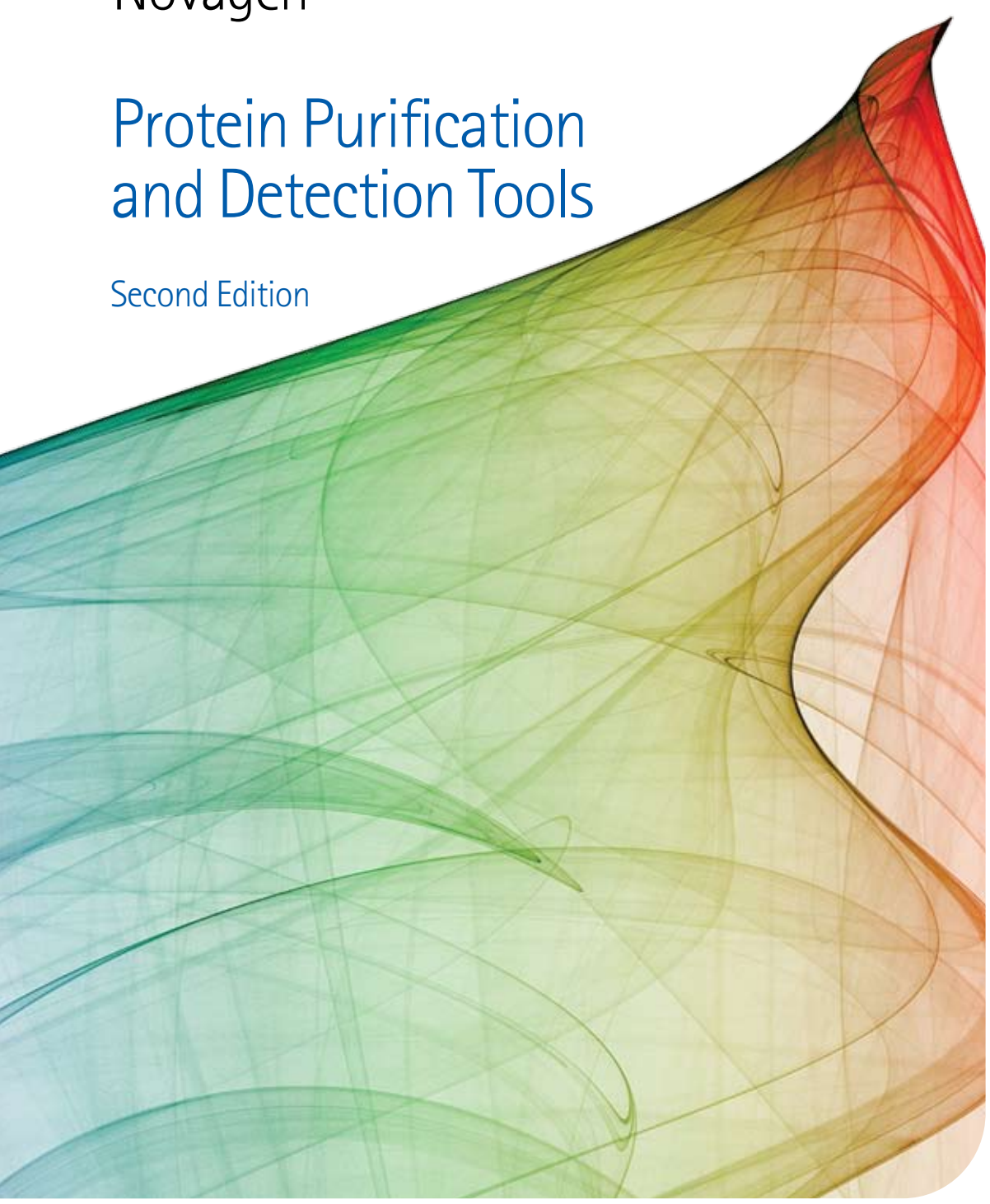
Novagen®

Protein Purification and Detection Tools

Second Edition



What's Inside

MERCK

Introduction

Affinity purification is based on the specific interaction of a target molecule with an immobilized ligand. Affinity technology can isolate specific molecules from a mixture, capture a desired molecule for interaction studies, or remove a component from a reaction. For recombinant proteins, the addition of fusion tags using appropriate expression vectors enables affinity purification by a number of strategies. Here we showcase products specifically designed for the purification or detection of fusion proteins containing His•Tag®, GST•Tag™, S•Tag™, Strep•Tag® II, HSV•Tag®, Nus•Tag™, and T7•Tag® sequences. These products are optimized for purification of proteins expressed in bacterial, yeast, insect, or mammalian systems. Reagents and kits are available in a variety of configurations, suitable for processing cultures from milliliter to liter scales in low- or high-throughput formats.

For maximal recovery of intact target proteins from cell cultures, a first step in purification should be an efficient, gentle extraction. In Part 1, Protein Extraction Reagents, we describe a variety of these detergent-based and enzymatic methods for convenient lysis and protein extraction from bacterial, yeast, insect, or mammalian cells. Part 2, Protein Purification and Detection, features a variety of affinity purification platforms, which provide options for conventional column or cartridge chromatography, rapid magnetic-based separations, or filtration methods for purifying milligram quantities in a high-throughput format. Premium quality fusion tag monoclonal antibodies and Western blot kits featured in this section allow highly specific, sensitive detection of fusion proteins with antibodies directed against GST•Tag, His•Tag, HSV•Tag, Nus•Tag, S•Tag, Strep•Tag II, T7•Tag, or Trx•Tag™ sequences. After the target protein is purified, the fusion tag may be removed with one of the site-specific proteases described in Part 3, Site-specific Proteases and Cleavage Capture Kits. Key accessory products for protein production, purification, and detection are highlighted in Part 4, Accessory Products. These products include iFOLD™ Protein Refolding Systems, D-Tube™ Dialyzers and the D-Tube Electroelution Accessory Kit, protease inhibitor cocktails, electrophoresis size standards, Western blot reagents, and protein quantification kits. Finally, Part 5, Technical Tips for Recombinant Protein Purification, offers sound advice on how to improve protein purification.

Part 1
Protein Extraction Reagents

Part 2
Protein Purification and Detection

Part 3
Site-specific Proteases and Cleavage Capture Kits

Part 4
Accessory Products

Part 5

Part 1
Protein Extraction Reagents

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

When purifying proteins from cells, the first step is to disrupt the 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 the need for gentle, but efficient, lysis methods, 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, plant, 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. Lysonase™ Bioprocessing Reagent combines the functional activities of rLysozyme and Benzonase Nuclease in an optimized, ready-to-use reagent that significantly increases protein extraction efficiency and facilitates processing of protein extracts. For ultimate convenience, BugBuster Master Mix combines BugBuster Protein Extraction Reagent with Benzonase Nuclease and rLysozyme Solution in a single pre-mixed reagent. 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 insect cells in suspension or on tissue culture plates. The reagent is compatible with Benzonase Nuclease to reduce viscosity, and with protease inhibitors. The improved method increases processing efficiency and target protein yields and is amenable to automated expression screening and affinity purification methods.

Protein Extraction Reagents Application Guide

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

Key: 1D PAGE = One-dimensional Polyacrylamide Gel Electrophoresis
2D PAGE = Two-dimensional Polyacrylamide Gel Electrophoresis
MS = Mass Spectrometry
IEF = Isoelectric Focusing

Protein Extraction Reagents Application Guide continued

Cell Type	Product	Starting Material		Applications								Comments
					Analysis							
		Total Culture	Cell Pellet	HT Compatible	1D PAGE	2D PAGE	IEF	MS	Western Blot	Activity Assay	Purification	
Insect												
	CytoBuster™ Protein Extraction Reagent		✓+		✓	✓*	✓*		✓	✓	✓	Gentle lysis of mammalian cells with retention of protein activity for assays and purification. Can use with monolayers or pellets derived from suspension cultures.
	Insect PopCulture® Reagent	✓		✓	✓				✓	✓	✓	Lysis of insect cells directly in serum-free medium. Ideal for expression screening of many small samples. Compatible with affinity purification.
Mammalian												
	CytoBuster Protein Extraction Reagent		✓+		✓	✓*	✓*		✓	✓	✓	Gentle lysis of mammalian cells with retention of protein activity for assays and purification. Can use with monolayers or pellets derived from suspension cultures.
Lysis and Extraction Enhancement												
Gram-negative bacteria (*E. coli*)	rLysozyme™ Solution	✓	✓	✓	✓				✓	✓	✓	Cleaves bond in peptidoglycan layer of *E. coli* cell wall. Use alone or combined with BugBuster® or PopCulture reagents for improved protein extraction. Use with Benzonase Nuclease to reduce sample viscosity and degrade nucleic acids.
	Lysonase™ Bioprocessing Reagent	✓	✓	✓	✓				✓	✓	✓	Convenient mixture of rLysozyme and Benzonase Nuclease minimizes pipetting steps
Gram-positive bacteria	Chicken Egg White Lysozyme Solution	✓	✓	✓	✓				✓	✓	✓	Cleaves bond in peptidoglycan layer of bacterial cell wall.
All cells	Benzonase® Nuclease	✓	✓	✓	✓				✓	✓	✓	Degrades all types of nucleic acids for more efficient protein extraction, faster chromatography, and reduced interference in assays.

