centrifuge/rotor compatibility of Amicon® Ultra devices.

Amicon® Ultra Centrifugal Filters

	Product	Amicon® Ultra-0.5	Amicon® Ultra-2	Amicon® Ultra-4	Amicon® Ultra-15
	Maximum initial sample volume (mL)	0.5	2	4	15
	Final concentrate (retentate) volume (µL)	15–20	15–70	30–70	150–300
MWCO	**Qty/Pk**				
3,000 MWCO	8	UFC500308		UFC800308	UFC900308
	24	UFC500324	UFC200324	UFC800324	UFC900324
	96	UFC500396		UFC800396	UFC900396
	500	UFC5003BK			
10,000 MWCO	8	UFC501008		UFC801008*	UFC901008*
	24	UFC501024	UFC201024	UFC801024*	UFC901024*
	96	UFC501096		UFC801096*	UFC901096*
	500	UFC5010BK			
30,000 MWCO	8	UFC503008		UFC803008	UFC903008
	24	UFC503024	UFC203024	UFC803024	UFC903024
	96	UFC503096		UFC803096	UFC903096
	500	UFC5030BK			
50,000 MWCO	8	UFC505008		UFC805008	UFC905008
	24	UFC505024	UFC205024	UFC805024	UFC905024
	96	UFC505096		UFC805096	UFC905096
	500	UFC5050BK			
100,000 MWCO	8	UFC510008		UFC810008	UFC910008
	24	UFC510024	UFC210024	UFC810024	UFC910024
	96	UFC510096		UFC810096	UFC910096
	500	UFC5100BK			

*Certified for clinical applications.

To use the online Amicon® selector tool to choose the perfect filter and view protocols visit: www.merckmillipore.com/AmiconSelect

Specialized Concentration Devices

Concentration of gDNA and Protein

Microcon® DNA Fast Flow Filter

Optimized for the concentration and recovery of genomic DNA with SDS buffer. The low nonspecific binding characteristics of the membrane and the other device components, coupled with its medical-grade o-ring seal, allows the device to accommodate several wash steps with minimal sample loss.

Microcon® DNA Fast Flow Advantages:

- High recovery for small volumes with reverse spin (concentration factor <20X)
- Low-binding Ultracel® membrane
- Fast processing

Microcon® Centrifugal Filters

Simply and efficiently concentrate and desalt solutions of any macromolecule with the low-binding Ultracel® membrane, using any centrifuge that can accept 1.5 mL tubes.

Microcon® Advantages:

- Typical recoveries of >95%, even for dilute solutions
- Reverse spin to maximize recovery, even in the smallest samples
- Convenient storage of filtrate or concentrated sample in standard microfuge tube
- Concentration factors up to 100X

Application Guidelines

	Microcon® Device		
Application	10K	30K	DNA Fast Flow
Peptide and growth factor concentration	●		
Protein concentration and desalting of columns eluates	●	●	
Protein concentration before electrophoresis or other assays	●	●	
Protein removal prior to HPLC	●	●	
Purification of macromolecular components found in tissue culture extracts and cell lysates	●	●	
Concentration of biological samples (antigens, antibodies, enzymes)		●	
Concentration of gDNA with or without SDS buffer		●	●
Concentration and desalting of nucleic acids (single-or double-stranded)	●	●	●
Removal of labeled nucleotides	●	●	●
Removal of labeled amino acids	●	●	●
Removal of primers from amplified DNA		●	●
Removal of linkers prior to cloning		●	●

Ordering Information

MWCO	Qty/Pk	Catalogue No.	Description	Volume, mL	Min. final concentrate volume, µL
10	100	MRCPRT010	Microcon® filter, Ultracel®-10 membrane, 10kDa	0.5	5-50
30	100	MRCF0R030	Microcon® filter, Ultracel®-30 membrane, 30kDa	0.5	5-50
-	100	MRCF0R100	Microcon® filter, Ultracel® DNA Fast Flow Membrane	0.5	5-50

Spin filters for clarification, filtration, and sterilization

Ultrafree®-MC and Ultrafree®-CL centrifugal filters remove particles and precipitates from aqueous and some solvent based samples. These fast filtration units provide highly reproducible performance for sample recovery. Ultrafree® centrifugal filters are ideal for use in protein and nucleic acid solutions.

