Novagen®

His•Tag®
GST•Tag™

Purification and Detection Tools

Advancing your life science discoveries™

EMD

Introduction

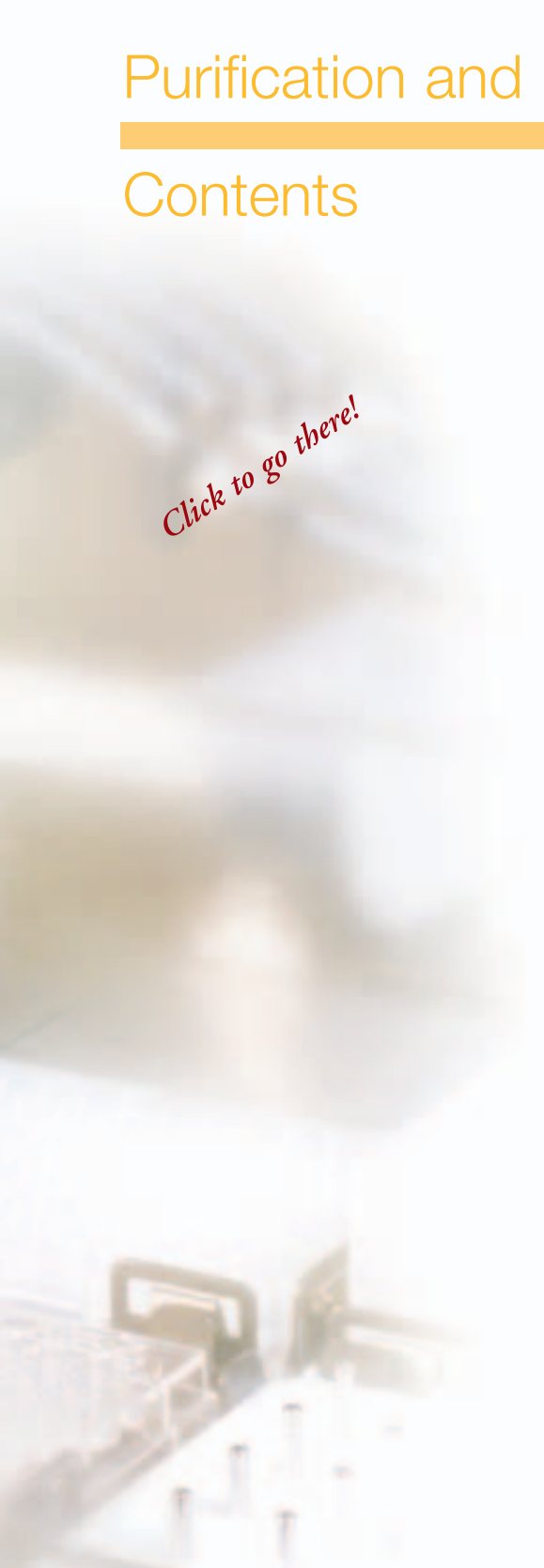
The His•Tag® and GST•Tag™ sequences are the most widely used fusion tags for the expression and purification of recombinant proteins. In this brochure, Novagen is pleased to showcase products specifically designed for the purification and detection of fusion proteins containing His•Tag and GST•Tag sequences. These products are optimized for purification of proteins expressed in bacterial, yeast, insect, or mammalian systems. Reagents and kits are offered in a variety of configurations suitable for processing milliliter- to liter-scale cultures in a low- or high-throughput environment.

As a first step in purification, efficient, gentle extraction is necessary for maximal recovery of intact target proteins from cell cultures. Part 1 describes a variety of detergent-based and enzymatic methods for convenient lysis and protein extraction from bacterial, yeast, insect, or mammalian cells.

Part 2 features a variety of affinity purification platforms, providing the options of conventional column chromatography, rapid magnetic-based separations, or filtration methods for purifying milligram quantities in a high-throughput environment. For the highly specific, sensitive detection of fusion proteins, antibodies directed against His•Tag or GST•Tag sequences may be used. Premium quality His•Tag and GST•Tag monoclonal antibodies and Western blot kits are also featured in Part 2.

After the target protein is purified, the fusion tag may be removed with one of the site-specific proteases described in Part 3. The final part of the brochure features key accessory products for protein purification and detection, including protease inhibitors, electrophoresis size standards, Western blot reagents, and protein quantification kits.

Contents



Click to go there!

Part 1 Contents

Click to go there!

Novagen®

Overview

Gentle, efficient, non-mechanical extraction of soluble proteins from bacteria, yeast, mammalian, and insect cells

When purifying proteins from cells or tissue sources, the first step is to disrupt cells in the sample and extract the relevant protein fraction. This step is critical because processing methods that require harsh mechanical and/or enzymatic treatments can directly affect the target protein's integrity and/or activity, or otherwise expose it to degradative conditions.

To address this problem, Novagen has introduced BugBuster®, YeastBuster™ and CytoBuster™ Protein Extraction Reagents, innovative combinations of detergents and other ingredients that enable gentle, efficient, non-mechanical extraction of soluble proteins from bacteria, yeast, mammalian, and insect cells. rLysozyme™ Solution increases the efficiency of bacterial lysis with BugBuster Reagent. Addition of Benzonase® Nuclease specifically degrades contaminating DNA and RNA for the preparation of non-viscous, nucleic acid-free extracts ready for target protein purification. Protease Inhibitor Cocktails are available to protect target protein against degradation in crude extracts (see Part 4).

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

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

BugBuster® Protein Extraction Reagents

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

BugBuster® Protein Extraction Reagent is formulated to gently disrupt the cell wall of *E. coli* and 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. At this point, Benzonase® Nuclease can be added to reduce the viscosity of the extract due to liberation of chromosomal DNA. The addition of rLysozyme™ Solution enhances the extraction efficiency, especially for larger proteins. Following a brief incubation, insoluble cell debris is removed by centrifugation. The clarified extract is ready to use and fully compatible with the affinity supports offered by Novagen, including GST•Bind™, GST•Mag™, His•Bind®, His•Mag™, and S•Tag™ Resins, or several other chromatography matrices. Following binding to affinity resin, excess BugBuster is easily removed by washing the column with the appropriate buffer. BugBuster 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 Reagent, BugBuster Plus Benzonase, BugBuster Purification Kits

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 bundled with 10,000 U Benzonase Nuclease (provided in a separate vial) for the preparation of low-viscosity extracts and/or removal of nucleic acids from protein preparations. BugBuster and Benzonase are compatible with common protease inhibitors. BugBuster Purification Kits (see Part 2) contain BugBuster, Benzonase, buffers and resins necessary for purification of His•Tag® and GST•Tag™ fusion proteins. rLysozyme Solution, a stable liquid formulation of recombinant lysozyme, is available separately.

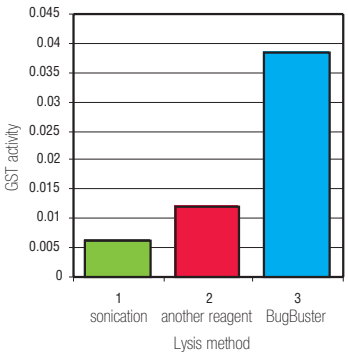
continued on next page

Product	Size	Cat. No.
BugBuster® Protein Extraction Reagent	100 ml	70584-3
	500 ml	70584-4
BugBuster Plus Benzonase® Nuclease	1 kit	70750-3

Components:

- 500 ml BugBuster Protein Extraction Reagent
- 10,000 U Benzonase Nuclease, Purity > 90%

Product listing continued on next page



Comparison of *E. coli* lysis methods

Fifty-milliliter sample 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 sec total. Samples in lysis reagent were treated according to their respective protocols. Extracts were clarified by centrifugation and assayed for GST enzymatic activity using Novagen's GST•Tag™ Assay Kit.

BugBuster® Protein Extraction Reagents *continued*

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 high-throughput protein purification applications.

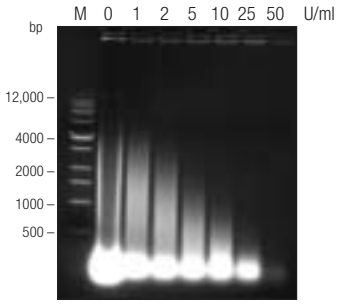
BugBuster 10X Protein Extraction Reagent

BugBuster 10X is a concentrated formulation of the proprietary detergents employed in BugBuster without the addition of buffer components. Concentrated BugBuster provides a flexible alternative to the ready-to-use standard 1X BugBuster, allowing user-defined dilution and addition of buffer components. BugBuster 10X has all of the bioprocessing benefits of standard BugBuster plus the freedom to control pH, reagent concentration, and buffer additives necessary for maximum extraction and activity of your target protein.

BugBuster (primary amine-free) Protein Extraction Reagent

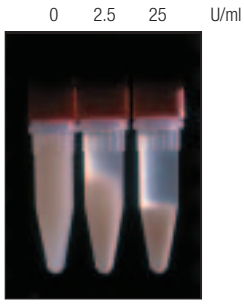
BugBuster (primary amine-free) is a special formulation of BugBuster designed for applications where primary amines would interfere if present in the protein extract, such as protein immobilization or cross-linking. The PIPPS buffer used in the primary amine-free formulation of BugBuster has a similar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

Product	Size	Cat. No.
BugBuster® HT Protein Extraction Reagent	100 ml	70922-3
	500 ml	70922-4
	1 L	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



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). Aliquots 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® Nuclease

E. coli BL21(DE3) cells containing a pET construct were suspended in BugBuster® Reagent (5 ml/g wet weight). Aliquots 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.

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 may 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 (1, 2). 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 (3). 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 need to separate cells from culture media
- No need to mechanically disrupt cells
- No need to clarify 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

Purification of His•Tag® GST expressed in *E. coli*

Purification Method	Yield[1]	Purity[2]
Standard His•Bind®	74	83
PopCulture™ His•Bind	111	89
PopCulture Ni-NTA His•Bind	170	85
PopCulture His•Mag™ [3]	128	94
Standard GST•Bind™	42	92
PopCulture GST•Bind	45	90
PopCulture GST•Mag™ [3]	40	94

1. Yield in micrograms of target protein purified per ml of culture, as determined by BCA protein assay.
2. % purity determined by scanning densitometry of Coomassie blue stained SDS polyacrylamide gels.
3. Data represent the average of 8 separate wells processed in parallel.

PopCulture Purification Kits

PopCulture Reagent is available bundled with His•Mag or 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 the RoboPop™ Purification Kits.

Product	Size	Cat. No.
PopCulture™ Reagent	15 ml	71092-3
	75 ml	71092-4
	250 ml	71092-5
PopCulture GST•Mag™ Purification Kit		71113-3

Components:

- 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

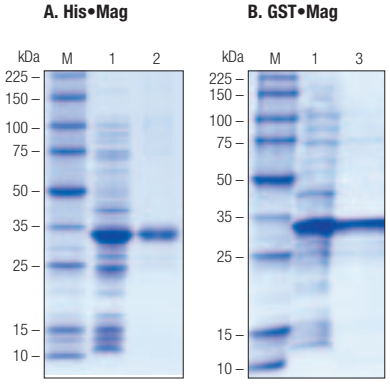
Product	Cat. No.
PopCulture His•Mag™ Purification Kit	71114-3

Components:

- 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

REFERENCES

1. Grabski, A., Drott, D., Handley, M., Mehler, M., and Novy, R. (2001) *in**Nova**tions* **13**, 1–4.
2. *in**Nova**tions* **15**, 18–19.
3. Grabski, A., Mehler, M., Drott, D., and Van Dinther, J. (2002) *in**Nova**tions* **14**, 2–5.



Lane Sample
M Perfect Protein™ Markers (10–225 kDa)
1. Crude extract (BugBuster® protocol)
2. PopCulture™ + His•Mag™ Agarose
3. 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 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.

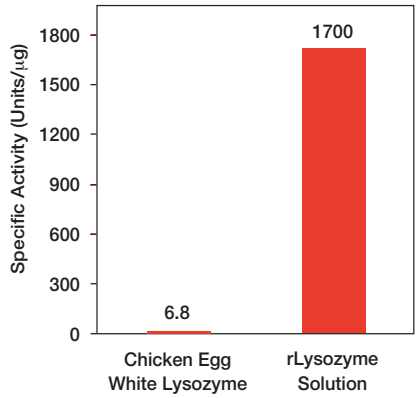
Bacterial Cell Lysis

rLysozyme™ Solution

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 (1,700 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 (3–5,000 U/gram 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 1.0 gram cell paste with 45–60 KU rLysozyme. 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 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 A_{450} units per minute at 25°C in a 1.0 ml suspension (1 mg/ml) of Tuner™(DE3) cells in 0.5X BugBuster diluted with 50 mM Tris-HCl, pH 7.5.



Comparison of chicken egg white lysozyme and rLysozyme activities

Activities were measured in a standard activity assay.

Product	Size	Cat. No.
rLysozyme™ Solution (30 KU/µl)	300 KU	71110-3
	1200 KU	71110-4
	6000 KU	71110-5

Components:

- 300 KU *or* 1200 KU *or* 6000 KU rLysozyme Solution
- 1 ml rLysozyme Dilution Buffer (71110-3 only)

Note: 1 KU = 1000 units

Nucleic Acid Removal

Benzonase® Nuclease

Effective viscosity reduction and removal of 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 increase yields of protein. For example, the enzyme is compatible with BugBuster and PopCulture Protein Extraction Reagents and can therefore be added along with these reagents to eliminate viscosity and remove 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°C to 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 HCl. 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 units/mg protein. The > 99% purity grade is tested for endotoxins and contains < 0.25 EU/1,000 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.
Benzonase® Nuclease, Purity > 90%	10 KU	70746-3
	2.5 KU	70746-4
Benzonase Nuclease HC, Purity > 90%	25 KU	71205-3
Benzonase Nuclease, Purity > 99%	10 KU	70664-3
Benzonase Nuclease HC, Purity > 99%	25 KU	71206-3

Note: 1 KU = 1000 units

YeastBuster™ Protein Extraction Reagent

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

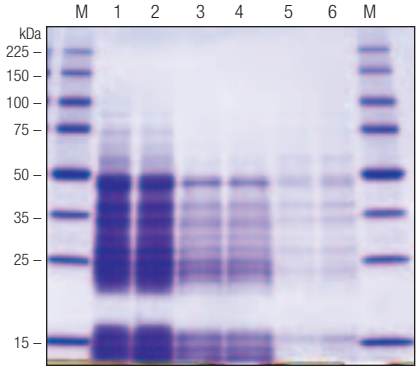
YeastBuster™ Protein Extraction Reagent is formulated for a fast, efficient and gentle extraction of soluble active proteins from *Saccharomyces cerevisiae* and *Pichia pastoris* cells. The reagent avoids harsh conditions of vigorous mechanical treatment that often result in heat and oxidative degradation of target proteins. The proprietary formulation utilizes a mix of mild detergent, protein stabilization buffer, and tris(hydroxypropyl)phosphine (THP) reducing agent (THP concentrate provided separately). 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. 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.