Key: 1D PAGE = One-dimensional Polyacrylamide Gel Electrophoresis
2D PAGE = Two-dimensional Polyacrylamide Gel Electrophoresis
✱ = Salt must be removed before IEF
MS = Mass Spectrometry
IEF = Isoelectric Focusing
R = Reporter Assay
G = Gel Shift
+=Monolayer

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, which hydrolyses N-acetylmuramide linkages in the cell wall, enhances the extraction efficiency, especially for larger proteins. Following a brief incubation, insoluble cell debris is removed by centrifugation. 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™, S•Tag™, Strep•Tactin®, and T7•Tag® Resins, or several other chromatography matrices. Following binding to the 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.

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 Nuclease are compatible with common protease inhibitors.

BugBuster® Master Mix

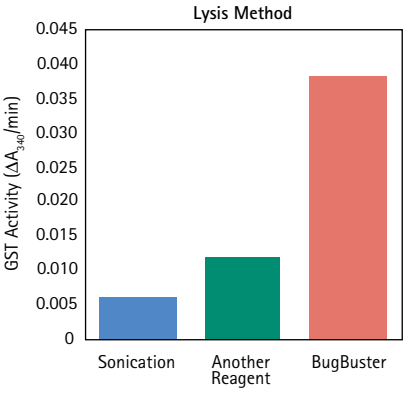
BugBuster Master Mix combines BugBuster Protein Extraction Reagent with Benzonase Nuclease and rLysozyme Solution in one convenient reagent. BugBuster Master Mix allows for maximum recovery of active, soluble protein from both Gram-negative and Gram-positive bacteria. With the Master Mix, there is no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

Product	Size	Cat. No.	Price
BugBuster® Protein Extraction Reagent	100 ml 500 ml	70584-3 70584-4	
BugBuster® Master Mix Extraction Reagent	100 ml 500 ml	71456-3 71456-4	
BugBuster® Plus Benzonase® Nuclease	1 kit	70750-3	

Components:

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

Note: 1 KU = 1000 units



Comparison of *E. coli* lysis methods
50 ml samples of an induced 500 ml culture of BL21(DE3) containing pET-41a(+) encoding GST were harvested by centrifugation and resuspended in 2 ml 1X PBS, another commercially available protein extraction reagent, or BugBuster Reagent. The sample in PBS was sonicated with 10 pulses at 50% duty for 30 s total. Samples in lysis reagent were treated according to their respective protocols. Extracts were clarified by centrifugation and assayed for GST enzymatic activity using the Novagen GST•Tag Assay Kit.

BugBuster® Plus Lysonase™ Kit

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

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. Compatible with rLysozyme Solution.

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.	Price
BugBuster Plus Lysonase™ Kit	1 kit*	71370-3	
	1 kit†	71370-4	
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® Extraction Reagent (primary amine-free)	100 ml	70923-3	
	500 ml	70923-4	

* Includes 100 ml BugBuster Protein Extraction Reagent and 0.2 ml Lysonase Bioprocessing Reagent, sufficient for protein extraction from 20 g cell paste.

† Includes 500 ml BugBuster Protein Extraction Reagent and 1 ml Lysonase Bioprocessing Reagent, sufficient for protein extraction from 100 g cell paste.

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 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.

Product	Size	Cat. No.	Price
PopCulture® Reagent	15 ml	71092-3	
	75 ml	71092-4	
	250 ml	71092-5	
PopCulture® GST•Mag™ Purification Kit	1 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

PopCulture® His•Mag™ Purification Kit		71114-3	

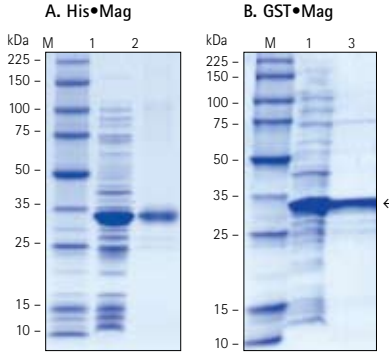
Components:
- 15 ml PopCulture Reagent
- 3 × 1 ml His•Mag Agarose Beads
- 80 ml 8X Binding Buffer
- 2 × 25 ml 8X Wash Buffer
- 50 ml 4X Elute Buffer
- 300 KU rLysozyme Solution
- 1 ml rLysozyme Dilution Buffer

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

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.



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.

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

rLysozyme™ Solution

Stabilized recombinant lysozyme

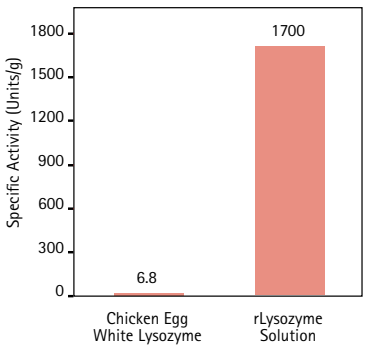
rLysozyme™ Solution contains a highly purified and stabilized recombinant lysozyme that can be used for lysis of *E. coli*. The enzyme catalyzes the hydrolysis of N-acetylmuramide linkages in bacterial cell walls. The specific activity of rLysozyme (1700 KU/mg) for *E. coli* lysis is 250 times greater than that of chicken egg white lysozyme. rLysozyme is optimally active at physiological pH. Very small amounts of rLysozyme (3–5 KU/gram cell paste) enhance the efficiency of protein extraction with BugBuster®, BugBuster HT Reagents and PopCulture® Reagents. In the absence of protein extraction reagents, direct lysis of *E. coli* can be achieved by treatment of 1 g cell paste with 45–60 KU rLysozyme. 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 Detergent. 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.

Product	Size	Cat. No.	Price
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



Comparison of chicken egg white lysozyme and rLysozyme activities
Activities were measured in a standard activity assay.

Lysonase™ Bioprocessing Reagent

Convenient blend of rLysozyme™ Solution and Benzonase® Nuclease

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

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

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

Chicken Egg White Lysozyme Solution

A ready-to-use, stabilized lysozyme solution

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

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

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

YeastBuster™ Protein Extraction Reagent

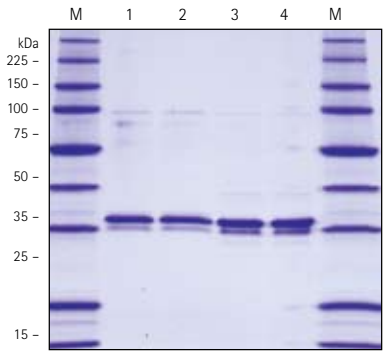
Efficient extraction of soluble protein from yeast and plants without mechanical disruption and enzymatic lysis

YeastBuster™ Protein Extraction Reagent is formulated for a fast, efficient, and gentle extraction of soluble active proteins from *Saccharomyces cerevisiae*, *Pichia pastoris*, *Aspergillus nidulans* cells, and plants. 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(hydroxy propyl)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 Reagent. 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 Ni-NTA His•Bind® immobilized metal affinity chromatography (IMAC) and GST•Bind™ affinity purification methods. The reagent is available in 100- and 500- ml sizes.

Features

- Gentle, rapid, efficient extraction of proteins from yeast cells, other fungi, and plants
- 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

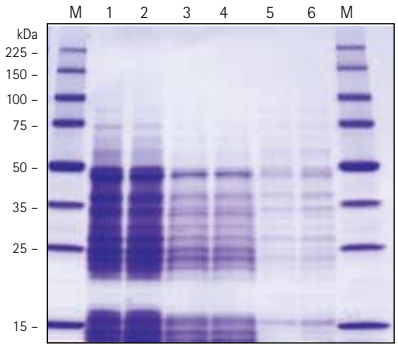
Product	Size	Cat. No.	Price
YeastBuster™ Protein Extraction Reagent	100 ml	71186-3	
	500 ml	71186-4	



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

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

A. SDS-PAGE



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

B. Protein and reporter assays

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

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

Panel A. SDS-PAGE analysis (4–20% gradient gel) and Coomassie blue staining of extracted proteins. *S. cerevisiae* cells containing a recombinant plasmid expressing a 35.6 kDa GST•Tag/His•Tag fusion protein were grown at 30°C, induced for expression, and harvested at OD_{600} of 1.2. Cells were collected by centrifugation at 3000 × *g* and resuspended in ice cold sterile water. Equal volumes of cells were dispensed into microcentrifuge tubes, and pelleted at 3000 × *g*. Cell pellets (~65 mg wet weight) were resuspended in 330 µl of the respective extraction reagents supplemented with 0.5 mM AEBSF and 15 µg/ml benzamidine. The YeastBuster Reagent also included 0.01 volume 100X THP Solution as directed in the protocol. 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 the Novagen BetaRed™ β–Gal Assay Kit. Data reflect the average of duplicate assays.