Ultrafree®-MC filter advantages:

- Five different pore sizes from 0.1 to 5.0 µm
- Pre-sterilized units also available
- Fast filtration and highly reproducible performance
- Use in fixed-angle rotors for 1.5 mL tubes

Ultrafree®-CL filter advantages:

- High recovery Durapore® (PVDF) and hydrophilic PTFE membranes
- Five different pore sizes from 0.1 to 5.0 µm
- Pre-sterilized units also available
- Fast filtration and highly reproducible performance
- Use in fixed-angle rotors for 15 mL tubes

Sterile Ultrafree®-MC and CL centrifugal filter units with microporous membrane

- Easy, pre-sterilized, centrifugal sample clarification units for either 0.5 mL (MC) or 2 mL (CL) maximum volumes
- High recovery Durapore® (PVDF) membrane
- Fast filtration and highly reproducible performance
- Use in fixed-angle rotors for 1.5 mL tubes (MC) or 15 mL tubes (CL)

Product	Ultrafree®-MC	Ultrafree®-CL
Maximum initial sample volume (mL)	0.5	2
Hold-up volume (µL)	5	10
Centrifugal force	12,000	5,000
Spin time	1 to 4 min.	1 to 4 min.
Pore Size (µm)	**Qty/Pk**	
0.1	25	UFC30VV25	UFC40VV25
	100	UFC30VV00	UFC40VV00
0.22	25	UFC30GV25	UFC40GV25
	100	UFC30GV00	UFC40GV00
	250	UFC30GVNB	
	5 x 10 sterile	UFC30GV0S	UFC40GV0S
0.45	25	UFC30HV25	UFC40HV25
	100	UFC30HV00	UFC40HV00
	250	UFC30HVNB	
0.65	25	UFC30DV25	UFC40DV25
	100	UFC30DV00	
	5 x 10 sterile	UFC30DV0S	
5	100/25	UFC30SV00	UFC40SV25

Concentrate high solute samples

Centriprep® centrifugal filters are disposable ultrafiltration devices used for purifying, concentrating, and desalting biological samples (2–15 mL volume range) and for filtration applications. Offering a high flow rate, these filters come complete and are easy to use with a twist-lock cap, a filtrate collector containing a low adsorptive Ultracel® regenerated cellulose membrane, plus an air-seal cap for sample isolation.

Centriprep® filter advantages and applications:

- Unique inverse flow mode of operation with large deadstop
- Concentrate and purify particle-laden solutions of high concentrations with Ultracel® membrane
- Fast sample processing
- Fits standard swinging-bucket rotor for 50 mL tubes
- Concentrate and purify particle-laden solutions or high concentrations
- Separate low MW solutes from fermentation broths, cell culture media, cell lysates

Ordering Information

MWCO	Qty/Pk	Catalogue No.	Description	Volume, mL	Min. final concentrate volume, µL
3	24	4302	Centriprep® YM-3, 3 kDa NMWL	15	700
3	96	4303	Centriprep® YM-3, 3 kDa NMWL	15	700
10	24	4304	Centriprep® YM-10,10 kDa NMWL*	15	700
10	96	4305	Centriprep® YM-10, 10 kDa NMWL*	15	700
30	24	4306	Centriprep® YM-30, 30 kDa NMWL*	15	700
30	96	4307	Centriprep® YM-30, 30 kDa NMWL*	15	700
50	24	4310	Centriprep® YM-50, 50 kDa NMWL	15	700
50	96	4311	Centriprep® YM-50, 50 kDa NMWL	15	700

* Centriprep® centrifugal filter devices with Ultracel® 10K and 30K membranes are approved for *in vitro* diagnostic use.

Clinical Ultrafiltration

Separate free from protein-bound solute

The Centrifree® filter was designed with the clinical laboratory in mind, these devices rapidly and efficiently separate free from protein-bound micro-solute in small volumes (0.15–1.0 mL) of serum, plasma, and other biological samples using ultrafiltration. Accurate partitioning occurs in minutes without dilution, change in physiologic pH, ion composition, or unbound microsolute concentration. These devices contain low-adsorptive hydrophilic membranes and O-rings without plasticizers to ensure excellent recovery.