Product	Size	Cat. No.
YeastBuster™ Protein Extraction Reagent (includes 100X THP Solution)	100 ml	71186-3
	500 ml	71186-4

Features

- Gentle, rapid, efficient extraction of proteins from yeast cells
- Eliminates the inconsistencies associated with abrasive grinding, ultrasonication and pressure disruption of yeast cells
- Higher yield of total and enzymatically active soluble proteins as compared with traditional mechanical or other commercially available methods of cell disruption
- Fully compatible with Ni-NTA His•Bind IMAC and GST•Bind affinity purification methods

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

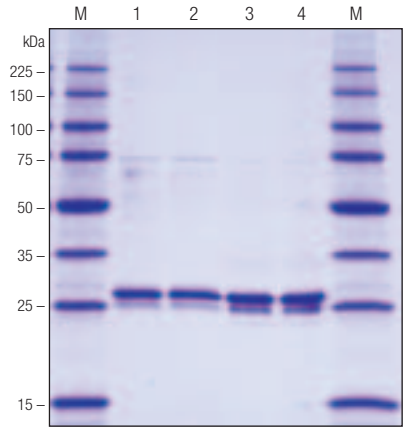
B. Protein and reporter assays

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

Figure 1. 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 grown at 30°C, induced for expression, and harvested at OD_{600} of 1.2. Cells were collected by centrifugation at 3,000 × g and resuspended in ice cold sterile water. Equal volumes of cells were dispensed into microcentrifuge tubes, and pelleted at 3,000 × 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. After initial resuspension of pellets by pipetting, YeastBuster and competitor reagent samples were agitated gently at room temperature for 20 min. Glass bead extraction was accomplished by resuspending the 65 mg pellet in lysis buffer containing 50 mM Tris-HCl, 250 mM LiCl, 100 mM $(NH_4)_2SO_4$, 1 mM DTT, and 2% glycerol, adding approximately 50 µl acid-washed glass beads (100–150 µm diameter), and vortexing the sample on high for 4 min with intermittent chilling on ice. 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 Non-Interfering Protein Assay™ Kit. GST activity was determined using 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 Novagen's 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

Figure 2. SDS-PAGE analysis of GST•Bind™ and Ni-NTA His•Bind® purified samples

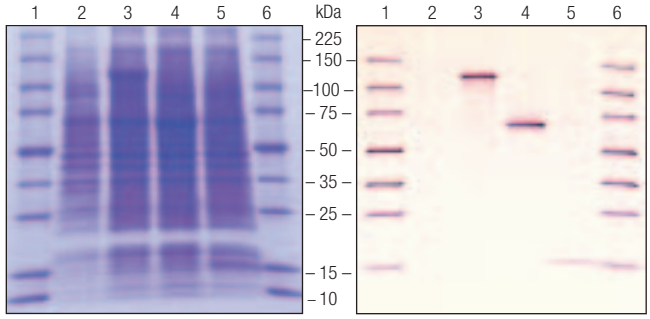
S. cerevisiae cells containing a recombinant plasmid expressing a 30.5 kDa GST•Tag™/His•Tag® fusion protein were grown and processed as described in Figure 1, panel A. The sample was centrifuged at 16,000 × g for 5 min and 4.5 ml aliquots of the supernatant were purified using GST•Bind or Ni-NTA His•Bind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

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 enables isolation of functionally active endogenous or expressed proteins without a need for secondary treatment such as sonication or freeze/thaw. CytoBuster 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.

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



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

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

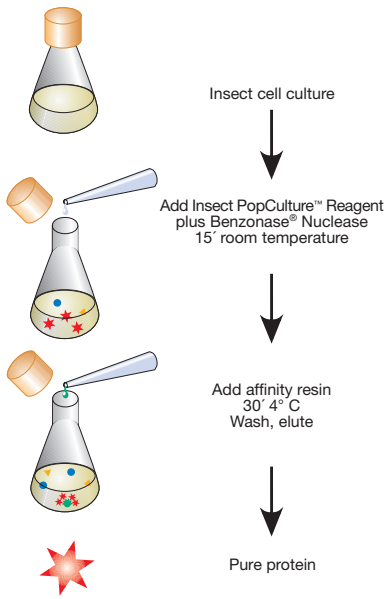
COS-1 cells were transfected with a pTriEx™ vector encoding the indicated S•Tag fusion proteins using GeneJuice™ Transfection Reagent. After 48 h the cells were treated with CytoBuster Protein Extraction Reagent and equal sample volumes analyzed by Coomassie stained SDS-PAGE (left panel) and Western 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.

Insect PopCulture™ Reagent

Protein extraction directly from insect cell cultures

Insect PopCulture™ Reagent is a new detergent-based lysis reagent that is 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 (1). It is amenable for automated expression-level screening and is fully compatible with Ni-NTA His•Bind® IMAC and GST•Bind™ affinity purification methods. Insect PopCulture reagent can be used for protein extraction from the insect cells grown in suspension and adherent cells grown on tissue culture plates.

Product	Size	Cat. No.
Insect PopCulture™ Reagent	50 ml	71187-3
	250 ml	71187-4



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 affinity resins from the total culture extract
- Compatible with protease inhibitor cocktails
- Ideal for high-throughput, expression-level screening and protein purification
- Compatible with baculovirus-infected cell cultures

REFERENCES

1. Loomis, K., Grabski, A., and Wong, S. C. (2002) *in**Nova**tions* **15**, 16–17.

Part 2 Contents

Click to go there!

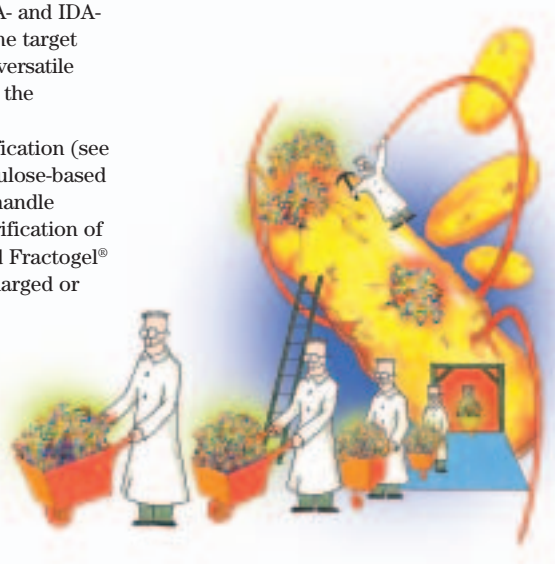
Novagen®

His•Bind® and His•Mag™ Purification Kits Overview

Purification of His•Tag fusion proteins by metal chelation chromatography

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

The various His•Bind supports cover many applications for fusion protein purification (see Affinity Resins and Buffers beginning on page 18). Choices include small scale cellulose-based columns and cartridges for convenient handling of multiple samples, bulk easy-to-handle agaroses for batch and gravity flow columns, His•Mag Agarose Beads for rapid purification of multiple samples with minimum handling time, and high flow rate Superflow™ and Fractogel® resins suitable for production scale purification. Supports are provided either uncharged or pre-charged with Ni^{2+}, and both NTA and IDA chemistries are available.



BugBuster® Ni-NTA His•Bind and His•Bind Purification Kits

BugBuster Protein Extraction Reagent is a ready-to-use solution that efficiently extracts soluble protein from *E. coli* without the need for mechanical disruption. The BugBuster Ni-NTA His•Bind Purification Kit and BugBuster His•Bind Purification Kits each combine BugBuster reagent with the respective resins for convenient preparation of soluble cell extracts and affinity purification of His•Tag fusion proteins. Please see page 17 for more information.

PopCulture™ His•Mag Purification Kit

PopCulture Reagent is a novel buffered detergent concentrate that extracts proteins from whole *E. coli* cultures without the need to harvest cells. The PopCulture His•Mag Purification Kit combines PopCulture with His•Mag Agarose Beads, buffers and rLysozyme™ Solution for convenient processing of small-scale cultures. Please see page 16 for more information.

RoboPop™ Ni-NTA His•Bind® Purification Kit

The RoboPop Ni-NTA His•Bind Purification Kit is designed for filtration-based 96-well format purification of His•Tag® fusion proteins directly from *E. coli* cultures without harvesting cells. The combination of PopCulture Extraction, Ni-NTA His•Bind purification, and a 2 ml filter plate allows high-throughput processing of up to 5 ml of *E. coli* culture per well. Please see page 14 for more information.

RoboPop His•Mag Purification Kit

The RoboPop His•Mag Purification Kit is configured for processing 96 × 1 ml cultures in a deep well plate (supplied in the kit). The combination of PopCulture™ Reagent and magnetic agarose beads enables the entire procedure, including both protein extraction and affinity purification, to be performed in the culture plate. Please see page 15 for more information.

Product	Culture scale	Processing method	Capacity[a]	Throughput level
BugBuster® Ni-NTA His•Bind® Purification Kit	Any	Gravity flow column chromatography	5–10 mg/ml of resin	Low
BugBuster His•Bind Purification Kit	Any	Gravity flow column chromatography	5–10 mg/ml of 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
RoboPop His•Mag Purification Kit	96 × 1 ml	Magnetic	125 µg/culture	High

[a] 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™ Ni-NTA His•Bind® Purification Kit

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

The RoboPop™ Ni-NTA His•Bind® Purification Kit is designed for filtration-based 96-well format purification of soluble His•Tag® fusion proteins directly from *E. coli* cultures without harvesting cells. The kit features PopCulture™ Reagent, rLysozyme™ Solution, and Benzonase® Nuclease for centrifuge-free cell lysis and extract preparation in one step. The combination of PopCulture extraction, Ni-NTA His•Bind Resin, and a 2 ml filter plate allows high-throughput processing of up to 5 ml of *E. coli* culture per well. Whereas the magnetic-based His•Mag™ kit purifies up to 125 µg target protein per 1 ml culture, the filtration-based kit purifies up to 1 mg His•Tag® fusion protein per 5 ml culture.

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 Packard-brand MultiPROBE® II liquid handling workstation from PerkinElmer Life Sciences. A 96-well Collection Plate (1 ml wells) with an air-tight aluminum foil sealer is provided for storage of the purified proteins.

Processing Protocol for RoboPop™ Ni-NTA His•Bind® Kit

1. Prepare *E. coli* cultures (3–5 ml in 24-well plate) under conditions for target protein production.
2. Add 0.1 culture volume PopCulture™ Reagent plus Benzonase® Nuclease and rLysozyme™ Solution to each well, mix, and incubate 10 min at room temperature.
3. (Optional) Take a sample from each well for screening expression levels of S•Tag™ fusion proteins using the FRETWorks™ S•Tag Assay, or by SDS-PAGE and Western blotting.
4. Add equilibrated Ni-NTA His•Bind® affinity resin, mix, and incubate 5 min at room temperature.
5. Transfer the mixture to the 96-well Filter Plate and separate the affinity resin from the extract with a vacuum manifold.
6. Wash the affinity resin by applying wash buffer to the 96-well Filter Plate followed by vacuum filtration.
7. Place the 96-well Collection Plate into the vacuum manifold, and elute the target protein using the appropriate elution buffer.

Product	Cat. No.
RoboPop™ Ni-NTA His•Bind® Purification Kit	71188-3

Components:

- 75 ml PopCulture™ Reagent
- 25 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 1 ml 96-well Collection Plate with Sealer
- 300 KU rLysozyme™ Solution
- 1 ml rLysozyme Dilution Buffer
- 10 KU Benzonase® Nuclease, Purity > 90%

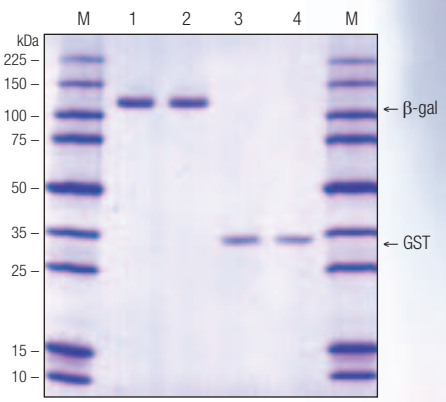
Note: 1 KU = 1000 units

Features

- High-throughput, 96-well protein purification directly from *E. coli* without harvesting cells
- Centrifuge-free cell lysis and extract preparation in one step
- Large-scale culture processing—up to 5 ml per well
- High protein yield—up to 1 mg per well
- Validated on the PerkinElmer MultiPROBE II HT robotic workstation

REFERENCES

1. Grabski, A., Mehler, M., Drott, D. and Van Dinther, J. (2002) *in**Nova**tions* **14**, 2–5.



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

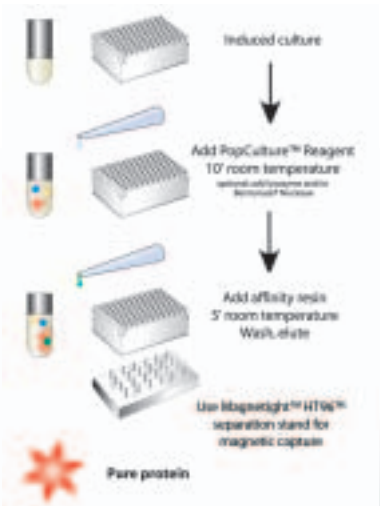
Robotic purification of His•Tag® β-galactosidase and GST with RoboPop™ Ni-NTA His•Bind® and GST•Bind™ Purification Kits

Duplicate induced cultures (4 ml) of *E. coli* expressing the indicated proteins were processed using the corresponding RoboPop Purification Kit with the recommended protocol and the PE MultiPROBE® II robot. Samples (2 µg) of the final elutions were analyzed by SDS-PAGE (10–20% gradient gel) and Coomassie blue staining. Lanes are indicated. Total yields averaged 800 µg His•Tag β-gal and 400 µg GST.

RoboPop™ His•Mag™ Purification Kit

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

The RoboPop™ His•Mag™ Purification Kit is designed for 96-well format purification of His•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 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 three 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 Novagen's Magnetight™ HT96™ Separation Stand (see page 28), which is recommended for efficient processing of magnetic affinity supports in deep well plates. The 96-well Deep Well Culture Plate with Sealers is available separately.

The RoboPop His•Mag Purification Kit will purify up to 12 mg of His•Tag fusion proteins per plate (up to 125 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. The RoboPop His•Mag Purification Kit has been validated for robotic sample processing with the MultiPROBE® II liquid handling workstation from PerkinElmer Life Sciences.