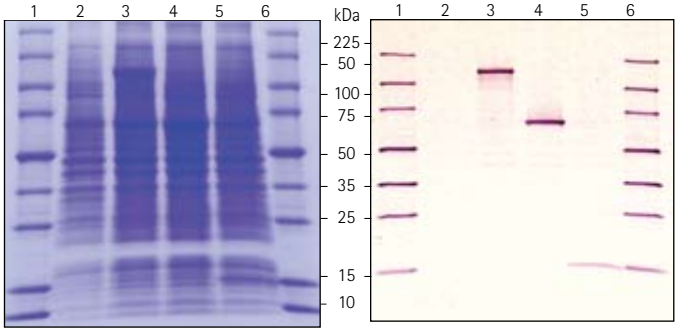
customer.service@merckbio.com
technical.service@merckbio.com
Visit our website www.merckbio.com

CytoBuster™ Protein Extraction Reagent

Simple extraction of soluble protein from mammalian and insect cells

The CytoBuster™ Protein Extraction Reagent is a proprietary formulation of detergents optimized for efficient extraction of soluble proteins from mammalian and insect cells. The gentle, non-ionic composition of CytoBuster Reagent enables isolation of functionally active endogenous or recombinant proteins without a need for secondary treatment such as sonication or freeze/thaw. CytoBuster Reagent has been specifically formulated for utilization in Western blotting, immunoprecipitation, and kinase/phosphatase assays. The reagent is compatible with protease inhibitors, kinase inhibitors, and phosphatase inhibitors. Store at room temperature.

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



Coomassie blue stained gel — Western blot

Lane	Sample
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 Marker

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

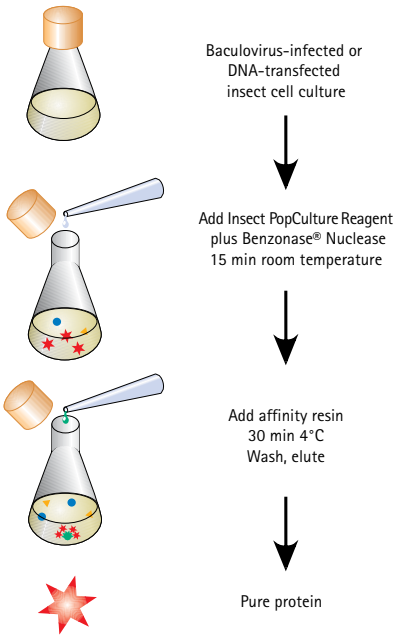
Protein extraction directly from insect cell cultures

Insect PopCulture® Reagent is a detergent-based lysis reagent specifically formulated for total insect cell culture extraction without the need for centrifugation. The improved method recovers both protein released into the medium and intracellular protein, increasing processing efficiency and target protein yields (1). It is amenable for automated expression-level screening and is fully compatible with the Ni-NTA His•Bind® affinity purification method. Insect PopCulture Reagent can be used for protein extraction from insect cells grown in suspension and adherent cells grown on tissue culture plates.

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

Features

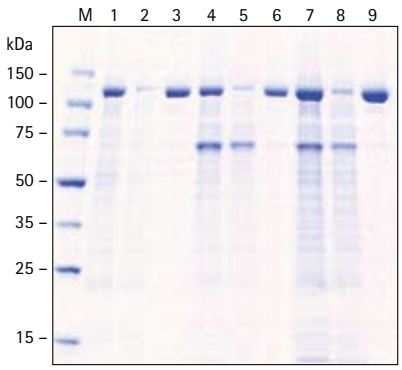
- No need to separate cells from culture media
- No need to clarify cell extracts prior to purification
- Higher protein yield due to target protein recovery from both media and cells
- Direct affinity adsorption of target proteins to Ni-NTA His•Bind and Ni•MAC™, Co-MAC™ Fractogel® Resin from the total culture extract
- Compatible with protease inhibitor cocktails
- Ideal for high-throughput, expression-level screening, and protein purification
- Compatible with transfected and baculovirus-infected insect cell cultures



Insect PopCulture method

Reference

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



Lane	Sample
M	Perfect Protein™ Markers, 15–150 kDa
1	Cell pellet, crude
2	Cell pellet, flow-through
3	Cell pellet, eluate
4	Medium, crude
5	Medium, flow-through
6	Medium, eluate
7	Insect PopCulture, crude
8	Insect PopCulture, flow-through
9	Insect PopCulture, eluate

Purification of His•Tag® β-galactosidase from baculovirus-infected insect cell cultures
The bacterial β-gal coding sequence, *lacZ*, was amplified by PCR and cloned into the pTriEx™-4 Ek/LIC Vector. Recombinant baculoviruses were generated by cotransfection using BacVector®-3000 Triple Cut Virus DNA (Cat. No. 70078-3) according to the recommended protocol. For protein expression, TriEx Sf9 cells (Cat. No. 71023-3) in a shaker culture in TriEx Insect Cell Medium (Cat. No. 71022-3) were infected with baculoviruses at MOI of 5. At 72 h post infection, 1 ml of the culture was used for direct in-media cell lysis with Insect PopCulture Reagent, and 1 ml was processed by standard extraction with CytoBuster™ Extraction Reagent. The His•Tag β-gal fusion protein was purified with the Ni-NTA His•Bind affinity purification method. Protein concentration of the pooled eluates was determined by the BCA method. The crude extract, flow-through, and pooled eluates were analyzed by SDS-PAGE and Coomassie blue staining.

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

customer.service@merckbio.com
technical.service@merckbio.com
Visit our website www.merckbio.com

Benzonase® Nuclease

Effective viscosity reduction and removal of nucleic acids from protein solutions

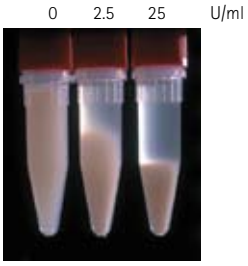
Benzonase® Nuclease is a genetically engineered endonuclease from *Serratia marcescens*. It degrades all forms of DNA and RNA (single stranded, double stranded, linear and circular) while having no proteolytic activity. It is effective over a wide range of conditions and possesses an exceptionally high 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 Nuclease 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. Addition of Benzonase Nuclease to eukaryotic extracts obtained with CytoBuster™ and Insect PopCulture Reagent also reduces extract viscosity and increases protein yields.

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/1000 units. The product is supplied as a 0.2 µm filtered solution in 50% glycerol. Store at –20°C.

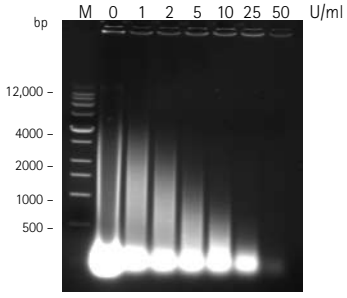
Unit definition: one unit is defined as the amount of enzyme that causes a ΔA_{260} of 1.0 in 30 minutes, which corresponds to complete digestion of 37 µg DNA.

Product	Size	Cat. No.	Price
Benzonase® Nuclease, Purity > 90%	10 KU 2.5 KU	70746-3 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



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 Nuclease for 10 min at room temperature, centrifuged at 350 × *g* for 3 min and photographed.



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

Part 2
Protein Purification and Detection

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 His•Tag Affinity Resins and Buffers beginning on page 24). 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, high flow rate Superflow™ and Fractogel® resins suitable for production scale purification, and Ni-MAC™, Co-MAC™, or u-MAC™ Fractogel Cartridges for use on liquid chromatography devices. Supports are provided either uncharged or precharged with Ni^{2+}, and both NTA and IDA chemistries are available.

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.

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 Kit 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 19 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 20 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 21 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 22 for more information.

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 Reagent. 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 2–mercaptoethanol). Use BugBuster His•Bind Purification Kit if you are planning to reuse His•Bind Resin many times.

Product	Cat. No.	Price
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

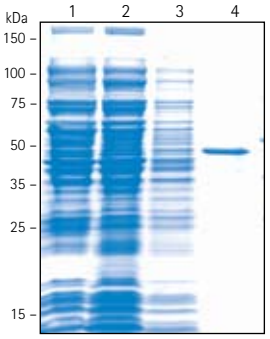
BugBuster® His•Bind® Purification Kit	70793-3	

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

Product	Size	Cat. No.	Price
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



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 clone 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.

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 the Novagen Magnetight™ Separation Stand. For 96-well processing using PopCulture Reagent, please refer to page 22 for more information about the RoboPop™ His•Mag Purification Kit.

Product	Cat. No.	Price
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

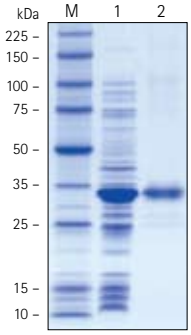
- No need to separate cells from culture media
- No need to mechanically disrupt cells
- No need to clarify cell extracts prior to purification
- Direct affinity adsorption of target proteins to resin from the total culture extract
- Ability to rapidly perform the entire cell growth and purification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

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.

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 Reagent, 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 Genesis® Freedom™ Workstation from Tecan and the MultiPROBE® II liquid handling work station 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.