Centrifree® filter advantages and applications:

- Separation of free from bound microsolute in serum, plasma, and other biological samples
- Determine free therapeutic drugs, testosterone, thyroxin
- Binding studies
- New drug investigations
- Deproteinization

Ordering Information

MWCO	Qty/Pk	Catalogue No.	Description	Volume, mL	Min. final concentrate volume, µL
10	50	4104	Centrifree® Ultrafiltration device with Ultracel® YM-T membrane	1	50

Concentrate Multiple Clinical Samples

Minicon® concentrators are non-sterile, disposable, multiwell ultrafiltration devices designed for concentrating macromolecules in clinical specimens such as urine, cerebrospinal fluid (CSF) or other biological solutions. The concentrators, which require no additional equipment and can be operated unattended, are used by researchers and clinical laboratories worldwide as a preparatory step to increase the sensitivity of subsequent tests.

Minicon® concentrator advantages and applications:

- Concentrate urine and cerebrospinal fluid to intensify proteins that indicate abnormal or pathological states prior to analysis by electrophoresis or immunoelectrophoresis (e.g., Bence Jones proteins in urine)
- Static concentrator, requiring no accessories
- Absorbent pulls solvent and salts through ultrafilter, concentrating sample

Ordering Information

MWCO	Qty/Pk	Catalogue No.	Description	Volume, mL	Min. final concentrate volume, µL
15	40	9031	Minicon® B15, 8 cells/unit	5	50
15	50	9051	Minicon® CS15, 10 cells/unit	2.5	30
15	50	9051	Minicon® CS15, 10 cells/unit	2.5	30

Large Volume Concentration

Convenient alternative to stirred cells

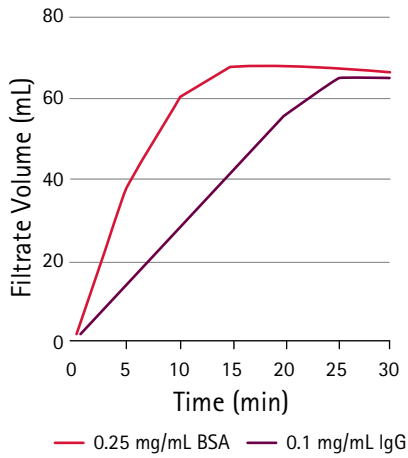
The Centricon® Plus-70 centrifugal filter is designed for rapid processing of aqueous biological solutions in volumes ranging from 15 to 70 mL. Centricon® filters concentrate most 70 mL solutions down to 350 µL in as little as 25 minutes. Samples are typically concentrated in the 50X to 200X range, depending on the sample type and starting sample volume. These units are a convenient alternative to dialysis, lyophilization, precipitation techniques or stirred cells.

Centricon® Plus-70 advantages and applications

- >90% typical recovery
- Low hold-up volume
- Polypropylene housing minimizes binding
- True dead stop prevents spinning to dryness
- Concentrating and desalting chromatography column eluates
- Concentrating monoclonal antibodies
- Concentrating proteins or viruses from culture supernatants
- Clarifying tissue homogenates and cell lysates

Performance

Spin time with respect to filtrate volume



Ordering Information

MWCO	Qty/Pk	Catalogue No.	Description	Volume, mL	Min. final concentrate volume, µL
10	8	UFC701008	Centricon® Plus-70 10K	70	350
30	8	UFC703008	Centricon® Plus-70 30K	70	350
100	8	UFC710008	Centricon® Plus-70 100K	70	330

Stirred Cells: 3 mL to 400 mL concentration

Amicon® stirred cells provide high flow rates with solutions up to 10% macrosolute concentration and are capable of rapid concentration, or salt removal followed by concentration in the same unit. For protein concentration, gas pressure is applied directly to ultrafiltration cell. Solutes above the membrane's molecular weight (MW) cut-off are retained in cell, while water and solutes below the cut-off pass into the filtrate and out of cell.

Advantages

- Gentle magnetic stirring minimizes concentration polarization and shear denaturation.
- All stirred cells can be autoclaved.
- Five different sizes to handle volumes from 3 mL to 400 mL
- High flow rates with solutions up to 10% macrosolute concentration

Applications

- Concentrate, diafilter, and exchange buffers for macromolecule solutions including proteins, enzymes, antibodies and viruses.