Product	Cat. No.
RoboPop His•Mag™ Purification Kit	71103-3

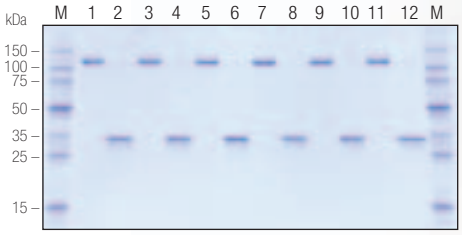
Components:

- 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
- 50 ml 4X Elute Buffer
- 300 KU rLysozyme™ Solution
- 1 ml rLysozyme Dilution Buffer
- 2500 U Benzonase® Nuclease, Purity > 90%

Note: 1 KU = 1000 units

Available separately:

Product	Size	Cat. No.
96-well Deep Well Culture Plate with Sealers	5 plates	71111-3



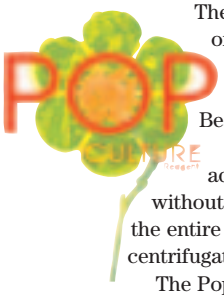
Lane	Sample
M	Perfect Protein™ Markers, 15–150 kDa
1, 3, 5, 7, 9, 11	His•Tag® β-galactosidase fusion protein
2, 4, 6, 8, 10, 12	His•Tag/GST•Tag™ fusion protein

RoboPop™ His•Mag™ purification

Induced cultures of *E. coli* strain BL21(DE3) containing either pET-30b(+), which encodes a His•Tag® β-gal fusion protein, or pET-41b(+), which encodes a His•Tag/GST•Tag™ fusion protein, were processed using RoboPop His•Mag Purification Kit. One sample was taken randomly from each row and analyzed by SDS-PAGE and Coomassie blue staining.

PopCulture™ His•Mag™ Purification Kit

PopCulture™ extraction and His•Mag purification from E. coli *cultures*



The PopCulture™ His•Mag™ Purification Kit is designed for purification of His•Tag® fusion proteins directly from *E. coli* cultures without harvesting cells. The procedure combines PopCulture total culture extraction with magnetic affinity purification using His•Mag Agarose Beads.

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 centrifugation. Use of His•Mag Agarose Beads enables the entire procedure to be carried out in a single tube without using columns or centrifugation.

The PopCulture His•Mag Purification Kit combines PopCulture Reagent, His•Mag Agarose Beads, corresponding buffers and rLysozyme™ Solution. This kit enables the processing of 40 × 3 ml cultures with yields up to 375 µg His•Tag® fusion protein per 3 ml culture, based on bead binding capacity. The kit is compatible with Novagen's Magnetight™ Separation Stand. (See page 28 for more information.) For 96-well processing using PopCulture, please refer to page 15 for more information about the RoboPop™ His•Mag Purification Kits.

Product	Cat. No
PopCulture™ His•Mag™ Purification Kit	71114-3

Components:

- 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

Features

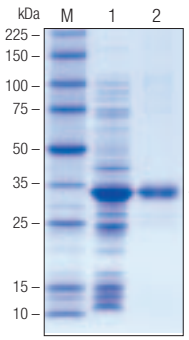
- No need to separate cells from culture media
- No need to mechanically disrupt cells
- No need to clarify cell extracts prior to purification
- Direct affinity adsorption of target proteins to resin from the total culture extract
- Ability to rapidly perform the entire cell growth and purification process in a single tube or well

Purification of His•Tag® GST expressed in *E. coli*

Purification Method	Yield[1]	Purity[2]
Standard His•Bind®	74	83
PopCulture™ His•Bind	111	89
PopCulture Ni-NTA His•Bind	170	85
PopCulture His•Mag™[3]	128	94

1. Yield in micrograms of target protein purified per ml of culture, as determined by BCA protein assay.
2. % purity determined by scanning densitometry of Coomassie blue stained SDS polyacrylamide gels.
3. Data represent the average of 8 separate wells processed in parallel.

Product	Average bead size	Binding capacity	Beads/ml culture	Form
His•Mag™ Agarose Beads	3 µm	5 µg/µl	25 µl settled beads (50 µl 50% v/v suspension)	Ni-charged IDA magnetic agarose



Lane Sample
M Perfect Protein™ Markers (10–225 kDa)
1. Crude extract (BugBuster® protocol)
2. PopCulture™ + His•Mag™ Agarose

← Target protein

PopCulture™ His•Mag™ purification

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

BugBuster® Ni-NTA His•Bind® and His•Bind Purification Kits

Convenient preparation of soluble extracts and affinity purification of His•Tag® fusion proteins

The BugBuster® Ni-NTA His•Bind® and BugBuster His•Bind Purification Kits combine Ni-NTA His•Bind or His•Bind Resin, respectively, His•Bind Buffer Kit (His•Bind Kit only), Benzonase® Nuclease, and BugBuster Protein Extraction Reagent for convenient preparation of soluble cell extracts and affinity purification of His•Tag® fusion proteins. BugBuster Protein Extraction Reagent is formulated for the gentle disruption of the cell wall of *E. coli* to liberate soluble proteins.

In practice, cells are harvested by centrifugation and suspended in BugBuster. At this point, Benzonase® Nuclease can be added to reduce the viscosity of the extract due to liberation of chromosomal DNA. The addition of rLysozyme™ Solution enhances extraction efficiency, especially for larger proteins. After a brief incubation, insoluble cell debris is removed by centrifugation. The clarified extract is ready to use and fully compatible with Ni-NTA His•Bind and His•Bind Resins. Following binding to affinity resin, excess BugBuster is easily removed by washing the column with the appropriate buffer.

Use BugBuster Ni-NTA His•Bind Purification Kit for the purification of proteins in a reducing environment (Ni-NTA His•Bind Resin is compatible with up to 20 mM β-mercaptoethanol). Use BugBuster His•Bind Purification Kit if you are planning to reuse His•Bind Resin many times.

Product	Cat. No.
BugBuster® Ni-NTA His•Bind® Purification Kit	70751-3

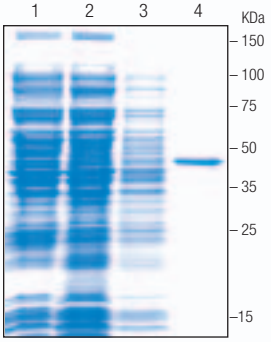
Components:

- 2 × 100 ml BugBuster Protein Extraction Reagent
- 10,000 U Benzonase® Nuclease, Purity > 90%
- 10 ml Ni-NTA His•Bind Resin
- pkg/4 Chromatography Columns

Product	Cat. No.
BugBuster His•Bind Purification Kit	70793-3

Components:

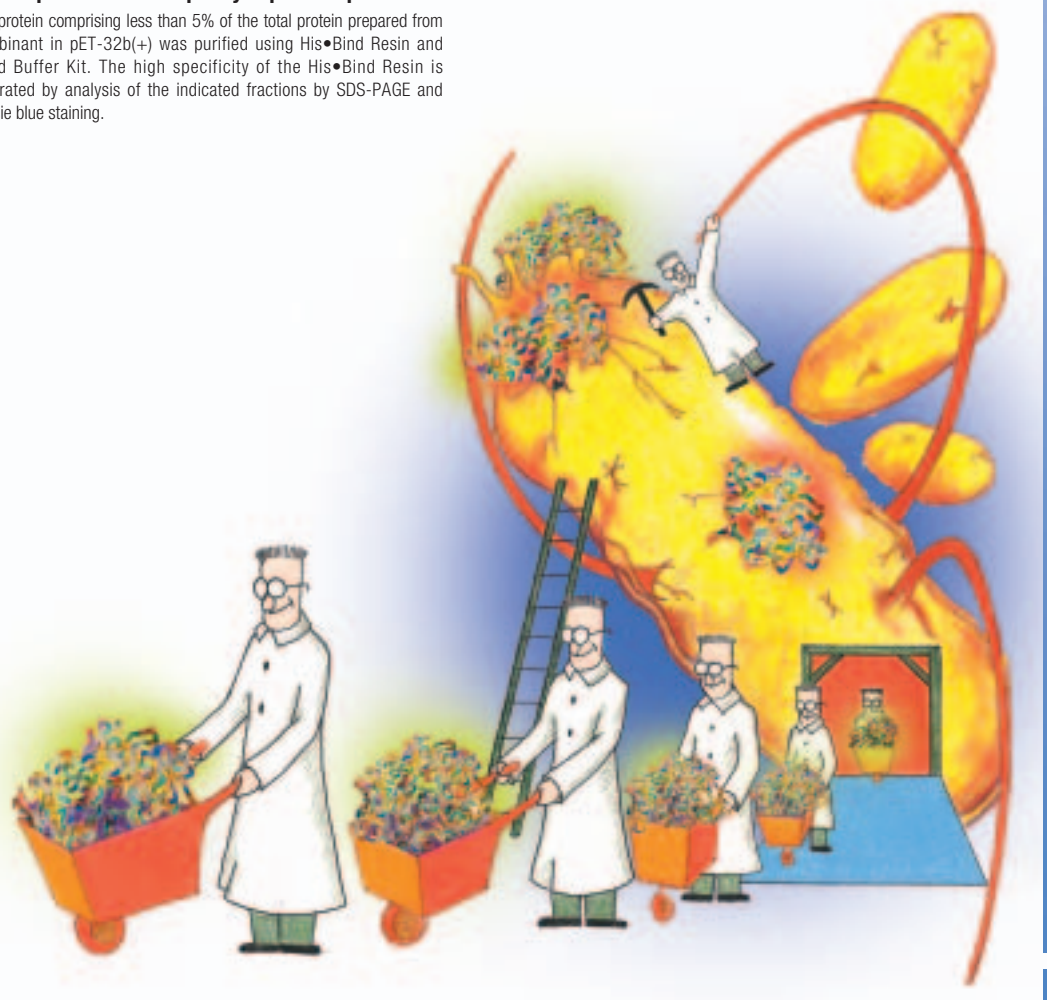
- 2 × 100 ml BugBuster Protein Extraction Reagent
- 10,000 U Benzonase Nuclease, Purity > 90%
- 10 ml His•Bind Resin
- 1 His•Bind Buffer Kit
- pkg/4 Chromatography Columns



Lane Sample
1. Starting material
2. Column flow-through
3. Wash 1
4. Elution

His•Bind® purification of a poorly expressed protein

A target protein comprising less than 5% of the total protein prepared from a recombinant in pET-32b(+) was purified using His•Bind Resin and His•Bind Buffer Kit. The high specificity of the His•Bind Resin is demonstrated by analysis of the indicated fractions by SDS-PAGE and Coomassie blue staining.



His•Tag® Affinity Resins and Buffer Kits

Purification of His•Tag fusion proteins by metal chelation chromatography

NTA and IDA Chemistries

With the His•Tag®/His•Bind® technology, purification is based on the affinity between the neighboring histidines of the His•Tag sequence and an immobilized metal ion (usually Ni^{2+} or Cu^{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 up to 20 mM β-mercaptoethanol 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 co-purification of non-specifically bound proteins.

continued on next page

Product	Size	Cat. No.
Ni-NTA His•Bind® Resin (resin pre-charged with Ni^{2+})	10 ml	70666-3
	25 ml	70666-4
	100 ml	70666-5
Ni-NTA His•Bind Superflow™ (resin pre-charged with Ni^{2+})	10 ml	70691-3
	25 ml	70691-4
	100 ml	70691-5
His•Bind Resin	10 ml	69670-3
	50 ml	69670-4
	100 ml	69670-5
His•Bind Columns (resin pre-charged with Ni^{2+})	pkg/5	70971-3
	pkg/25	70971-4
His•Bind Fractogel® (S), 20–40 µm	25 ml	70692-3
His•Bind Fractogel (M), 40–90 µm	25 ml	70693-3
His•Bind Quick Columns (resin pre-charged with Ni^{2+}, requires vacuum processing)	pkg/12	70159-3
	pkg/60	70159-4
His•Bind Quick 300 Cartridges (resin pre-charged with Ni^{2+})	pkg/10	70155-3
	pkg/50	70155-4
His•Bind Quick 900 Cartridges (resin pre-charged with Ni2+)	pkg/10	70156-3
	pkg/50	70156-4
His•Mag™ Agarose Beads (resin pre-charged with Ni^{2+})	2 ml	71002-3
	10 ml	71002-4

Available separately:

Product	Size	Cat. No.
Chromatography Columns	pkg/4	69673-3

Product listing continued on next page

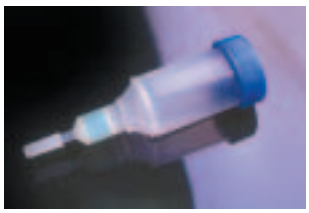
His•Bind and His•Mag Matrix Selection Guide

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 β-ME	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 β-ME High flow rates and pressures	Small to production scale FPLC or gravity flow column Recommended for eukaryotic extracts
His•Bind Resin	Uncharged IDA agarose	8 mg/ml	Reusable many times Compatible with His•Bind Buffer Kit	Small to medium scale Gravity flow column or batch mode
His•Bind Column	Ni-charged IDA agarose, prepacked column	10 mg	Pre-packed column Compatible with His•Bind Quick Buffer Kit	Convenient purification Gravity flow column
His•Bind Fractogel (S)	Uncharged Tentacle IDA methacrylate	> 10 mg/ml	20–40 µm particle size High flow rates and pressures	Small to production scale FPLC or gravity flow column High resolution separations
His•Bind Fractogel (M)	Uncharged Tentacle IDA methacrylate	> 10 mg/ml	40–90 µm particle size High flow rates and pressures	Small to production scale FPLC or gravity flow column
His•Bind Quick 300 Cartridge	Ni-charged IDA cellulose packed cartridge	0.5 mg	Luer fitting on both ends Compatible with His•Bind Quick Buffer Kit	Syringe-driven processing Vacuum Manifold processing Rapid purification
His•Bind Quick 900 Cartridge	Ni-charged IDA cellulose packed cartridge	2 mg	Luer fitting on both ends Compatible with His•Bind Quick Buffer Kit	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	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	Rapid small scale purification Magnetic separation HT compatible
Note: as with any affinity matrix, the cleanest separations are achieved when a His•Bind resin is used near its binding capacity				

* manufactured by QIAGEN

His•Tag® Affinity Resins and Buffer Kits *continued*

His•Bind® Columns



Designed for convenience, the single-use His•Bind Columns are pre-packed with 1.25 ml bed volume of Ni^{2+}-charged His•Bind Resin. Top and bottom frits ensure even buffer flow and minimal disturbance when loading and running the column. Optimal performance is achieved with bacterial lysates prepared using BugBuster® plus Benzonase® Nuclease.