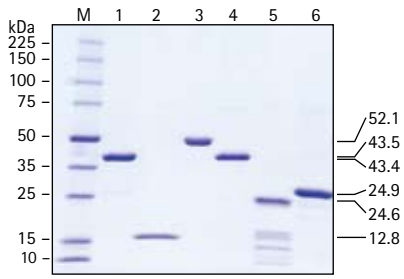
Product	Cat. No.	Price
RoboPop™ Ni-NTA His•Bind® Purification Kit	71188-3	

Components:

- 75 ml PopCulture Reagent
- 25 ml Ni-NTA His•Bind Resin
- 125 m 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%

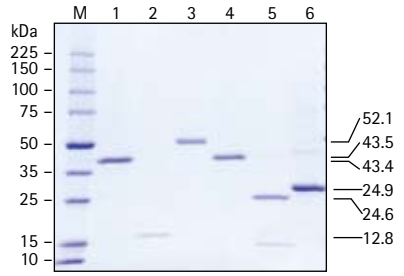
Note: 1 KU = 1000 units

A. Filtration-based affinity purification



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

B. Magnetic-based affinity purification



Automated purification of His•Tag fusion proteins using Novagen magnetic- and filtration-based affinity purification kits and Tecan® workstation

Cultures of *E. coli* strain BL21(DE3) containing various pET vector constructs were incubated at 30°C and target protein expression was induced by Overnight Express™ Autoinduction System 1 (Cat. No. 71300). Following further incubation for approximately 16 h at 30°C, the cultures were processed robotically according to the RoboPop purification protocols. Panel A (approximately 1 µg protein load) and panel B (approximately 2 µg protein load) show purified protein samples analyzed by SDS-PAGE (10–20% gradient gel with Coomassie blue staining). The entire purification process after cell culture and induction was performed automatically by the Tecan Genesis 200. Protein assays were performed by the Bradford method and purity was determined by densitometry of the scanned gel. Lane M: Perfect Protein™ Markers, 10–225 kDa.

RoboPop™ 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 Reagent 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 the Novagen Magnetight™ HT96™ Separation Stand 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 kit has been validated for robotic sample processing with the Genesis® Freedom™ Workstation from Tecan and the MultiPROBE® II liquid handling work station from PerkinElmer Life Sciences.

Product	Cat. No.	Price
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

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

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

Rapid purification of recombinant proteins from 96-insect cell cultures is possible with the Insect RoboPop™ Ni-NTA His•Bind® Purification Kit. This kit includes Insect PopCulture® Reagent for protein extraction from total cultures, Benzonase® Nuclease for viscosity reduction, Ni-NTA His•Bind Resin and buffers, 2-ml 96-well Filter Plate, and Collection Plate with Sealer. The kit is configured for robotic processing of 10-ml suspension cultures and purifies up to 400 µg His•Tag® fusion protein per culture based on binding capacity of the resin. Protein yields ranging from 60 to 140 µg per 10-ml culture were obtained with β-gal, Fluc, MAP kinase, and cdc2 kinase expressed in the pIEx™ transient protein expression system (1).

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

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

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

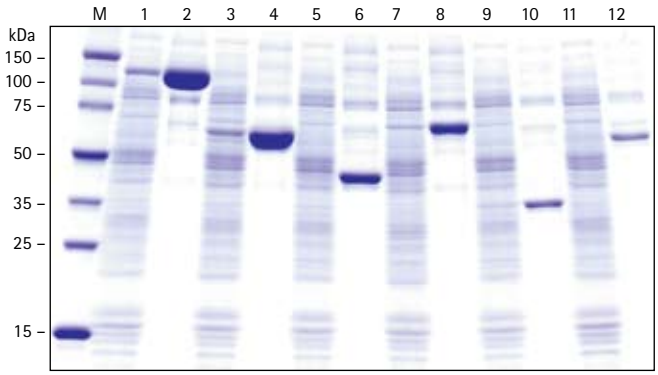
Components:

- 50 ml Insect PopCulture Reagent
- 10 KU Benzonase Nuclease, Purity > 90%
- 10 ml Ni-NTA His•Bind Resin
- 125 ml 4X Ni-NTA Bind Buffer
- 2 × 125 ml 4X Ni-NTA Wash Buffer
- 50 ml 4X Ni-NTA Elute Buffer
- 1 2-ml 96-well Filter Plate
- 1 Collection Plate with Aluminum Plate Sealer

Note: 1 KU = 1000 units

Reference

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



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

Target protein expression levels and purification from transfected Sf9 cells
Sf9 cells in 10-ml suspension cultures (1×10^6 cells/ml) were transfected with 20 µg of the indicated pIEx™ recombinants using Insect GeneJuice® Transfection Reagent (Cat. No. 71259). Total culture extracts were prepared 48 h later by the addition of Insect PopCulture Reagent (500 µl) followed by the addition of Benzonase Nuclease (5 µl). Samples were taken at this point to represent the total cell protein. Ni-NTA His•Bind Resin (50 µl per culture) was then added to the extracts. Samples were processed robotically using a Genesis Freedom workstation from Tecan. Target protein was eluted in a volume of 150 µl. Crude and purified fractions (10 µl) were analyzed in adjacent lanes (10–20% SDS-PAGE, stained with Coomassie blue). Purified protein yields were determined by BCA assay.

customer.service@merckbio.com
technical.service@merckbio.com
Visit our website www.merckbio.com

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 Co^{2+}). The metal is held by chelation with reactive groups covalently attached to a solid support. The most commonly used chelators include nitriloacetic acid (NTA*) and iminodiacetic acid (IDA), which have four and three sites available for interaction with metal ions, respectively. The two chemistries confer different properties to the affinity support and conditions used for binding, washing, and elution of target proteins for both native and denaturing conditions. In practice, the additional chelation site available with NTA minimizes leaching of the metal during the purification and is compatible with up to 20 mM 2-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 copurification of non-specifically bound proteins.

Product	Size	Cat. No.	Price
Ni-NTA His•Bind® Resin (resin pre-charged with Ni^{2+})	10 ml 25 ml 100 ml	70666-3 70666-4 70666-5	
Ni-NTA His•Bind® Superflow™ (resin pre-charged with Ni^{2+})	10 ml 25 ml 100 ml	70691-3 70691-4 70691-5	
His•Bind® Resin	10 ml 50 ml 100 ml	69670-3 69670-4 69670-5	
His•Bind® Resin, Ni Charged (resin pre-charged with Ni^{2+})	10 ml 25 ml 100 ml	71035-3 71035-4 71035-5	
His•Bind® Columns (resin pre-charged with Ni^{2+})	pkg/5 pkg/25	70971-3 70971-4	
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 pkg/60	70159-3 70159-4	
His•Bind® Quick 300 Cartridges (resin pre-charged with Ni^{2+})	pkg/10 pkg/50	70155-3 70155-4	
His•Bind® Quick 900 Cartridges (resin pre-charged with Ni^{2+})	pkg/10 pkg/50	70156-3 70156-4	
His•Mag™ Agarose Beads (resin pre-charged with Ni^{2+})	2 ml 10 ml	71002-3 71002-4	

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 and 1 mM THP Compatible with Ni-NTA Buffer Kit	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 and 1 mM THP Compatible with Ni-NTA Buffer Kit 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 Compatible with 1 mM THP	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 Compatible with 1 mM THP	Convenient purification Gravity flow column
His•Bind Resin, Ni Charged	Ni-charged IDA agarose	8 mg	Pre-charged bulk resin Compatible with His•Bind Quick Buffer Kit Compatible with 1 mM THP	Convenient purification Gravity flow column
His•Bind Fractogel (M) Resin	Uncharged Tentacle IDA methacrylate	30 mg/ml	40–90 µm particle size High flow rates and pressures Compatible with 1 mM THP	Small to production scale FPLC or gravity flow column
His•Bind Quick 300 Cartridge	Ni-charged IDA cellulose packed cartridge	0.5 mg	Luer fitting on both ends Compatible with His•Bind Quick Buffer Kit Compatible with 1 mM THP	Syringe-driven processing Vacuum Manifold processing Rapid purification
His•Bind Quick 900 Cartridge	Ni-charged IDA cellulose packed cartridge	2 mg	Luer fitting on both ends Compatible with His•Bind Quick Buffer Kit Compatible with 1 mM THP	Syringe-driven processing Vacuum Manifold processing Rapid purification
His•Bind Quick Column	Ni-charged IDA cellulose packed cartridge	5 mg	Luer fitting on one end Compatible with His•Bind Quick Buffer Kit Compatible with 1 mM THP	Vacuum Manifold processing Rapid purification of multiple samples
His•Mag Agarose Beads	Ni-charged IDA magnetic agarose	5 mg/ml	3 µm magnetic agarose beads Compatible with 1 mM THP	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
2-ME: 2-mercaptoethanol
* Manufactured by QIAGEN

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 10 × 2.5 ml columns. The His•Bind Quick Buffer Kit contains the same components except that the 8X Charge Buffer is not included.

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.	Price
His•Bind® Purification Kit	70239-3	
Components: • 10 ml His•Bind Resin • 1 His•Bind Buffer Kit • pkg/4 Chromatography Columns		
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		
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).		
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		

NEW Ni-MAC™, Co-MAC™, and u-MAC™ Fractogel® Cartridges

Metal Affinity Chromatography (MAC) Resins and Cartridges

Advantages of Fractogel® Resin

The Fractogel® Matrix is a durable synthetic methacrylate-based resin with long polymer chain "tentacles" covalently bonded to hydroxyl groups on the bead surface. The steric accessibility of the ligands attached to the tentacles allows high protein binding capacity. With low steric hindrance, biomolecules bind more readily during the separation process, leading to higher purification yields. Like the bead surface, the tentacles and functional ligands are stable in the presence of regeneration buffers, so the resins can be reused many times.

Ni-MAC™, Co-MAC™, and u-MAC™ Cartridges are pre-packed with 1 ml His•Bind® Fractogel Resin charged with nickel (Ni-MAC), cobalt (Co-MAC), or provided as uncharged (u-MAC) for charging with the metal of choice for rapid affinity purification of His•Tag® fusion proteins. The cartridges are available separately in packages of 2 or 5 and include Luer Lock Adaptors. The Purification Kits contain a set of concentrated phosphate-based (Ni-MAC) or tris-based (Co-MAC) buffers and 5 ready-to-use cartridges.