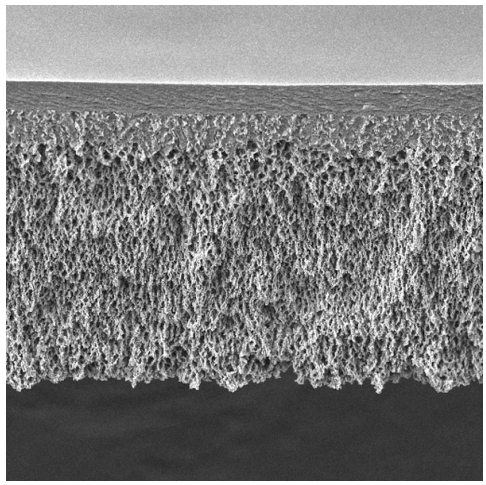
Available in five sizes

Min. Volume	Max. Volume	Catalogue No.
0.075 mL	3 mL	5125
1.0 mL	10 mL	5121
2.5 mL	50 mL	5122
5.0 mL	200 mL	5123
10 mL	400 mL	5124

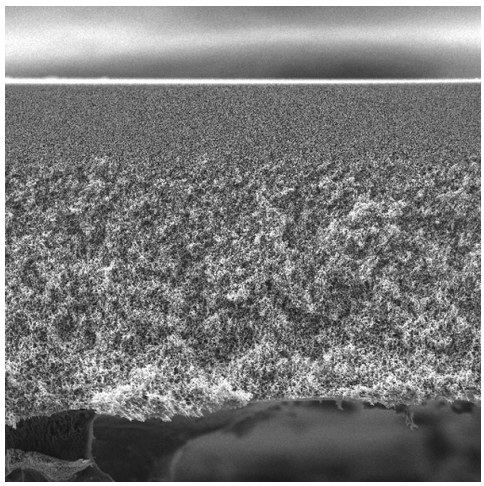
Ultracel® Ultrafiltration Discs for Use in Stirred Cells

To concentrate or desalt dilute solutions, use Ultracel® regenerated cellulose membranes. The hydrophilic, tight microstructure of Ultracel® membranes assures the highest possible retention with the lowest possible adsorption of protein, DNA or other macromolecules.

- Membranes available in 1, 3, 5, 10, 30 and 100 kDa nominal molecular weight limit (NMWL).
- Filter diameters available in 25, 44.5, 47, 63.5, 76, 90 and 150 mm.



Ultracel® regenerated cellulose ultrafiltration membrane.



Biomax® polyethersulfone ultrafiltration membrane.

To concentrate or desalt higher volumes of more concentrated samples (recommended for protein concentrations greater than 1.0 µg/mL), use Biomax® polyethersulfone (PES) membranes. These membranes are recommended for samples such as serum, plasma, or conditioned tissue culture media.

- Membranes available in 300 kDa nominal molecular weight limit (NMWL).
- Filter diameters available in 25, 44.5, 47, 63.5, 76, 90 and 150 mm.

For ordering information, please visit
www.merckmillipore.com/psp

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Drive your research forward with IR-based quantitation.

With the Direct Detect® spectrometer, the first infrared (IR)-based biomolecular quantitation system, there's no sample prep, messy cuvettes or waste—with one-time standard curves. The Direct Detect® system distinguishes proteins and peptides from sample components, such as lipids, that interfere with classical quantitation methods. Now you can achieve truly accurate results without the pitfalls of colorimetric assays, even for most lysates and complex samples.

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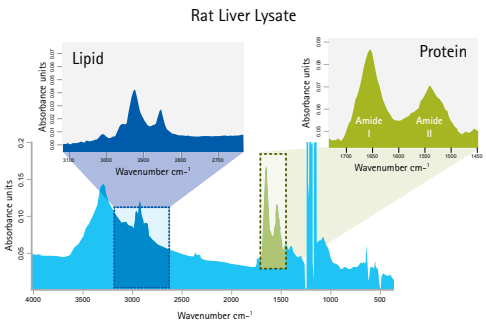

Accurate IR-based protein quantitation in a lipid-rich lysate. The most intense regions of lipid absorbance are distinct from the protein's Amide I signal.

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