His•Bind and His•Bind Quick Buffer Kits

The His•Bind Buffer Kit is a set of pre-tested buffers designed for use with IDA-based His•Bind Resin for convenient, rapid one-step purification of proteins by metal chelation chromatography. Solutions are included for Ni^{2+} charging, binding, washing and elution of up to ten 2.5 ml columns. The His•Bind Quick Buffer Kit contains the same components except that the 8X Charge Buffer is not included (the resin is provided pre-charged with Ni^{2+}).

Ni-NTA Buffer Kit

The Ni-NTA Buffer Kit provides a convenient set of buffers optimized for purification of His•Tag® fusion proteins under native conditions on Ni-NTA His•Bind Resin. These phosphate-buffered solutions differ from the Tris-based solutions used in the His•Bind Buffer Kit. Carefully prepared 4X concentrates are included for binding, washing and elution according to recommended protocols.

Product	Cat. No.
His•Bind® Purification Kit	70239-3

Components:
- 10 ml His•Bind Resin
- 1 His•Bind Buffer Kit
- pkg/4 Chromatography Columns

Product	Cat. No.
His•Bind Buffer Kit	69755-3

Components:
- 2 × 80 ml 8X Binding Buffer
- 25 ml 8X Wash Buffer
- 50 ml 4X Elute Buffer
- 50 ml 4X Strip Buffer
- 20 ml 8X Charge Buffer

Available separately:

Product	Size	Cat. No.
8X Binding Buffer	80 ml	69754
8X Wash Buffer	25 ml	69756
4X Elute Buffer	50 ml	69757
4X Strip Buffer	50 ml	69758
8X Charge Buffer	20 ml	69759

Product	Cat. No.
His•Bind Quick Buffer Kit	70665-3

Components are the same as 69755-3 except that only one bottle of 8X Binding Buffer is included and 8X Charge Buffer is omitted (His•Bind Quick resins are pre-charged).

Product	Cat. No.
Ni-NTA Buffer Kit	70899-3

Components:
- 2 × 125 ml 4X Ni-NTA Bind Buffer
- 125 ml 4X Ni-NTA Wash Buffer
- 50 ml 4X Ni-NTA Elute Buffer

His•Tag® Monoclonal Antibody

Sensitive, specific detection of His•Tag fusion proteins

The His•Tag® Monoclonal Antibody is a mouse monoclonal antibody (IgG_1) directed against the His•Tag sequence encoded by many of Novagen's expression vectors as well as many other commercially available vectors. The antibody recognizes five consecutive histidine residues regardless of the surrounding amino acid context. The high affinity ($K_d = 5 \times 10^{-8} - 1 \times 10^{-9}$ M) enables sensitive, specific detection of His•Tag fusion proteins at antibody concentrations of 0.1 to 0.2 µg/ml. The His•Tag Monoclonal Antibody binds to N-terminal, C-terminal and internal His•Tag sequences. This antibody also detects the recombinant marker bands in the Perfect Protein™ and Trail Mix™ Western Markers for convenient visualization of accurate internal standards on Western blots*.

For lowest background in Western blotting applications, Alkali-Soluble Casein (Cat. No. 70955-3) is recommended as a blocking agent. Please see His•Tag Western and LumiBlot™ Reagents on page 21 for reagents specifically configured for Western detection of the His•Tag Monoclonal Antibody.

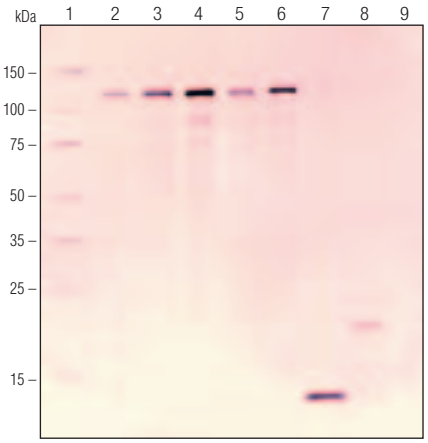
The 100 µg package size provides enough purified antibody for up to 1000 ml of working solution.

For a list of our secondary antibodies and other Western blot reagents, see page 36.

* Not recommended for HRP-based detection of Trail Mix Western Markers

Product	Size	Cat. No.
His•Tag® Monoclonal Antibody	100 µg	70796-3
	3 µg	70796-4

Specificity	HisHisHisHisHis; N-terminal, C-terminal or internal
Species/Isotype	Mouse monoclonal IgG_1
Cross-reactivity	Negligible with bacterial, yeast, insect, or mammalian cell lysates
Sensitivity	2 ng (Western blot developed with chromogenic substrates)
Applications	Western blot, immunoprecipitation, and immunolocalization
Form	Lyophilized, BSA-free
Working dilution	1:1,000–1:2,000 of antibody working solution [lyophilized antibody should be dissolved in 15 µl (3 µg) or 500 µl (100 µg) sterile water prior to dilution]
Stability	Lyophilized: 1 year at 2–8°C; in solution: 3 months at 2–8°C, 6 months at –20°C



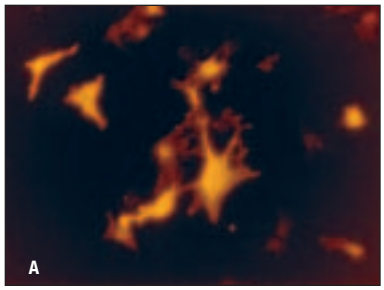
Lane	Vector	I, N or C	Sequence context
1.	(Perfect Protein™ Western Markers)		
2.	pET-15b	N	MGSSHHHHHHSSGLVPRGS...
3.	pET-16b	N	MGHHHHHHHHHHSSGHIEGR...
4.	pET-19b	N	GHHHHHHHHHHSSGHIDDDDK...
5.	pET-28b(+)	N	MGSSHHHHHHSSGLVPRGS...
6.	pET-30b(+)	N	MHHHHHHSSGLVPRGS...
7.	pET-31b(+)	C	...HACQMLLEHHHHHH
8.	pET-32a(+)	I	...GSGSGHMHHHHHHSSGLVPRGS...
9.	(negative control extract)		

I Internal His•Tag
N N-terminal His•Tag
C C-terminal His•Tag

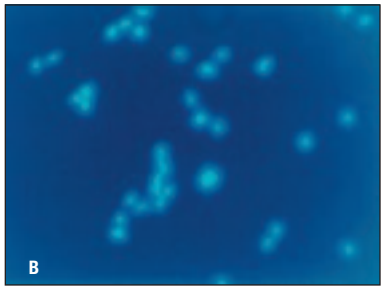
Detection of internal, N- and C-terminal His•Tag® sequences

Various pET recombinants were grown at 37°C, induced with IPTG, and harvested by centrifugation. Cells were resuspended in SDS sample buffer and roughly equivalent amounts run on an SDS-polyacrylamide gel, followed by electrophoretic transfer to nitrocellulose. The blot was incubated with a 1:1000 dilution of the His•Tag Monoclonal Antibody followed by Goat Anti-Mouse AP Conjugate and chromogenic detection with NBT/BCIP substrates. Vectors and context of the His•Tag sequence are indicated. The target protein was a His•Tag β-galactosidase fusion protein in lanes 2–6.

His•Tag fusion protein detection



Nuclear staining



Immunohistochemical detection of His•Tag® fusion proteins in transfected COS-1 cells

pTriEx™ plasmid DNA encoding a His•Tag firefly luciferase (Fluc) fusion protein was transiently transfected into COS-1 cells with GeneJuice™ Transfection Reagent. Twenty-four hours after transfection, cells were fixed, blocked with BSA and horse serum, and then exposed to His•Tag Monoclonal Antibody (1:1000 dilution of 0.2 mg/ml) followed by a Cy3 conjugated Goat Anti-Mouse IgG. Hoechst 33258 was used for visualization of cell nuclei. A, Immunofluorescent staining of His•Tag Fluc; B, Hoechst staining of the same field as in A showing both transfected and non-transfected cells.

His•Tag® Western and LumiBlot™ Reagents

Sensitive detection of His•Tag fusion proteins

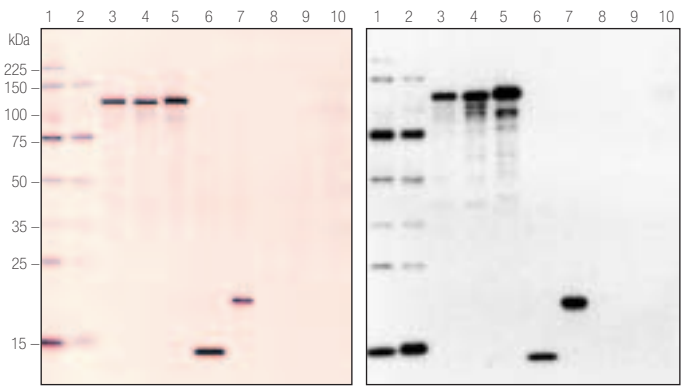
The His•Tag® Reagents are kits containing optimized components for blot detection using the His•Tag Monoclonal Antibody. The kits feature concentrated buffers for dilution and incubation, Alkali-Soluble Casein blocking protein, Anti-Mouse IgG AP or HRP conjugate, and choice of colorimetric or chemiluminescent substrates. Use of these reagents with the His•Tag Monoclonal Antibody (sold separately, see page 20) ensures optimal sensitivity and low backgrounds in Western blot applications.

Product	Size	Cat. No.
(kits do not include His•Tag Monoclonal Antibody)		
His•Tag® AP Western Reagents (colorimetric)	25 blots	70972-3
His•Tag AP LumiBlot™ Reagents (luminescent)	25 blots	70973-3
His•Tag HRP LumiBlot Reagents (luminescent)	25 blots	70974-3

Components:

- 125 ml — 20X TBS
- 2 × 250 ml — 10X TBST
- 225 ml — 5% Alkali-Soluble Casein
- 40 µl — Goat Anti-Mouse IgG AP *or* HRP Conjugate (H+L)
- Development substrates:
  (70972-3: BCIP, NBT, 20X AP Buffer)
  (70973-3: CDP-Star® Substrate with Nitro-Block™ II)
  (70974-3: SuperSignal® Substrate)
- pkg/25 — gLOCATOR™ Luminescent Labels (70973-3, 70974-3 only)
- pkg/25 — Development Folders (70973-3, 70974-3 only)
- 25 lanes — TrailMix™ Western Markers (AP kits) *or* Perfect Protein™ Western Markers (HRP kit)

A. His•Tag AP Western blot **B. His•Tag AP LumiBlot**



Lane Sample
1. Trail Mix™ Western Markers
2. Perfect Protein™ Western Markers
3. N-term. His•Tag® β-gal, pET-16b
4. N-term. His•Tag β-gal, pET-28b(+)
5. N-term. His•Tag β-gal, pET-30b(+)
6. C-term. His•Tag KSI, pET-31b(+)
7. Internal His•Tag Trx, pET-32a(+)
8. Bacterial control extract
9. Sf9 insect cell control extract
10. COS-1 mammalian cell extract

Colorimetric and chemiluminescent Western blot detection of His•Tag® fusion proteins

BL21(DE3) cells were transformed with appropriate pET vectors encoding proteins with the His•Tag sequence in an N-terminal, internal, or C-terminal configuration. Samples from induced cultures were combined with a 10X protein excess of uninduced culture extracts prior to loading. Insect cells and mammalian COS-1 cell extracts were made with CytoBuster™ Extraction Reagent. Samples (~ 5 µg protein) were run on 4–20% SDS-polyacrylamide gels, and proteins were transferred from the gels to nitrocellulose membranes. Western detection was performed using a 1:1000 dilution of the His•Tag Monoclonal Antibody and the respective His•Tag Western Reagents Kit. Development times were 5 min for Panel A and 40 sec for Panel B.

His•Tag® Antibody Plate

For reliable and specific immobilization of His•Tag fusion proteins

The His•Tag® Antibody Plate is a 96-well ELISA-compatible plate containing immobilized His•Tag Monoclonal Antibody. The antibody is covalently immobilized to the surface using a method that retains maximal binding activity. The antibody specifically recognizes five consecutive histidines, and so will bind with high affinity (K_d = 5 × 10^{-8} – 1 × 10^{-9} M) to virtually any His•Tag fusion protein in which the tag is exposed. This plate has outstanding binding characteristics, with a capacity of > 100 ng His•Tag fusion protein per well and low non-specific binding. Well-to-well variability is less than 5% and stability is greater than two years when stored dry at 4°C. The His•Tag Antibody Plate can be used in a variety of binding assays where reliable, specific immobilization of His•Tag fusion proteins is required.

Product	Size	Cat. No.
His•Tag® Antibody Plate	1 plate	71184-3
	5 plates	71184-4

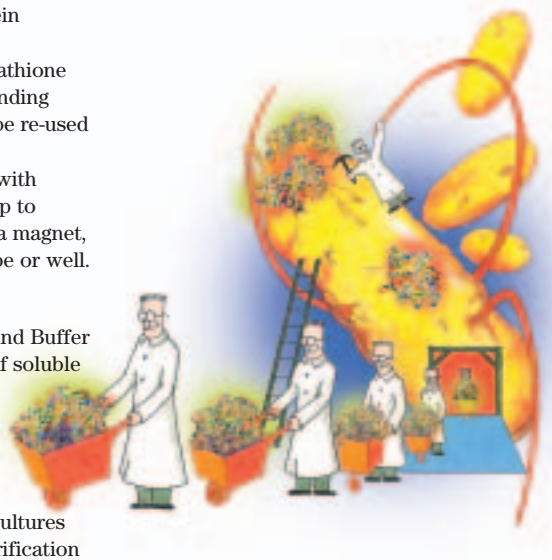
GST•Bind™ and GST•Mag™ Purification Kits Overview

Affinity purification of GST fusion proteins

The GST•Bind™ and GST•Mag™ purification systems are based on the widely recognized affinity of glutathione-S-transferase (GST•Tag™) fusion proteins for immobilized glutathione. Proteins are quickly and easily purified to near homogeneity in a single chromatographic step. Glutathione-resin based purifications require that the GST domain is soluble and properly folded. The gentle elution condition (10 mM reduced glutathione) avoids target protein denaturation.

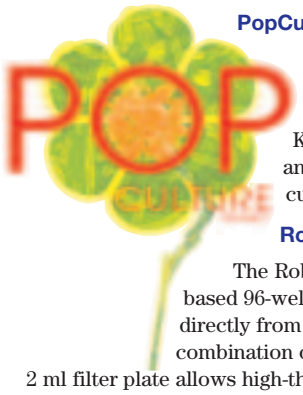
GST•Bind Resin utilizes an 11-atom spacer arm to covalently attach reduced glutathione via a sulfide linkage. The high degree of substitution of glutathione ensures a high binding capacity with yields of GST fusion proteins of 5–8 mg/ml settled resin. The resin can be re-used several times without loss of capacity.