Connect to FPLC system with M6 fittings

Features

- Rapid affinity purification of His•Tag fusion proteins
- Each cartridge packed with 1 ml Fractogel resin
- Cartridge dimensions: 2.1 cm × 0.8 cm (Fractogel bed height x diameter)
- Compatible with automated liquid chromatography systems
- Resin precharged with Ni^{2+} or Co^{2+}, or uncharged to use with choice of metal ion
- High binding capacity—up to 30 mg protein/ml
- Very low non-specific protein binding
- High mechanical and chemical stability
- Pressure stability—up to 20 bar
- High flow rates—up to 800 cm/h (~7 ml/min)
- Stable resin permits efficient regeneration
- Economical, can be reused at least 10 times

Product	Size	Cat. No.	Price
Ni-MAC™ Purification Kit	1 kit	71658-3	
Ni-MAC™ Fractogel® Cartridges	2 cartridges	71649-3	
	5 cartridges	71649-4	
Co-MAC™ Purification Kit	1 kit	71659-3	
Co-MAC™ Fractogel® Cartridges	2 cartridges	71650-3	
	5 cartridges	71650-4	
u-MAC™ Cartridges	5 cartridges	71651-3	

Components

Cat. No. 71658
- 5 Ni-MAC Cartridges (with Luer Lock Adaptors)
- 2 × 75 ml 4X MAC Wash Buffer, Phosphate
- 2 × 100 ml 4X MAC Bind Buffer, Phosphate
- 75 ml 4X MAC Elute Buffer, Phosphate

Cat. No. 71659
- 5 Co-MAC Cartridges (with Luer Lock Adaptors)
- 2 × 80 ml 8X Bind Buffer
- 3 × 25 ml 8X Wash Buffer
- 3 × 25 ml 4X Elute Buffer

Properties of the Fractogel His•Bind tentacle affinity resins

Type of Chromatography	Immobilized Metal Affinity Chromatography (IMAC)
Matrix	Fractogel crosslinked polymethacrylate
Particle size	40-90 µm
Functional group	Iminodiacetic acid (IDA)
Functional group attachment	Via long polymer chains (tentacles)
Metal ion binding capacity	80 µmol/ml of resin
Protein binding capacity	30 mg/ml of resin
Elution conditions	Increasing concentration of imidazole, free histidine, EDTA, decreasing pH
Reducing agent	Compatible with 1 mM THP
pH stability range	pH 1 to 12
Pressure limit	20 bar
Linear flow rate	up to 800 cm/h (7 ml/min)
Operating temperature	4˚C to room temperature
Storage conditions	150 mM NaCl, 20% EtOH

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 the Novagen 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) is recommended as a blocking agent. Please see His•Tag Western and LumiBlot™ Reagents on page 29 for reagents specifically configured for Western detection of the His•Tag Monoclonal Antibody.

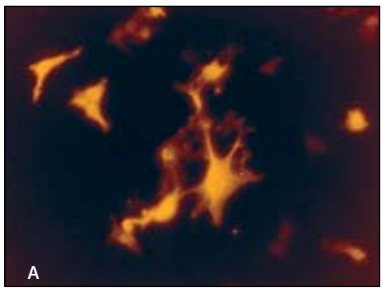
The 100-µg package size provides enough purified antibody for up to 1000 ml of working solution.

For a list of our secondary antibodies and other Western blot reagents, see page 69.

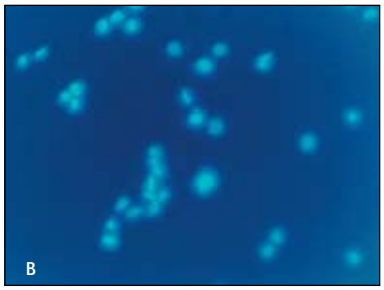
* Not recommended for HRP-based detection of Trail Mix Western Markers

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

His•Tag fusion protein detection



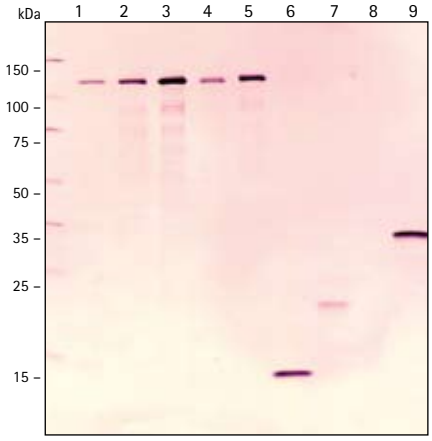
Nuclear staining



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. 24 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.

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	1:1000–1:2000 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

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-gal fusion protein in lanes 2–6.



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

customer.service@merckbio.com
technical.service@merckbio.com
Visit our website www.merckbio.com

NEW His•Tag® Antibody HRP Conjugate

Sensitive, specific & quick detection of His•Tag® fusion proteins

The His•Tag® Antibody HRP Conjugate Kit contains a horseradish peroxidase-conjugated His•Tag Monoclonal Antibody that is useful as a single detection reagent, thus eliminating cross-reactivity associated with secondary antibody reagents. It recognizes five consecutive histidine residues regardless of the surrounding amino acid context. The purified antibody conjugate produces a strong signal at a 1:1000-1:2000 dilution on Western blots and dot blots. The package size provides enough conjugate for 25-50 blots and includes a 5% Alkali-Soluble Casein Solution as the blocking reagent.

Product	Size	Cat. No.	Price
His•Tag® Antibody HRP Conjugate Kit	1 kit	71840-3	

Components

- 125 µl His•Tag Antibody Conjugate
- 150 ml 5% Alkali-soluble Casein

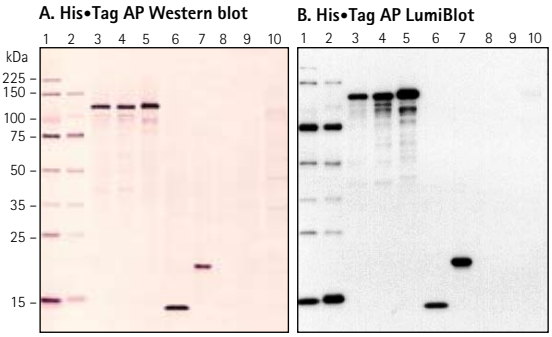
Features:

- No need for secondary antibody
- High specificity to 5X His regardless of surrounding amino acid context
- Binds to N-terminal, C-terminal, and internal His•Tag sequences
- Cross-reactivity to bacterial, insect, and mammalian proteins is negligible
- Detects the recombinant protein marker bands in the Perfect Protein™ Western Markers

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 26) ensures optimal sensitivity and low backgrounds in Western blot applications.



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 s for Panel B.

Product	Size	Cat. No.	Price
(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 Trail Mix™ Western Markers (AP kits) *or* Perfect Protein™ Western Markers (HRP kit)

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 ($K_d = 5 \times 10^{-8} - 1 \times 10^{-9}$ 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.	Price
His•Tag® Antibody Plate	1 plate	71184-3	
	5 plates	71184-4	

customer.service@merckbio.com
technical.service@merckbio.com
Visit our website www.merckbio.com

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 conditions with reduced glutathione avoids target protein denaturation.

GST•Bind Resin is ideal for batch purification or in gravity flow columns. GST•Mag Agarose Beads are available for rapid purification of multiple samples with minimum handling time, and are easily collect with a magnet which enables binding, wash, and elution procedures to be carried out in a single tube or well.

GST•Bind Fractogel Cartridges are designed for rapid, single step purification utilizing liquid chromatography devices or syringe.

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.

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 34 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 34 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 32 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 33 for more information.

NEW GST•Bind™ Fractogel® Cartridges

Rapid, single step purification of GST Fusion proteins

The Fractogel® matrix is a durable synthetic methacrylate-based resin with long polymer chain "tentacles" covalently bonded to hydroxyl groups on the bead surface. The steric accessibility of the ligands attached to the tentacles allows high protein binding capacity. With low steric hindrance, biomolecules bind more readily during the separation process, leading to higher purification yields. Like the bead surface, the tentacles and functional ligands are stable in the presence of regeneration buffers, so the resins can be reused many times.

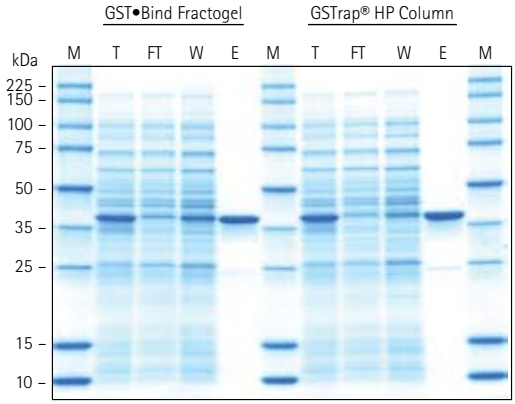
The GST•Bind™ Fractogel® Cartridges are designed for rapid affinity purification of Glutathione-S-transferase (GST•Tag™) fusion proteins which is based on the widely recognized affinity of Glutathione-S-transferase for immobilized glutathione. Each cartridge is packed with 1 ml GST•Bind Fractogel resin, which has has glutathione immobilized along the length of the flexible tentacles. The GST•Bind Fractogel Cartridges can be used with a syringe or liquid chromatography devices. Each cartridge binds up to 10 mg protein and can be reused up to 10 times without significant loss of capacity. The cartridges are provided in packages of 2 or 5 and include luer lock adaptors. The cartridges are compatible with the GST•Bind Buffer Kit (see page 35).