GST•Mag Agarose Beads are available for rapid purification of multiple samples with minimum handling time. The 3 µm (average diameter) beads have binding capacity up to 2 mg/ml of settled resin as measured with GST protein and are easily collected with a magnet, which enables binding, wash, and elution procedures to be carried out in a single tube or well.



BugBuster® GST•Bind Purification Kit

The BugBuster GST•Bind Purification Kit combines the GST•Bind Resin, GST•Bind Buffer Kit reagents and BugBuster Protein Extraction Reagent for convenient preparation of soluble cell extracts and affinity purification of GST•Tag fusion proteins. Please see page 25 for more information.

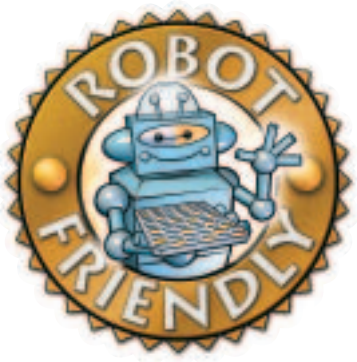


PopCulture™ GST•Mag Purification Kit

PopCulture Reagent is a novel buffered detergent concentrate that extracts proteins from whole *E. coli* cultures without harvesting cells. The PopCulture GST•Mag Purification Kit combines PopCulture with GST•Mag Agarose Beads, buffers and rLysozyme™ Solution for convenient processing of small-scale cultures. Please see page 25 for more information.

RoboPop GST•Bind Purification Kit

The RoboPop GST•Bind Purification Kit is designed for filtration-based 96-well format purification of GST•Tag fusion proteins directly from *E. coli* cultures without harvesting cells. The combination of PopCulture extraction, GST•Bind Resin, and a 2 ml filter plate allows high-throughput processing of up to 5 ml of *E. coli* cell culture per well. Please see page 23 for more information.



RoboPop™ GST•Mag Purification Kit

The RoboPop GST•Mag Purification Kit is designed for 96-well purification of GST•Tag™ fusion proteins directly from *E. coli* cultures without harvesting cells. The kit is configured for processing of 96 × 1 ml cultures in a deep well plate (supplied in the kit). The combination of PopCulture Reagent and magnetic agarose beads enables protein extraction and affinity purification to be performed in the culture plate. Please see page 24 for more information.

Product	Culture scale	Processing method	Capacity[a]	Throughput level
BugBuster® GST•Bind™ Purification Kit	Any	Gravity flow column chromatography	5–8 mg/ml of resin	Low
PopCulture™ GST•Mag™ Purification Kit	3 ml	Magnetic	150 µg/culture	Low
RoboPop™ GST•Bind Purification Kit	96 × 5 ml	Filtration	0.8 mg/culture	High
RoboPop GST•Mag Purification Kit	96 × 1 ml	Magnetic	50 µg/culture	High

[a] 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™ GST•Bind™ Purification Kit

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

The RoboPop™ GST•Bind™ Purification Kit is designed for filtration-based 96-well format purification of soluble GST•Tag™ fusion proteins directly from *E. coli* cultures without harvesting cells. The kit features PopCulture™ Reagent, rLysozyme™ Solution, and Benzonase® Nuclease for centrifuge-free cell lysis and extract preparation in one step. The combination of PopCulture extraction, GST•Bind Resin, and a 2 ml filter plate allows high-throughput processing of up to 5 ml of *E. coli* culture per well. Whereas the magnetic-based GST•Mag kit purifies up to 50 µg target protein per 1 ml culture, the filtration-based kit purifies up to 0.8 mg GST•Tag fusion protein per 5 ml culture.

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 Packard-brand MultiPROBE® II liquid handling workstation from PerkinElmer Life Sciences. A 96-well Collection Plate (1 ml wells) with an air-tight aluminum foil sealer is provided for storage of the purified proteins.

Processing Protocol for RoboPop™ GST•Bind™ Kit

1. Prepare *E. coli* cultures (3–5 ml in 24-well plate) under conditions for target protein production.
2. Add 0.1 culture volume PopCulture™ Reagent plus Benzonase® Nuclease and rLysozyme™ Solution to each well, mix, and incubate 10 min at room temperature.
3. (Optional) Take a sample from each well for screening expression levels of S•Tag™ fusion proteins using the FRETWorks™ S•Tag Assay, or by SDS-PAGE and Western blotting.
4. Add equilibrated GST•Bind affinity resin, mix, and incubate 5 min at room temperature.
5. Transfer the mixture to the 96-well Filter Plate and separate the affinity resin from the extract with a vacuum manifold.
6. Wash the affinity resin by applying wash buffer to the 96-well Filter Plate followed by vacuum filtration.
7. Place the 96-well Collection Plate into the vacuum manifold, and elute the target protein using the appropriate elution buffer.

Product	Cat. No.
RoboPop™ GST•Bind™ Purification Kit	71189-3

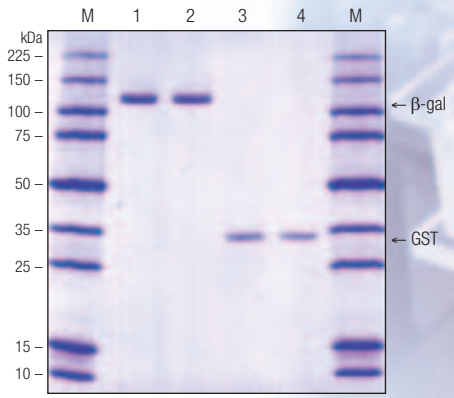
Components:

- 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%

Note: 1 KU = 1000 units

REFERENCES

1. Grabski, A., Mehler, M., Drott, D. and Van Dinther, J. (2002) *in**Nova**tions* **14**, 2–5.



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

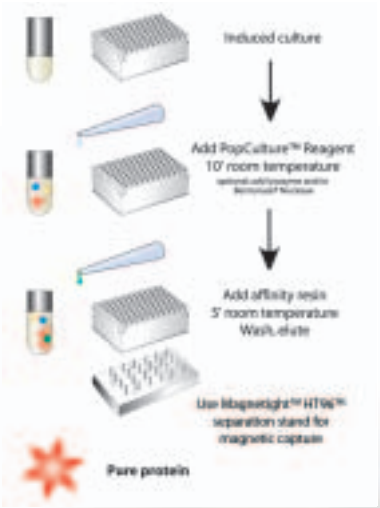
Robotic purification of His•Tag® β-galactosidase and GST with RoboPop™ Ni-NTA His•Bind® and GST•Bind™ Purification Kits

Duplicate induced cultures (4 ml) of *E. coli* expressing the indicated proteins were processed using the corresponding RoboPop Purification Kits with the recommended protocol and the PE MultiPROBE® II robot. Samples (2 µg) of the final elutions were analyzed by SDS-PAGE (10–20% gradient gel) and Coomassie blue staining. Lanes are indicated. Total yields averaged 800 µg His•Tag β-gal and 400 µg GST.

RoboPop™ GST•Mag™ Purification Kit

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

The RoboPop™ GST•Mag™ Purification Kit is designed for 96-well format purification of GST•Tag™ fusion proteins directly from *E. coli* cultures without harvesting cells. The kit features PopCulture™ Reagent for extraction of proteins from total cultures without the need for centrifugation, and 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 kit contains one 96-well Deep Well Culture Plate (2 ml wells) with three 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 Novagen's Magnetight™ HT96™ Separation Stand (see page 28), which is recommended for efficient processing of magnetic affinity supports in deep well plates. The 96-well Deep Well Culture Plate with Sealers is available separately (at right).

The RoboPop GST•Mag Purification Kit will purify 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. The RoboPop His•Mag Purification Kit has been validated for robotic sample processing the the MultiPROBE® II liquid handling workstation from PerkinElmer Life Sciences.

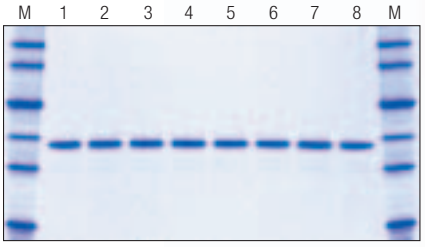
Product	Cat. No.
RoboPop™ GST•Mag™ Purification Kit	71102-3

Components:

- 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 Glutathione, Reduced
- 300 KU rLysozyme™ Solution
- 1 ml rLysozyme Dilution Buffer
- 2500 U Benzonase® Nuclease, Purity > 90%

Available separately:

Product	Size	Cat. No.
96-well Deep Well Culture Plate with Sealers	5 plates	71111-3



Lane	Sample
M	Perfect Protein™ Markers, 15–150 kDa
1–8	His•Tag®/GST•Tag™ fusion protein

RoboPop™ GST•Mag™ purification

Induced cultures of *E. coli* strain BL21(DE3) containing pET-41b(+), which encodes a His•Tag®/GST•Tag™ fusion protein, were processed using RoboPop GST•Mag Purification Kit. One sample was taken randomly from each row and analyzed by SDS-PAGE and Coomassie blue staining.

PopCulture™ GST•Mag™ Purification Kit

PopCulture extraction and GST•Tag™ fusion protein purification from E. coli *cultures*

The PopCulture™ GST•Mag™ Purification Kit is designed for purification of GST•Tag™ fusion proteins directly from *E. coli* cultures without harvesting cells. The procedure combines PopCulture total culture extraction with magnetic affinity purification using GST•Mag Agarose Beads.

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 centrifugation. Use of GST•Mag Agarose Beads enables the entire procedure to be carried out in a single tube without using columns or centrifugation.

The PopCulture GST•Mag Purification Kit combines PopCulture Reagent, GST•Mag Agarose Beads, corresponding buffers and rLysozyme™ Solution. This kit enables processing of 40 × 3 ml cultures with yields up to 150 µg GST•Tag fusion protein per 3 ml culture, based on bead binding capacity. The kit is compatible with Novagen's Magnetight™ Separation Stand. (See page 28 for more information.) For 96-well processing using PopCulture, please refer to the RoboPop™ Purification Kits.

Product	Cat. No.
PopCulture™ GST•Mag™ Purification Kit	71113-3

Components:

- 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

Features

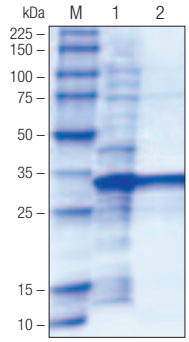
- No need to separate cells from culture media
- No need to mechanically disrupt cells
- No need to clarify cell extracts prior to purification
- Direct affinity adsorption of target proteins to resin from the total culture extract
- Ability to rapidly perform the entire cell growth and purification process in a single tube or well

Purification of His•Tag® GST expressed in *E. coli*

Purification Method	Yield[1]	Purity[2]
Standard GST•Bind™	42	92
PopCulture GST•Bind	45	90
PopCulture GST•Mag™[3]	40	94

1. Yield in micrograms of target protein purified per ml of culture, as determined by BCA protein assay.
2. % purity determined by scanning densitometry of Coomassie blue stained SDS polyacrylamide gels.
3. Data represent the average of 8 separate wells processed in parallel.

GST•Mag



Lane Sample
M Perfect Protein™ Markers (10–225 kDa)
1. Crude extract (BugBuster® protocol)
2. PopCulture™ + GST•Mag™ Agarose

PopCulture™ GST•Mag™ purification

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

Product	Average bead size	Binding capacity	Beads/ml culture	Form
GST•Mag™ Agarose Beads	3 µm	up to 2 µg/µl	25 µl settled beads (50 µl 50% v/v suspension)	Glutathione-derivatised magnetic agarose

BugBuster® GST•Bind™ Purification Kit

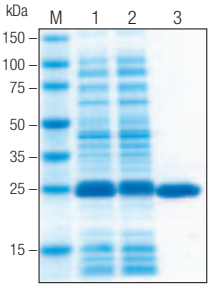
Convenient preparation of soluble cell extracts and affinity purification of GST•Tag fusion proteins

The BugBuster® GST•Bind™ Purification Kit combines the GST•Bind Resin, GST•Bind Buffer Kit reagents and BugBuster Protein Extraction Reagent for convenient preparation of soluble cell extracts and affinity purification of GST•Tag™ fusion proteins. BugBuster Protein Extraction Reagent is formulated for the gentle disruption of the cell wall of *E. coli*, resulting in the liberation of soluble protein. Cells are harvested by centrifugation as usual, followed by suspension in BugBuster reagent. During a brief incubation, soluble proteins are released. The extract is clarified by centrifugation, which removes cell debris and insoluble proteins. The clarified extract is ready to apply to GST•Bind Resin.

Product	Cat. No.
BugBuster® GST•Bind™ Purification Kit	70794-3

Components:

- 2 × 100 ml BugBuster Protein Extraction Reagent
- 10,000 U Benzonase® Nuclease, Purity > 90%
- 10 ml GST•Bind Resin
- pkg/4 Chromatography Columns
- 2 × 100 ml 10X GST Bind/Wash Buffer
- 40 ml 10X Glutathione Reconstitution Buffer
- 1 g Glutathione, Reduced



Lane Sample
M Perfect Protein™ Markers, 15–150 kDa
1. BugBuster® extract
2. Flow-through
3. Eluate

GST•Bind™ purification

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

GST•Tag™ Affinity Resins and Buffer Kit

For glutathione resin-based purifications

The GST•Bind™ and GST•Mag™ purification systems are based on the widely recognized affinity of glutathione-S-transferase (GST•Tag™) fusion proteins for immobilized glutathione. Proteins are quickly and easily purified to near homogeneity in a single chromatographic step. Glutathione resin-based purifications require that the GST domain is soluble and properly folded. The gentle elution condition (10 mM reduced glutathione) avoids target protein denaturation.

GST•Bind Resin

GST•Bind Resin utilizes an 11-atom spacer arm to covalently attach reduced glutathione via a sulfide linkage. The high degree of substitution of glutathione ensures a high binding capacity with yields of GST fusion proteins of 5–8 mg/ml settled resin. The resin can be re-used several times without loss of capacity.

GST•Mag Agarose Beads

GST•Mag Agarose Beads are available for rapid purification of multiple samples with minimum handling time. The 3 µm (average diameter) beads have binding capacity up to 2 mg/ml of settled resin as measured with GST protein and are easily collected with a magnet, which enables binding, wash, and elution procedures to be carried out in a single tube or well.

GST•Bind Buffer Kit

The GST•Bind Buffer Kit contains a set of pre-tested buffers for binding, washing and elution of GST•Tag fusion proteins from GST•Bind Resin or GST•Mag Agarose Beads. Sufficient components are provided to run a minimum of ten 2.5 ml GST•Bind columns.

Product	Size	Cat. No.
GST•Bind™ Resin	10 ml	70541-3
	50 ml	70541-4
	25 ml	70541-5
GST•Mag™ Agarose Beads	2 × 1 ml	71084-3
	10 × 1 ml	71084-4
GST•Bind Buffer Kit		70534-3

Components:

- 2 × 100 ml 10X GST Bind/Wash Buffer
- 40 ml 10X Glutathione Reconstitution Buffer
- 1 g Glutathione, Reduced

Available separately:

Product	Size	Cat. No.
Chromatography Columns	pkg/4	69673-3

GST•Tag™ Assay Kit

Quantitative assay of GST•Tag fusion proteins

The GST•Tag™ Assay Kit is designed to perform quantitative colorimetric enzymatic assays of glutathione-S-transferase or GST fusion proteins (1). The kit is useful for the quantification of GST activity in crude samples or purified fractions. The suitability of this assay for crude samples allows expression conditions to be evaluated and rapidly optimized by comparing GST activity levels. The GST activity assay is simple to perform using the supplied 1-chloro-2, 4-dinitrobenzene (CDNB) substrate. A sample is combined with CDNB substrate in the supplied reaction buffer and the absorbance of the reaction is monitored at 340 nm. The rate of change in A_{340} is proportionate to the amount of GST activity present in the sample. The assay has sufficient sensitivity to detect as little as 8 pmol of functional GST, which corresponds to approximately 250 ng of unfused GST.

Product	Size	Cat. No.
GST•Tag™ Assay Kit	100 assays	70532-3

Components:

- 2 × 5 ml 10X GST•Tag Assay Buffer
- 1.2 ml 100 mM CDNB
- 1 g Glutathione, Reduced
- 50 µg GST•Tag Standard

REFERENCE

1. Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7130–7139.

GST•Tag™ Monoclonal Antibody

Sensitive, specific detection of GST•Tag fusion proteins

The GST•Tag™ Monoclonal Antibody is a mouse monoclonal antibody (IgG_1) with high affinity for the 26 kDa glutathione-S-transferase (GST) domain from *Schistosoma japonicum*. This highly purified antibody is superior for detecting fusion proteins containing the GST•Tag expressed with the pET-41 or pET-42 vector series or other GST-encoding vectors.

The 50 µg package size provides enough purified antibody to perform 50 Western blots (10 cm × 10 cm).

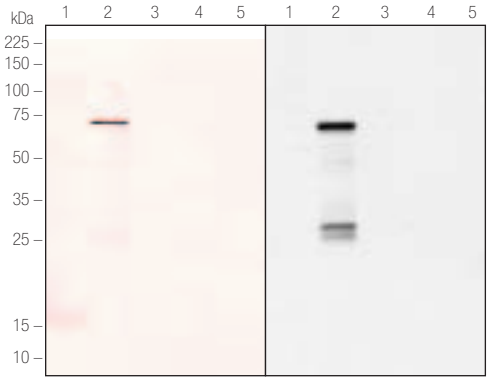
Please see the Western Blot Reagents on page 36 for conjugates and substrates compatible with the determination of GST•Tag and other Novagen monoclonal and polyclonal antibodies.

Product	Size	Cat. No.
GST•Tag™ Monoclonal Antibody	50 µg	71097-3
	250 µg	71097-4

REFERENCES

1. Smith, D. B., and Johnson, K. S. (1988) *Gene* **67**, 31–40.
2. Toye, B., Zhong, G. M., Peeling, R., and Brunham (1990) *Infect. Immunol.* **58**, 3909–3913.
3. Fikrig, E. Barthold, S. W., Kantor, F. S., and Flavell, R. A. (1990) *Science* **250**, 553–556.
4. Beekman, J. M., Cooney, A. J., Elliston, J. F., Tsai, S. Y., and Tsai, M. J. (1994) *Gene* **146**, 285–289.
5. Poon, R. Y., and Hunt, T. (1994) *Anal. Biochem.* **218**, 26–33.

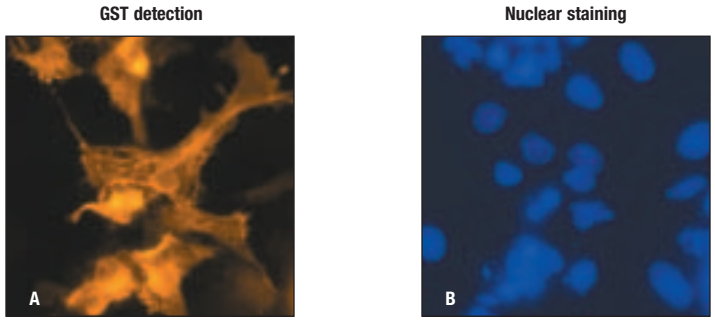
Specificity	220 aa GST protein; precise epitope not determined
Species/Isotype	Mouse monoclonal IgG_1
Cross-reactivity	Negligible with bacterial, yeast, insect, or mammalian cell lysates
Sensitivity	2.5–5 ng (Western blot developed with chromogenic substrates) < 1 ng (AP or HRP conjugate developed with chemiluminescent substrates)
Applications	Western blot, immunoprecipitation, and immunolocalization
Form	Stabilized solution of 1 mg/ml pure antibody in PBS, 50% glycerol
Working dilution	1:10,000 for Western blotting



1. Trail Mix™ Protein Markers
2. pET-41a(+) GFP, induced extract, 100 ng
3. *E. coli* BL21(DE3) extract, 100 ng
4. Sf9 insect cell control extract, 5 µg
5. CHO-K1 mammalian cell extract, 5 µg

Western blot detection of a GST•Tag™ GFP fusion protein

Two parallel blots were incubated with GST•Tag Monoclonal Antibody, then incubated with Anti-Mouse IgG AP or HRP Conjugate and processed by colorimetric (left panel) or chemiluminescent (right panel) detection.



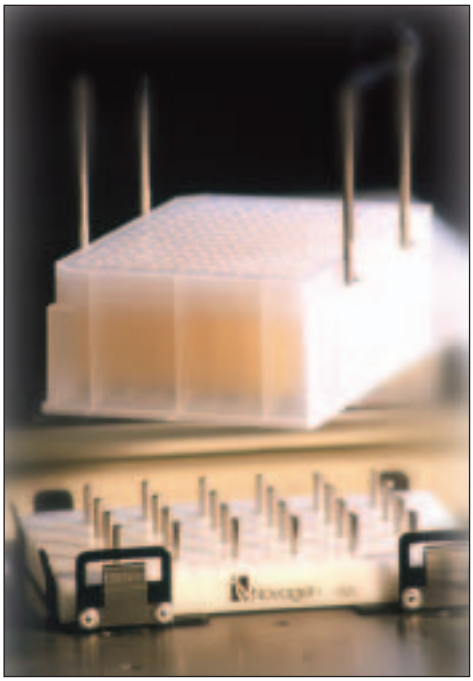
Immunohistochemical detection of GST expressed in transfected COS-1 cells

A pTriEx™ vector expressing GST was transiently transfected into COS-1 cells with GeneJuice™ Transfection Reagent. Twenty-four hours after transfection, cells were fixed, blocked with BSA and horse serum, and then exposed to GST•Tag™ Monoclonal Antibody (1:10,000 dilution) followed by a Cy3 conjugated Goat Anti-Mouse IgG. Hoechst 33258 was used for visualization of cell nuclei. A, Immunofluorescent staining of GST; B, Hoechst staining of the same field as in A showing both transfected and non-transfected cells.

Magnetight™ HT96™ Stand

Powerful magnetic separation for 96-well plates

The Magnetight™ HT96™ Stand is designed for high-throughput bioseparations using magnetic beads in a 96-well plate format. The stand uses 24 permanent, extremely strong, rare earth magnet rods arranged to fit between the wells of 2 ml 96-well deep well plates and 300 µl flat-bottom 96-well microplates. Each magnet rod pulls the beads in four adjacent wells to the side of the wells to allow for efficient buffer removal with a manual or automated pipetting device. For washing and elution steps, plates are removed from the magnet and beads are easily resuspended in the absence of the magnetic field. This stand is ideal for use with the RoboPop™ His•Mag™ and GST•Mag™ Purification Kits (see pages 15 and 24, respectively).



Product	Cat. No.
Magnetight™ HT96™ Stand	71101-3

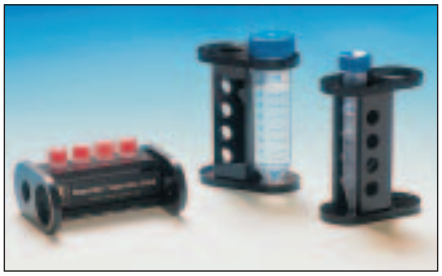
Magnetight™ Separation Stand and Multitube Rack

Powerful magnetic separation for 1.5 ml, 15 ml and 50 ml tubes

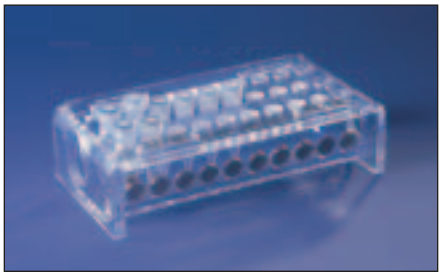
The versatile Magnetight™ Separation Stand allows efficient magnetic separations using 1.5 ml, 15 ml or 50 ml centrifuge tubes. The stand uses permanent, extremely strong, rare earth magnets embedded in the body and protected by a nylon polymer housing. The configuration of holes allows tube walls to come in the closest possible proximity to the magnets, which enables efficient separations in a minimum amount of time. With its compact design, the stand and inserted tube can be easily held in one hand while pipetting solutions away from magnetized pellets with the other. The stand contains four places for 1.5 ml tubes and one place each for 15 ml and 50 ml tubes.

The Magnetight Multitube Rack is designed for use with multiple 1.5 ml centrifuge tubes. The rack features a 10-place removable magnet holder that enables rapid separation of Magnetight or MagPrep® particles and beads. The rack holds up to 30 tubes, conveniently spaced for easy handling.

Product	Cat. No.
Magnetight™ Separation Stand	69964-3
Magnetight Multitube Rack	70747-3



Separation Stand



Multitube Rack

Vacuum Manifold

Convenient simultaneous processing of up to 12 samples

Novagen's Vacuum Manifold system for sample processing consists of a clear, rugged glass chamber to which a vacuum is applied, a chemical-resistant polypropylene lid, and a set of accessories for convenience in sample handling. The manifold is designed for consistent processing and elution of up to 12 samples simultaneously. Loading, washing, and elution steps can be performed rapidly, and all fractions can be collected in individual tubes because of the unique design of the rack used in the vacuum chamber. Fractions can be collected in either 1.5 to 2.0 ml tubes or in 15 ml conical tubes. Placement of the large reservoir in the glass chamber enables large volume collection of up to 1 liter.

The Vacuum Manifold can be used to draw a sample through any medium or column configured with compatible Luer-type fittings. The adjustable rack placed in the glass vacuum chamber will accommodate a variety of sample collection vessels. The manifold is ideal for use with Novagen's His•Bind® Quick Cartridges (see page 18).

An external vacuum source controls the vacuum level (along with the pressure release valve); individual stopcocks for each port enable single column control. The rugged glass chamber and polypropylene lid are rated to withstand vacuum levels of up to 20 inches of Hg.

The system also includes vacuum chamber, gauge/valve assembly, lid with gasket, 12 Teflon needles, collection rack package, 12 nylon/polypropylene stopcocks, and reservoir liner.

Product	Cat. No.
Vacuum Manifold	70147-3

Part 3 Contents

Click to go there!

Restriction Grade and Biotinylated Thrombin

Highly efficient, specific cleavage of fusion proteins

Thrombin, Restriction Grade

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.

Unit definition: one unit is defined as the amount of enzyme needed to cleave 1 mg of fusion protein in 16 hours at 20°C in a 200 µl reaction containing 20 mM Tris-HCl pH 8.4, 150 mM NaCl, 2.5 mM $CaCl_2$, 50 µg fusion protein and enzyme.

Biotinylated Thrombin

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. Novagen's preparation is tested for activity using the same assay as for unmodified thrombin, and for greater than 99% binding to Streptavidin Agarose (see below).

Product	Size	Cat. No.
Thrombin, Restriction Grade	50 U	69671-3
Biotinylated Thrombin	50 U	69672-3

Components:

- 50 U — Thrombin *or* Biotinylated Thrombin
- 1 ml — 10X Thrombin Cleavage Buffer
- 2 ml — 1X Thrombin Dilution/Storage Buffer
- 10 µg — Cleavage Control Protein

Thrombin Cleavage Capture Kit

Highly efficient, specific cleavage of fusion proteins

The Thrombin Cleavage Capture Kit is designed for cleavage of fusion proteins followed by convenient and quantitative removal of thrombin protease. The method is based on the use of Biotinylated Thrombin for digestion and its subsequent removal with Streptavidin Agarose. The kit is suitable for use with any fusion protein that contains a thrombin recognition sequence. A Cleavage Control Protein is included in the kit to monitor performance of cleavage conditions. It is cleaved into 2 fragments, which are easily visualized by SDS-PAGE.

The Cleavage Control Protein is also available separately to monitor performance of either thrombin or enterokinase cleavage conditions. The 48 kDa control protein is cleaved into two proteolytic fragments of 35 kDa and 13 kDa, which are easily visualized by SDS-PAGE. The Cleavage Control Protein also features an amino terminal S•Tag™ sequence enabling sensitive detection of the 16 kDa proteolytic product with Western blot reagents.

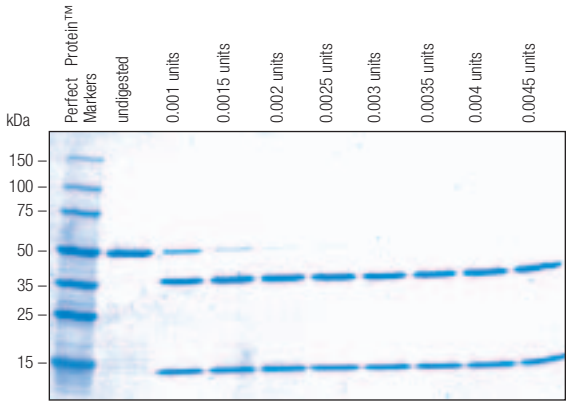
Product	Cat. No.
Thrombin Cleavage Capture Kit	69022-3

Components:

- 50 U — Biotinylated Thrombin
- 5 × 1 ml — 10X Thrombin Cleavage Buffer
- 2 ml — 1X Thrombin Dilution/Storage Buffer
- 2 × 0.4 ml — Streptavidin Agarose
- 10 µg — Cleavage Control Protein
- pkg/10 — Spin Filters, 2 ml capacity

Available separately:

Product	Size	Cat. No.
Streptavidin Agarose	5 ml	69203-3
Cleavage Control Protein	10 µg	69069-3
Spin Filter, 2 ml	pkg/10	69072-3



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-units lane represents a 2.25-fold overdigestion.