Connect to FPLC system with M6 fittings

Features

- Binding capacity up to 10 mg of GST
- Gentle elution conditions
- Compatible with automated liquid chromatography systems, such as GE Healthcare ÄKTA™
- Reusable

Product	Size	Cat. No.	Price
GST•Bind™ Fractogel® Cartridges	2 cartridges	71749-3	
	5 cartridges	71749-4	



Purification of a human Phosphohistidine phosphatase-GST•Tag fusion protein with a GST•Bind Fractogel Cartridge and a GSTrap® HP Column

A GST-PHPT1 (Glutathione S transferase - human phosphohistidine phosphatase fusion protein) fusion protein was expressed in BL21(DE3) and purified by chromatography. Glutathione affinity chromatography was performed according to manufacturers' protocols for GST•Bind Fractogel and GSTrap HP (GE Healthcare) 1 ml cartridges. Total soluble protein (T), flow through (FT), wash (W) and elute (E) fractions were collected and analyzed by SDS-PAGE. (M) Perfect Protein™ Markers 10-225 kDa.

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 Genesis® Freedom™ Workstation from Tecan and the MultiPROBE® II liquid handling work station 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.

References

1. Grabski, A., Mehler, M., Drott, D., and Van Dinther, J. (2002) *inNovations* **14**, 2.

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

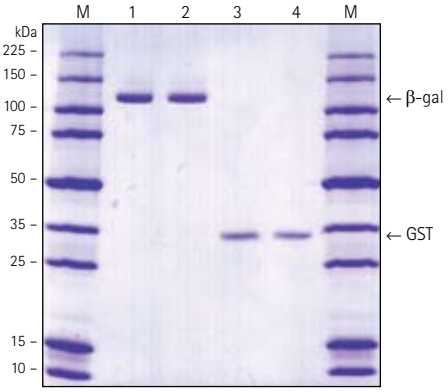
Components:

- 75 ml PopCulture Reagent
- 25 ml GST•Bind Resin
- 100 ml 10X 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

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 ambient 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.



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–gal 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 Perkin Elmer 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 Reagent 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.

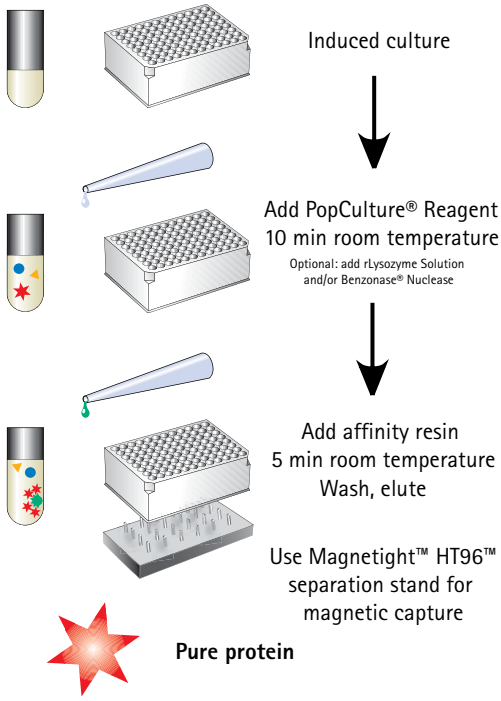
The Culture Plate is compatible with the Novagen Magnetight™ HT96™ Separation Stand (see page 50), which is recommended for efficient processing of magnetic affinity supports in deep well plates.

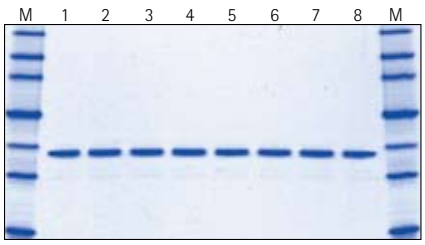
The RoboPop GST•Mag Purification Kit will purify up to 4.8 mg of GST•Tag fusion proteins per plate (up to 50 µg/well). Stated yields are based on 1-ml cultures and binding capacities of the beads, and will vary with the folding properties, expression levels, and solubility of individual fusion proteins. The RoboPop His•Mag Purification Kit has been validated for robotic sample processing with the Genesis® Freedom™ Workstation from Tecan and the MultiPROBE® II liquid handling work station from PerkinElmer Life Sciences.

Product	Cat. No.	Price
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%





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 the Novagen Magnetight™ Separation Stand. (See page 50 for more information.) For 96-well processing using PopCulture, please refer to the RoboPop™ Purification Kits.

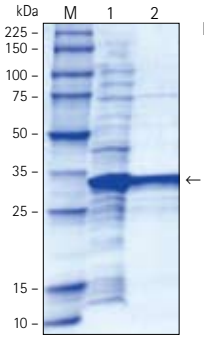
Features

- 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 vessel

Product	Cat. No.	Price
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

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.

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.

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.	Price
BugBuster® GST•Bind™ Purification Kit	70794-3	

Components:
- 2 × 100 ml BugBuster Protein Extraction Reagent
- 10 KU 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

GST•Tag™ Affinity Resins and Buffer Kit

Affinity purification of GST•Tag 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 conditions with 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

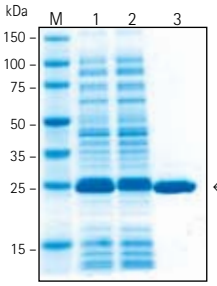
GST•Mag Agarose Beads are available for rapid purification of multiple samples with minimum handling time. The 3 µm (average diameter) beads have binding capacity up to 2 mg/ml of settled resin as measured with GST protein and are easily collected with a magnet, which enables binding, wash, and elution 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 10 × 2.5 ml GST•Bind columns.

Product	Size	Cat. No.	Price
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



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™ 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.	Price
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.

customer.service@merckbio.com
technical.service@merckbio.com
Visit our website www.merckbio.com

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 69 for conjugates and substrates compatible with the determination of GST•Tag and other Novagen monoclonal and polyclonal antibodies.

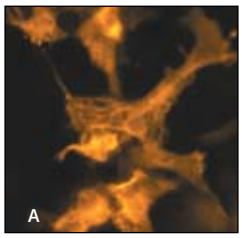
References

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.

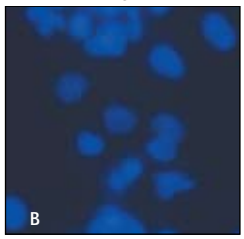
Specificity	220 aa GST protein; precise epitope not determined
Species/Isotype	Mouse monoclonal IgG_1
Cross-reactivity	Negligible with bacterial, yeast, insect, or mammalian cell lysates
Sensitivity	2.5–5 ng (Western blot developed with chromogenic substrates) < 1 ng (AP or HRP conjugate developed with 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

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

GST detection



Nuclear staining



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. 24 h 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.

GST•Tag™ Antibody Plate

Immobilized antibody in a 96-well format

The GST•Tag Antibody Plate is a 96-well, ELISA-compatible plate in an 8-well strip format containing immobilized GST•Tag Monoclonal Antibody with high affinity for the 26-kDa glutathione-S-transferase (GST) domain of *Schistosoma japonicum*. The antibody will bind with high affinity to virtually any GST•Tag fusion protein with an exposed epitope. These plates carry a binding capacity of > 50 ng GST•Tag fusion protein per well with < 10% CV among wells. The GST•Tag Antibody Plate can be used in any application in which reliable, specific immobilization of GST•Tag fusion proteins is required.

Product	Size	Cat. No.	Price
GST•Tag™ Antibody Plate	1 plate 5 plates	71437-3 71437-4	

S•Tag™ Purification Kits

Rapid affinity purification of S•Tag™ fusion proteins

The S•Tag™ Purification Kits enable rapid analytical- to medium-scale affinity purification of S•Tag fusion proteins produced from appropriate vectors. Sufficient reagents are provided for purification of up to 1 mg target protein under native or denaturing conditions. Crude samples are incubated with S-protein Agarose, which specifically retains S•Tag fusion proteins (1, 2). After unbound proteins are washed away, the target protein is released by incubation of the agarose beads with site-specific protease, leaving the S•Tag peptide bound to the resin. As alternative elution strategies, bound proteins can be dissociated from the S-protein Agarose in the presence of 3 M NaSCN, 3 M $MgCl_2$, or 0.2 M citrate, pH 2.

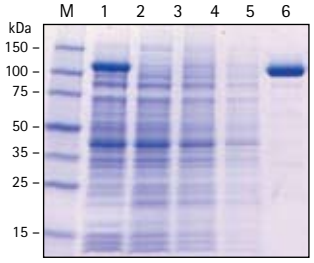
The S•Tag Thrombin Purification Kit includes Biotinylated Thrombin as the site-specific protease used to release proteins from the S-protein Agarose. The Biotinylated Thrombin can then be removed using Streptavidin Agarose. Streptavidin Agarose provides a convenient solid phase for the capture of biotinylated molecules. This preparation is qualified for use with proteins, DNA, and RNA, and has a binding capacity of 85 nmol biotin per milliliter of settled resin.