Restriction Grade Factor Xa

Specific cleavage of fusion proteins

Restriction Grade Factor Xa is a highly purified enzyme isolated from bovine plasma and activated with Russell's viper venom. Novagen's preparation is purified to near homogeneity and shows no secondary cleavage from contaminating proteases. The preparation is also functionally tested for activity with fusion proteins.

Like enterokinase, Factor Xa cleaves at the C-terminal side of its recognition sequence (IleGluGlyArg↓) and can therefore be used for removing all vector-encoded sequences from appropriately designed constructs.

Unit definition: one unit of Restriction Grade Factor Xa cleaves 50 µg Xa Cleavage Control Protein to > 95% completion in 16 hours at 21°C in a buffer containing 50 mM Tris-HCl pH 8.0, 100 mM NaCl, and 5 mM $CaCl_2$.

Product	Size	Cat. No.
Factor Xa, Restriction Grade	400 U	69036-3

Components:

- 400 U Restriction Grade Factor Xa
- 2 ml Factor Xa Dilution/Storage Buffer
- 1 ml 10X Factor Xa Cleavage Buffer
- 10 µg Cleavage Control Protein

Factor Xa Cleavage Capture Kit

Specific cleavage of fusion proteins

The Factor Xa Cleavage Capture Kit is designed for highly specific cleavage of fusion proteins followed by convenient affinity-based capture and removal of Factor Xa. After cleavage of the target protein, Factor Xa is removed with greater than 95% efficiency from the reaction by affinity capture on Xarrest™ Agarose. Following capture of Factor Xa, the agarose is removed by spin-filtration. No buffer changes are necessary because the same buffer conditions are used for both cleavage and capture. The kit also includes a Cleavage Control Protein for conducting control digests in parallel with experimental samples, or to test cleavage under customized buffer conditions. The 49 kDa Xa Cleavage Control Protein is cleaved into two proteolytic fragments of 32 kDa and 17 kDa, which are easily visualized by standard SDS-PAGE followed by Coomassie blue staining (see figure below). The Xa Cleavage Control Protein also features an amino terminal S•Tag™ sequence enabling sensitive detection of the 17 kDa proteolytic product with Western blot reagents.

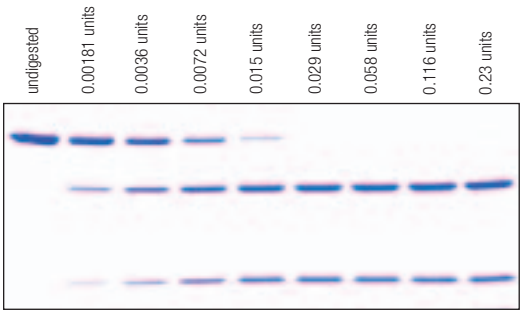
Product	Cat. No.
Factor Xa Cleavage Capture Kit	69037-3

Components:

- 400 U Restriction Grade Factor Xa
- 2 ml Factor Xa Dilution/Storage Buffer
- 5 ml 10X Factor Xa Cleavage Buffer
- 2 × 2.5 ml Xarrest™ Agarose
- 10 µg Xa Cleavage Control Protein
- pkg/10 Spin Filters, 2 ml capacity

Available separately:

Product	Size	Cat. No.
Xarrest Agarose	5 ml	69038-3
Xa Cleavage Control Protein	10 µg	69051-3
Spin Filter, 2 ml	pkg/10	69072-3



Factor Xa cleavage

The Xa Cleavage Control Protein (3 µg) was digested with increasing amounts of Factor Xa in separate reactions under standard assay conditions. Samples were analyzed by SDS-PAGE (4–20% gradient gel) followed by staining with Coomassie blue. The 0.015-units lane corresponds to 0.25 units enzyme per 50 µg target protein, which exhibits > 95% cleavage.

Recombinant Enterokinase

Highly specific cleavage of fusion proteins

Recombinant Enterokinase (rEK) is a highly purified preparation of the catalytic subunit of bovine enterokinase, which recognizes the identical cleavage site as the native enzyme (i.e., AspAspAspAspLys↓) and has similar enzymatic activity. rEK exhibits superior rates of cleavage of fusion proteins containing the recognition sequence when compared to the native enzyme (1). Novagen's rEK is purified to near homogeneity and, unlike some preparations of native bovine enterokinase, exhibits no secondary cleavage arising from contaminating proteases. The preparation is also functionally tested for activity with fusion proteins.

Unit definition: one unit is defined as the amount of enzyme needed to cleave 50 µg fusion protein in 16 hours at 23°C in a buffer containing 20 mM Tris-HCl pH 7.4, 50 mM NaCl, and 2 mM $CaCl_2$.

REFERENCES

1. Collins-Racie, L. A., McColgan, J. M., Grant, K. L., DiBlasio-Smith, E. A., McCoy, J. M., and LaVallie, E. R. (1995) *Bio/Technology* **13**, 982–987.

Product	Size	Cat. No.
Recombinant Enterokinase	50 U	69066-3

Components:

- 50 U Recombinant Enterokinase
- 2 ml 1X rEK Dilution/Storage Buffer
- 1 ml 10X rEK Cleavage Buffer
- 10 µg Cleavage Control Protein

Enterokinase Cleavage Capture Kit

Highly specific cleavage of fusion proteins

The Enterokinase Cleavage Capture Kit is designed for highly specific cleavage of fusion proteins followed by rapid, affinity-based capture and removal of enterokinase.

Following cleavage of the target protein, rEK is removed with > 99% efficiency from the reaction by affinity capture on EKapture™ Agarose. Following capture of rEK, the EKapture Agarose is removed by spin filtration. Because the same buffer conditions are used for both cleavage and capture, no buffer changes are necessary.

The kit also includes a Cleavage Control Protein for conducting control digests in parallel with experimental samples, or to test cleavage under customized buffer conditions. The 48 kDa Cleavage Control Protein is cleaved into two proteolytic fragments of 32 kDa and 16 kDa, which are easily visualized by standard SDS-PAGE followed by Coomassie blue staining (see figure below). The Cleavage Control Protein also features an amino terminal S•Tag™ sequence enabling sensitive detection of the 16 kDa proteolytic product with Western blot reagents.

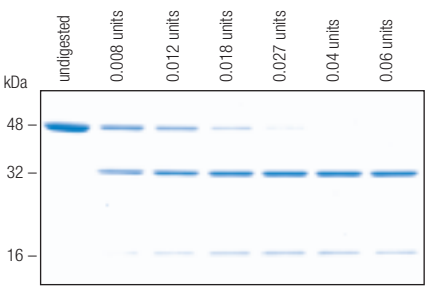
Product	Cat. No.
Enterokinase Cleavage Capture Kit	69067-3

Components:

- 50 U Recombinant Enterokinase
- 2 ml 1X rEK Dilution/Storage Buffer
- 5 ml 10X rEK Cleavage Buffer
- 1.5 ml EKapture™ Agarose
- 10 µg Cleavage Control Protein
- pkg/10 Spin Filters, 2 ml capacity

Available separately:

Product	Size	Cat. No.
EKapture Agarose	1.5 ml	69068-3
	10 ml	69068-4
Cleavage Control Protein	10 µg	69069-3
Spin Filter, 2 ml	pkg/10	69072-3

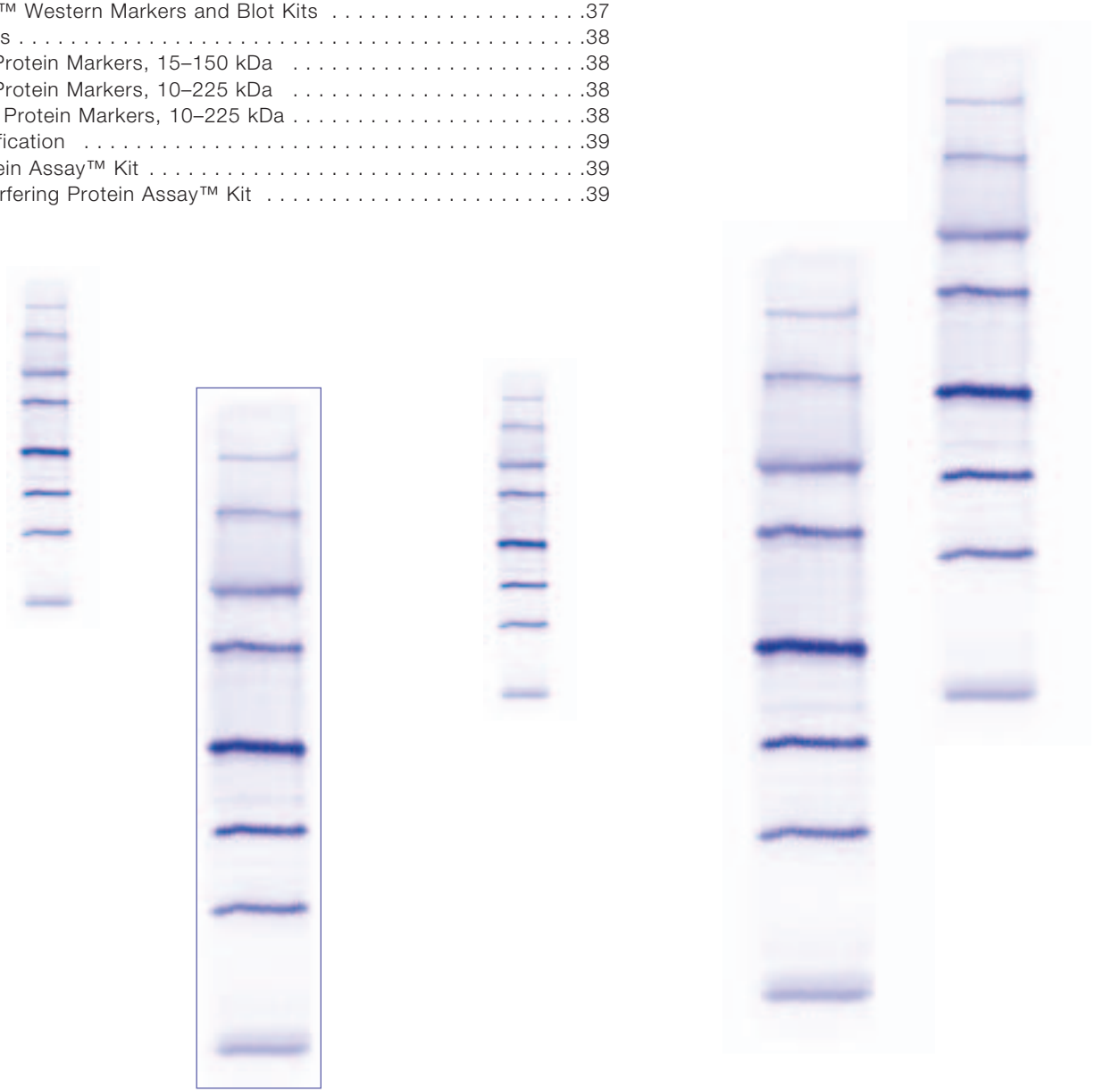


rEK cleavage

The Cleavage Control Protein (3 µg) was digested with increasing amounts of rEK in separate reactions under standard assay conditions. Samples were analyzed by SDS-PAGE (4–20% gradient gel) followed by staining with Coomassie blue. The 0.06-units lane corresponds to 1 enzyme unit per 50 µg target protein, which exhibits > 95% cleavage.

Part 4 Contents

Click to go there!



Novagen®

Protease Inhibitors

Protection against proteolysis during purification

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 configuration: 10 × 1 vial or as a single vial. Each vial, when reconstituted with 1 ml of water, will generate 1 ml of 100X stock solution.

When reconstituted, each vial of Protease Inhibitor Cocktail Set I contains 50 mM AEBSF, 15 mM 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. *Risk and Safety Statements:* R: 22-36/37/38; S: 26-36

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 5 ml stock solution. Slight turbidity in the reconstituted solution is normal. When reconstituted, each vial contains 20 mM AEBSF, 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. Five milliliters is recommended for the inhibition of proteases extracted from 20 g *E. coli*. *Risk and Safety Statements:* R: 36/37/38; S: 26-36

Protease Inhibitor Cocktail Set III (without EDTA)

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, 80 µM Aprotinin, 5 mM Bestatin, 1.5 mM E-64, 2 mM Leupeptin, and 1 mM Pepstatin A as a solution in DMSO. **Contains no metal chelators.** One milliliter is recommended for the inhibition of proteases extracted from 20 g of bovine liver or 20 g *E. coli*. *Risk and Safety Statements:* R: 36/37/38; S: 26-36

Protease Inhibitor Cocktail Set IV (without EDTA)

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. 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. *Risk and Safety Statements:* R: 25-36/37/38-50/53; S: 26-36-45-60-61

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. 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. *Risk and Safety Statements:* R: 36/37/38; S: 26-36

Product	Size	Cat. No.
Protease Inhibitor Cocktail Set I (with EDTA)	1 vial 10 vials	539131
Protease Inhibitor Cocktail Set II (with EDTA)	1 set 5 sets	539132
Protease Inhibitor Cocktail Set III (without EDTA)	1 ml 5 × 1 ml	539134
Protease Inhibitor Cocktail Set IV (without EDTA)	1 set	539136
Protease Inhibitor Cocktail Set V, EDTA-Free	1 vial 10 vials	539137

Anti-Mouse IgG AP and HRP Conjugates

Highest quality conjugates for detection of antibodies

Anti-IgG AP and HRP Conjugates

Goat Anti-Mouse IgG AP and HRP Conjugates are optimized for maximal signal:noise in Western blotting and plaque/colony screening applications. The conjugates are prepared from affinity-purified anti-IgG. For ELISA applications, the optimal working dilution is higher than for blots (e.g., up to 1:50,000).

Product	Size	Cat. No.
Goat Anti-Mouse IgG AP Conjugate (H + L)	40 µl	69266-3
Goat Anti-Mouse IgG HRP Conjugate (H + L)	40 µl	71045-3

Specificity	**Goat Anti-Mouse IgG AP Conjugate**: Mouse IgG, H + L chains **Goat Anti-Mouse IgG HRP Conjugate**: Mouse IgG, H + L chains
Cross-reactivity	Minimal with bacterial, insect or mammalian cell lysates
Form	Stabilized solutions. Store Anti-Mouse IgG AP and HRP at –20°C
Working dilution	**Goat Anti-Mouse IgG AP Conjugate**: 1:5,000–1:10,000 (up to 1:50,000 for ELISA) **Goat Anti-Mouse IgG HRP Conjugate**: 1:5,000–1:10,000 (up to 1:50,000 for ELISA)

AP and HRP Blot Development Substrates

Optimal performance and convenience for Western and dot blot applications

The quality of the substrates used for signal development is critical to achieve the required sensitivity and low background in Western and dot blots. Novagen's substrates are tested for compatibility and reproducibility with all of our detection kits and components.

The AP Detection Reagent Kit includes standardized solutions of 3-bromo-4-chloro-5-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT), plus 20X AP Buffer, for sensitive chromogenic detection of alkaline phosphatase conjugates. With the NBT/BCIP system, positive bands turn a deep blue-violet color that resists fading.

For very high sensitivity, chemiluminescent detection is recommended. Both the CDP-Star® AP Substrate and SuperSignal® HRP Substrate enable subnanogram sensitivity in a convenient ready-to-use format. The CDP-Star Substrate also includes Nitro-Block™ II signal enhancer for increased signal-to-noise ratios with standard nitrocellulose membranes.

Product	Size	Cat. No.
AP Detection Reagent Kit (NBT, BCIP, 20X AP Buffer)	1X 5X	69264-3 69264-4
CDP-Star® AP Substrate	40 ml	69086-3
SuperSignal® HRP Substrate	50 ml	69059-3

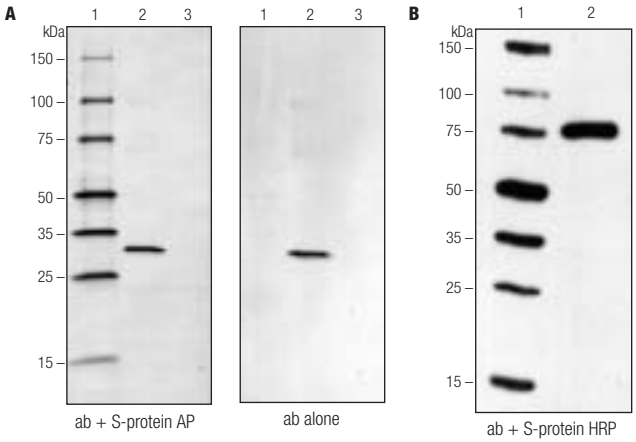
Perfect Protein™ Western Markers and Blot Kits

Accurate size markers detectable on any Western blot

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

Features

- Detect markers simply by adding the His•Tag Monoclonal Antibody or S-protein (AP or HRP Conjugate) to the same incubation used for sample detection
- S-protein conjugate (included in kits) 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.
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 for AP and HRP Kits:
- 25 lanes Perfect Protein Western Markers
- 50 µl S-protein AP *or* HRP Conjugate

Available separately:

Product	Size	Cat. No.
S-protein AP Conjugate	50 µl	69598-3
S-protein HRP Conjugate	50 µl	69047-3

Perfect Protein AP and HRP Western blots

A: Two parallel blots were incubated with primary antibodies for an expressed protein, then incubated with Anti-Mouse IgG AP Conjugate and processed using chemiluminescent detection. The S-protein AP Conjugate was included with secondary antibody for the blot on the left. Lanes: 1, 5 µl Perfect Protein™ Western Markers; 2, 10 ng induced cell extract; 3, 1 µg uninduced cell extract.
B: Blot contains the Perfect Protein Western Markers plus purified GUS expressed in insect cells incubated with a GUS-specific monoclonal antibody, then incubated with Anti-Mouse IgG HRP Conjugate and S-protein HRP Conjugate and detected with chemiluminescent HRP substrate. Lanes: 1, 5 µl Perfect Protein Western Markers; 2, 800 ng purified GUS protein.

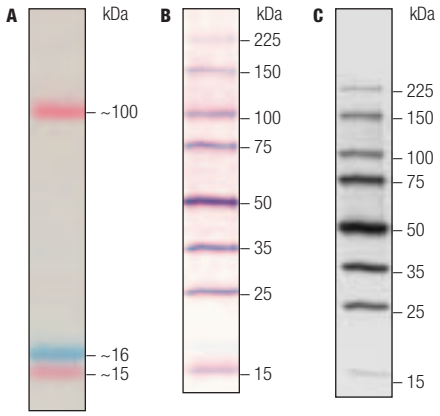
Trail Mix™ Western Markers and Blot Kits

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. Trail Mix Western Markers and Western Blot Kits have all of the features of Perfect Protein Western Markers and Western Blot Kits, plus easy to track prestained bands at 15, 16 and 100 kDa. This mix also contains an additional Perfect Protein Western Marker at 225 kDa for improved sizing accuracy of very large proteins.

Features

- Trail Mix contains three prestained indicator proteins plus eight unstained Perfect Protein Western Markers.
- Perfect Protein Western Marker concentrations are optimized for blot detection.
- Each Perfect Protein Western Marker carries a His•Tag® and S•Tag™ fusion peptide.
- 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.



Product	Size	Cat. No.
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 for AP and HRP Kits:
- 25 lanes Trail Mix Western Markers
- 50 µl S-protein AP *or* HRP Conjugate

Available separately:

Product	Size	Cat. No.
S-protein AP Conjugate	50 µl	69598-3
S-protein HRP Conjugate	50 µl	69047-3

A Unstained gel and Western transfer
B AP Western blot (S-protein AP conjugate) colorimetric detection
C AP Western blot (S-protein AP conjugate) chemiluminescent detection

Perfect Protein™ Markers

Precisely sized, conveniently spaced for accurate protein size determination

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.). Additionally, each protein marker carries the His•Tag sequence and will be visulaized with Western blot analysis when using His•Tag antibody.

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 (50 µg per band except for 100 µg of the 50 kDa band as a high-intensity reference).

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 (50 µg per band except for 100 µg of the 50 kDa band as a high-intensity reference).

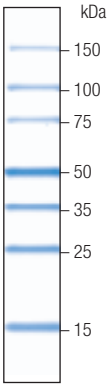
The 4X SDS Sample Buffer is a standard formulation commonly used for SDS-PAGE analysis of proteins. The solution includes DTT for complete denaturation of disulfide bonds. The buffer can be used at a final concentration of 2X for most applications.

Product	Size	Cat. No.
Perfect Protein™ Markers, 15–150 kDa	100 lanes	69149-3
Perfect Protein Markers, 10–225 kDa	100 lanes	69079-3

Available separately:

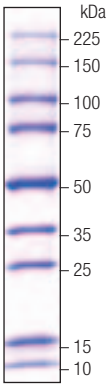
Product	Size	Cat. No.
4X SDS Sample Buffer	2 ml	70607-3

15–150 kDa



Coomassie blue
4–20% SDS-polyacrylamide gel

10–225 kDa



Coomassie blue
4–20% SDS-polyacrylamide gel

Trail Mix™ Protein Markers

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

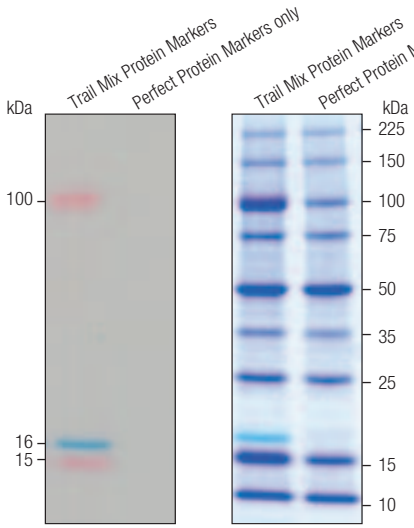
Trail Mix™ Protein Markers are a mixture of Novagen's Perfect Protein™ Markers and three prestained indicator proteins that together allow direct visualization of protein migration during electrophoresis. Unlike other marker sets in which the entire ladder is prestained, Trail Mix uses only three reference 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 prestained bands in Trail Mix do not affect the migration or band sharpness of the Perfect Protein Markers.

When stained with Coomassie blue, 10 bands appear, ranging from 10 kDa to 225 kDa. Besides the prestained bands, the 50 kDa marker serves as a landmark on stained gels, due to its higher concentration in the mixture relative to 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. Additionally, each protein marker carries the His•Tag sequence and will be visualized with Western blot analysis when using His•Tag antibody.

Product	Size	Cat. No.
Trail Mix™ Protein Markers	100 lanes	70980-3

Available separately:

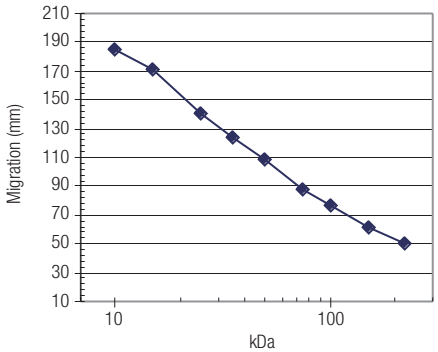
Product	Size	Cat. No.
4X SDS Sample Buffer	2 ml	70607-3



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

Coomassie blue
4–20% SDS-PAGE

Mobility plot



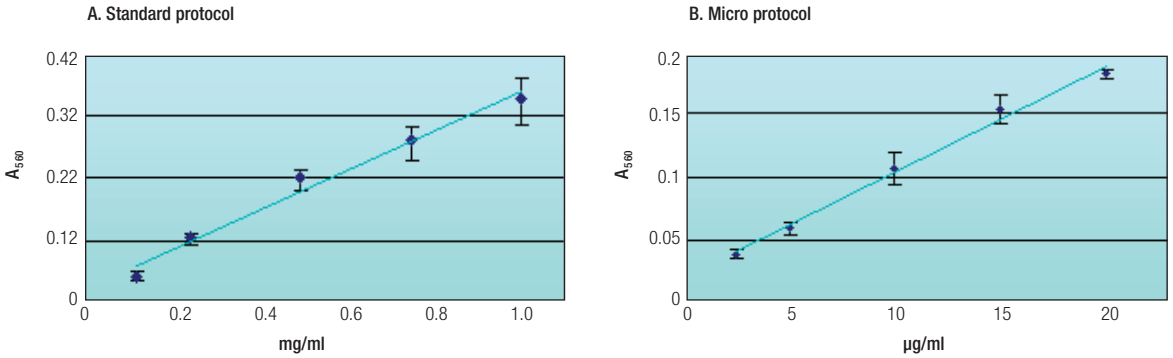
Novagen®

CB-Protein Assay™ Kit

An improved Coomassie dye-based protein assay for the simple and rapid estimation of protein concentration

The CB-Protein Assay™ Kit, a simple and rapid method for the estimation of protein concentration, offers an improvement over the well-known Bradford Coomassie dye-binding assay. This protein assay reaches an endpoint in 5 minutes and is compatible with reducing reagents, but is not suitable for use with solutions containing detergents. Each kit contains CB-protein dye and an albumin standard solution. Note: one kit is sufficient for up to 500 protein determinations. *Risk and Safety Statements:* R: 36/37/38; S: 26-36

Product	Size	Cat. No.
CB-Protein Assay™ Kit	1 kit	219468



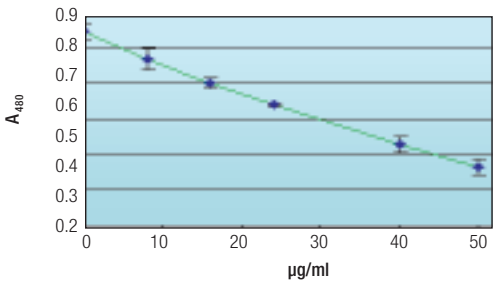
Standard curves generated with the CB-Protein Assay™ Kit

Non-Interfering Protein Assay™ Kit

Easy to use; overcomes interference of agents found in protein solutions

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, etc. The Universal Protein Precipitating Agent (UPPA™) 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. Note: one kit is sufficient for 500 individual protein determinations. *Risk and Safety Statements:* R: 36/37/38; S: 26-36

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



Standard curve generated using 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. BSA was used as the standard.

Risk and Safety Statements Key:

Risk Statements:	R22	Harmful if swallowed.
	R20/21/22	Harmful by inhalation, in contact with skin and if swallowed.
	R36/37/38	Irritating to eyes, respiratory system and skin.
Safety Statements:	S26	In case of contact with eyes, rinse immediately with plenty of water.
	S36	Wear suitable protective clothing.
	S36/37	Wear suitable protective clothing and gloves.

United States and Canada

EMD Biosciences, Inc.
441 Charmany Drive
Madison, WI 53719

Ordering:
Tel: 608 238 6110
Tel: 800 526 7319
Fax: 608 238 1388
e-mail: customerservice@novagen.com
web: www.novagen.com

Technical Service:
Tel: 800 207 0144
e-mail: novatech@novagen.com

VWR International

Ordering:
Tel: 800 932 5000
web: www.vwr.com

Germany

Merck Biosciences GmbH
Postfach 1167, 65796 Bad Soden
Ober der Röth 4, 65824 Schwalbach/Ts.

Ordering:
Freecall: 0800 69 31 000
Freefax: 0800 62 36 100
e-mail: customer.service@merckbiosciences.de
web: www.merckbiosciences.de

Technical Service:
Freecall: 0800 100 34 96
e-mail: techservice@merckbiosciences.de

VWR International GmbH
D-64271 Darmstadt

Ordering:
Tel: 0180 570 2000
Fax: 0180 570 2222
e-mail: biotech@de.vwr.com
web: www.vwr.com

United Kingdom

Merck Biosciences Ltd.
Boulevard Industrial Park
Padge Road, Beeston
Nottingham NG9 2JR

Ordering:
Freephone: 0800 622935
Tel: 0115 943 0840
Fax: 0115 943 0951
e-mail: customer.service@merckbiosciences.co.uk
web: www.merckbiosciences.co.uk

VWR International Ltd.
Hunter Boulevard
Magna Park
Lutterworth LE17 4XN

Ordering:
Freephone: 0800 22 33 44
Fax: 01455 55 85 86
e-mail: uk.sales@uk.vwr.com
web: www.vwr.com

Republic of Ireland

Merck Biosciences Ltd.
Boulevard Industrial Park
Padge Road, Beeston
Nottingham NG9 2JR

Ordering:
Toll-free: 1800 409445
Fax: +44 115 943 0951
e-mail: customer.service@merckbiosciences.co.uk
web: www.merckbiosciences.co.uk

Novagen®

A Brand of EMD Biosciences, Inc.
An Affiliate of Merck KGaA, Darmstadt, Germany

220007-1 INTL