The S•Tag rEK Purification Kit enables target protein release by incubation of the agarose beads with Recombinant Enterokinase (rEK), leaving the S•Tag peptide bound to the resin. Residual rEK can then be removed efficiently using EKapture™ Agarose beads.

References

1. Ho, I.-C., Hodge, M. R., Roone, J. W., and Glimcher, L. H. (1996) *Cell* **85**, 973–983.
2. Raines, R. T., McCormick, M., Van Oosbree, T. R., and Mierendorf, R. C. (2000) *Meth. Enzymol.* **326**, 362–376.

Product	Size	Cat. No.	Price
S•Tag™ Thrombin Purification Kit		69232-3	
Components:			
• 2 ml	S-protein Agarose		
• 3 × 5 ml	10X Bind/Wash Buffer		
• 3 × 1 ml	10X Thrombin Cleavage Buffer		
• 50 U	Biotinylated Thrombin		
• 2 × 0.4 ml	Streptavidin Agarose		
• pkg/2	Spin Filters, 5-ml capacity		
S•Tag™ rEK Purification Kit		69065-3	
Components:			
• 2 ml	S-protein Agarose		
• 3 × 5 ml	10X Bind/Wash Buffer		
• 2 ml	1X rEK Dilution/Storage Buffer		
• 50 U	Recombinant Enterokinase		
• 1.5 ml	EKapture Agarose		
• pkg/2	Spin Filters, 5-ml capacity		
S-protein Agarose	2 ml	69704-3	
	5 × 2 ml	69704-4	



Lane	Sample
M	Perfect Protein™ Markers, 15–150 kDa
1	Crude extract
2	Flow through
3	Wash 1
4	Wash 2
5	Eluate + Biotinylated Thrombin
6	Eluate after Biotinylated Thrombin removal

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

S-protein Conjugates

Detection of S•Tag™ fusion proteins on blots, plates, or in cells

The 104–amino acid (aa) S-protein binds with high affinity (K_d = 10^{-9} M) and specificity to the 15-aa S•Tag™ fusion tag expressed from many Novagen vectors (1). Background is virtually absent in bacterial or eukaryotic extracts and there is no interference with other affinity reagents. Highly purified S-protein is available conjugated with biotin, alkaline phosphatase (AP), or horseradish peroxidase (HRP). These reagents are optimized for maximum signal without loss in S-protein binding activity, and enable detection of S•Tag fusion proteins in a variety of formats, including Western blot, dot blot, and ELISA.

Additionally, we offer an S-protein fluorescein isothiocyanate (FITC) conjugate that can be used to detect fusion proteins expressed in insect or mammalian cells. S-protein Conjugates are also useful for detection of the Novagen Perfect Protein™ and Trail Mix™ Western Markers for convenient, accurate protein size determination on Western blots. See the Western Blot Kits and Reagents section for complete S•Tag Western Blot Kits.

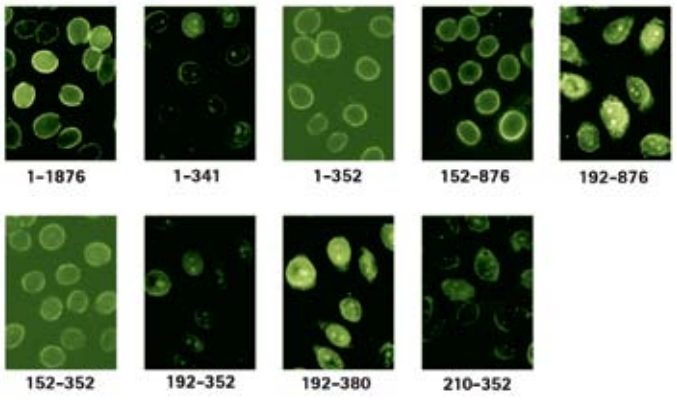
References

1. Raines, R. T., McCormick, M., VanOosbree, T. R., and Mierendorf, R. C. (2000) *Meth. Enzymol.* **326**, 362-376.

Product	Size	Cat. No.	Price
Biotinylated S-protein	250 µl	69218-3	
S-protein AP Conjugate	50 µl	69598-3	
S-protein HRP Conjugate	50 µl	69047-3	
S-protein FITC Conjugate	200 µl	69060-3	

Specificity	S•Tag peptide LysGluThrAlaAlaAlaLysPheGluArgGlnHisMetAspSer; N-terminal, C-terminal, or internal
Species	104-aa S-protein fragment of bovine RNase A (enzymatically inactive)
Cross-reactivity	Negligible with bacterial and eukaryotic cell lysates
Sensitivity	1–10 ng (Western blot developed with chromogenic substrates) < 1 ng (AP or HRP conjugate developed with chemiluminescent substrates, or biotin conjugate developed with Streptavidin AP and chromogenic substrates). Also suitable for histochemistry
Form	Stabilized solution of conjugate in 50% glycerol
Working dilutions	See table to the right

Conjugate	Recommended working dilution	Volume of working solution in 1X size
Biotinylated S-protein	1:10,000	2.5 L (250 blots)
S-protein AP	1:5000	250 ml (25 blots)
S-protein HRP	1:5000	250 ml (25 blots)
S-protein FITC	1:2000	400 ml (200 slides)



Binding of importin β to permeabilized cells
S•Tag fusion proteins of fragments of importin β were incubated on permeabilized MDBK cells grown on glass coverslips. The fusion proteins were detected with S-protein FITC and viewed by epi-fluorescence microscopy kindly provided by Stephen Adam, Northwestern University, *inNovations* (2001) **13**, 5–6.

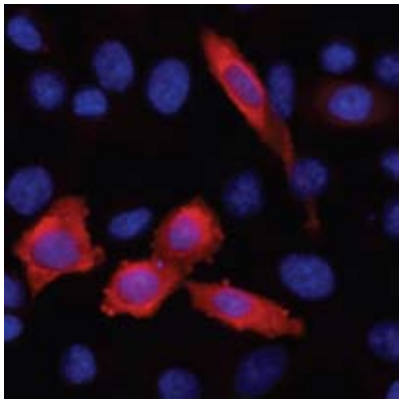
S•Tag™ Monoclonal Antibody

Sensitive detection of S•Tag™ containing proteins

The S•Tag™ Monoclonal Antibody is a mouse monoclonal antibody (IgG_{2b}) directed against the 15-amino acid S•Tag peptide expressed by many Novagen vectors. This antibody can be used for sensitive and specific detection of S•Tag fusion proteins and is suitable for immunoblotting, immunoprecipitation, and immunfluorescence. Purified by protein G affinity chromatography and supplied at 1 mg/ml. The S•Tag Monoclonal Antibody can detect as little as 1 ng of S•Tag fusion protein in immunoblots and displays negligible cross-reactivity with bacterial, mammalian and insect lysates. The S•Tag Monoclonal Antibody can also be used to detect the Novagen Perfect Protein™ and Trail Mix™ Western Markers by immunoblotting.

Product	Size	Cat. No.	Price
S•Tag™ Monoclonal Antibody	50 µg	71549-3	

Specificity	S•Tag sequence (LysGluThrAlaAlaAlaLysPheGluArgGlnHisMetAspSer);
Species/Isotype	Mouse monoclonal IgG_{2b}
Cross-reactivity	Negligible with bacterial, insect, or mammalian cell lysates
Sensitivity	5 ng (immunoblot developed with chromogenic substrates) < 1 ng (immunoblot developed with HRP conjugate and chemiluminescent substrates.)
Form	Liquid with 50% glycerol in 1 x PBS
Working dilution	1:5000 for immunoblotting, 1:2000 for immunofluorescence



Immunofluorescent staining using the S•Tag Monoclonal Antibody

COS-7 cells were transfected with a pTriEx™-4 expression vector encoding an S•Tag/β-galactosidase fusion protein. The fusion protein was detected with the S•Tag Monoclonal Antibody at 1:200 dilution followed by Cy3®-conjugated goat anti-mouse antibody. DNA was visualized by staining with Hoechst 33258 (1 µg/ml).

S•Tag™ Western Blot and LumiBlot™ Kits

Colorimetric and chemiluminescent blot detection of S•Tag™ fusion proteins

S•Tag™ fusion proteins can be quickly detected on blots using the S•Tag Western Blot and LumiBlot™ Kits. Blots are simply incubated with the appropriate S-protein conjugate, followed by development with colorimetric or chemiluminescent substrates. The S-protein conjugates have been optimized to give the highest signal-to-noise ratio when detecting S•Tag fusion proteins even when in low abundance in a complex protein mixture. They enable detection of target proteins without the need for potentially cross-reacting secondary antibodies or streptavidin/avidin-based systems. There is also negligible cross-reactivity with endogenous bacterial, insect, or mammalian proteins.

The kits include Perfect Protein™ Western Markers, a mixture of seven S•Tag fusion proteins of precise sizes (15–150 kDa) that serve as accurate standards for determining the sizes of unknown samples on blots.

S•Tag™ AP Western Blot Kit

The S•Tag AP Western Blot Kit enables colorimetric detection of S•Tag fusion proteins using NBT/BCIP substrates. The optimized protocol takes only 30 minutes for all incubations and washing steps, and enables detection of subnanogram amounts of target proteins.

S•Tag™ LumiBlot™ Kits

The S•Tag LumiBlot Kits are designed for ultrasensitive detection of S•Tag fusion proteins. Both kits offer an enhanced chemiluminescent substrate that enables high signal intensity and short exposure times in a ready-to-use format.

The chemiluminescent substrates offer several advantages, such as low background, subnanogram sensitivity levels, rapid results, and long-lived signal enabling multiple exposures to film for optimization of results.

The S•Tag HRP LumiBlot Kit contains SuperSignal® chemiluminescent substrate for horseradish peroxidase (HRP), enabling sensitive detection of S•Tag fusion proteins with the S-protein HRP conjugate. The SuperSignal enhanced substrate consists of two components: a Luminol/Enhancer Solution, and a Stable Peroxide Solution, which are simply mixed together for easy use.

The S•Tag AP LumiBlot Kit contains CDP-*Star*® chemiluminescent substrate for alkaline phosphatase (AP). Highly specific detection at subnanogram levels is possible after as little as one minute exposure time. The CDP-*Star* Substrate includes Nitro-Block-II™, a signal enhancer optimized for use with nitrocellulose membranes.

Product	Size	Cat. No.	Price
S•Tag™ AP Western Blot Kit	25 blots	69213-3	

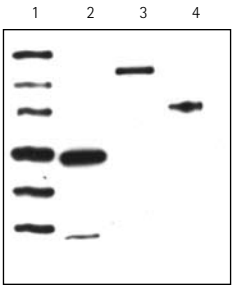
Components:

- 50 µl S-protein AP Conjugate
- 40 ml 10% Gelatin
- 35 ml 20% TWEEN® 20
- 1.5 ml BCIP
- 1.5 ml NBT
- 20 ml 20X AP Buffer
- 25 lanes Perfect Protein™ Western Markers

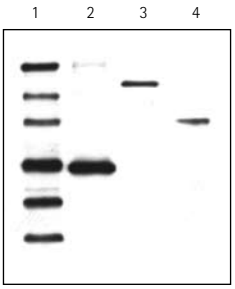
S•Tag™ HRP LumiBlot™ Kit	25 blots	69058-3	
S•Tag™ AP LumiBlot™ Kit	25 blots	69099-3	

Components:

- 50 µl S-protein HRP or AP Conjugate
- 4 × 50 ml 10X TBST Wash Buffer
- 25 g Blocking Reagent
- 25 lanes Perfect Protein Western Markers
- 50 ml SuperSignal Substrate or
- 40 ml CDP-*Star* Substrate (includes NitroBlock-II signal enhancer)
- pkg/25 gLOCATOR™ Luminescent Labels
- pkg/25 Development Folders



S•Tag HRP LumiBlot



S•Tag AP LumiBlot

Lane	Sample
1	Perfect Protein Western Markers
2	Protein expressed from pET-32
3	β–galactosidase produced *in vitro* in an STP3® reaction
4	β-glucuronidase expressed from virus transfer plasmid

Detection of S•Tag fusion proteins
S•Tag fusion proteins were detected in crude cell extracts using the indicated kits. Exposure times were one minute for HRP and four minutes for AP.

FRETWorks™ S•Tag™ Assay Kit

Ultrasensitive, homogeneous, fluorescent assay of S•Tag™ fusion proteins

The S•Tag™ fusion sequence encodes a 15–amino acid (aa) peptide that binds with high affinity to the 104-aa S-protein derived from subtilisin treatment of pancreatic ribonuclease A (1, 2). This interaction was first characterized by Richards and colleagues (3), who observed that the subtilisin cleavage products, S-protein: S-peptide complex (ribonuclease S), maintained enzymatic activity, but neither component had activity by itself. The unique property of reconstituting enzymatic activity by the S•Tag peptide:S-protein interaction enables sensitive quantitative measurement of any fusion protein by a simple assay (2). The S•Tag peptide is encoded by many of the Novagen expression vectors, and is extremely useful as an affinity tag for this assay, as well as for detection on Western blots and in purification (4).

The FRETWorks™ S•Tag Assay is a FRET-based method that enables extremely sensitive detection of S•Tag fusion proteins in minutes with a homogeneous format. The interaction of the 15–amino acid S•Tag fusion peptide with purified S-protein reconstitutes RNase activity, which is measured using the FRET ArUAA substrate. The substrate consists of a short, mixed ribo/deoxy-ribooligonucleotide having a fluorophore on the 5′-end and a quencher on the 3′-end (5). Fluorescence of the uncleaved molecule is inhibited by the interaction of the fluorophore and quencher; upon cleavage of the intervening ribonucleotide residue, the fluorophore is released and becomes highly fluorescent. The specificity of the substrate permits this assay to be performed with crude extracts. No reagent injection is necessary, and the signal is stable for > 6 hours. The FRETWorks S•Tag Assay Kit is compatible with the PopCulture® Reagent (including the additives Benzonase® Nuclease and rLysozyme™ Solution), making these reagents an ideal combination for rapid, automatable screening of protein expression.

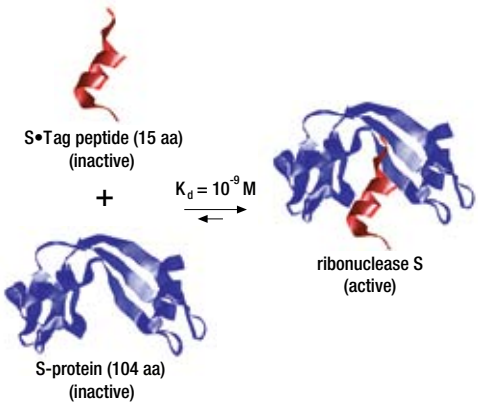
References

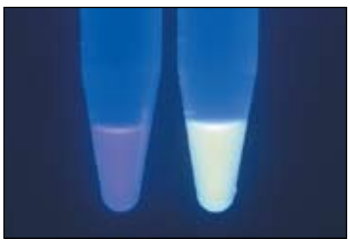
1. Kim, J. and Raines, R. T. (1993) *Protein Sci.* **2**, 348–356.
2. McCormick, M. and Mierendorf, R. (1994) *inNovations* **1**, 4a–7.
3. Richards, F. M. and Wyckoff, H. W. (1971) *The Enzymes*, **Vol IV**, P. D. Boyer, ed. (Academic Press, New York) pp. 647–806.
4. Raines, R. T., McCormick, M., Van Oosbree, T. R., and Mierendorf, R. C. (2000) *Meth. Enzymol.* **326**, 362-376.
5. Keleman, B. R., Klink, T. A., Behlke, M. A., Eubanks, S. R., Leland, P. A., and Raines, R. T. (1999) *Nucleic Acids Res.* **27**, 3696–3701.

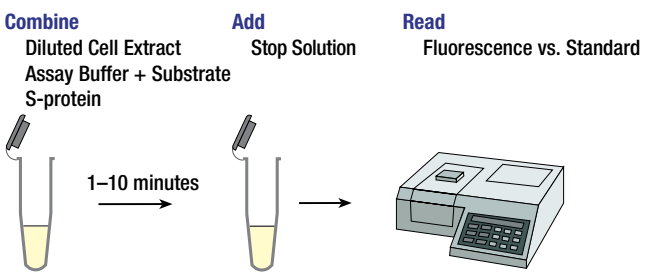
Product	Size	Cat. No.	Price
FRETWorks™S•Tag™ Assay Kit	100 assays	70724-3	
	1000 assays	70724-4	

Components:

- 1 or 5 × 1 ml S•Tag Grade S-protein
- 1 or 10 × 0.2 ml FRET ArUAA Substrate
- 1 or 10 × 2.5 ml 10X FRET Assay Buffer
- 1 or 10 × 2.5 ml 10X Stop Solution
- 1 or 10 × 50 µl S•Tag Standard







FRETWorks S•Tag Assay

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

customer.service@merckbio.com
technical.service@merckbio.com
Visit our website www.merckbio.com

S•Tag™ Rapid Assay Kit

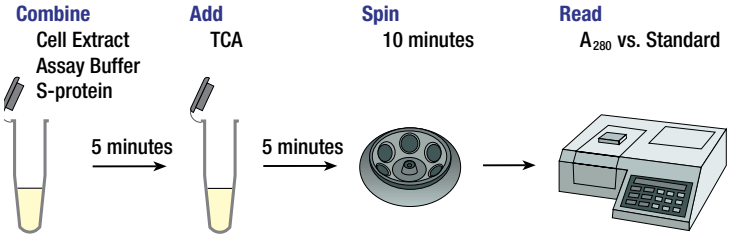
Sensitive colorimetric quantification of S•Tag™ fusion proteins

With the S•Tag™ Rapid Assay, a sample is added to a buffer containing the ribonuclease substrate poly(C). The reaction is started by adding purified S-protein. After a 5-minute incubation, the reaction is stopped with trichloroacetic acid. The resulting precipitate is removed by centrifugation. Activity is measured by reading the absorbance of the supernatant at 280 nm, which increases as the poly(C) is broken down into acid-soluble nucleotides by the enzyme. By comparing the results with a known S-peptide standard included in the kit, the molar concentration of target protein in the sample can be determined. As little as 20 fmol of target protein can be detected in a 5-minute incubation with the S•Tag Rapid Assay. Samples can be crude extracts prepared with BugBuster®, PopCulture®, CytoBuster™, or Reportasol™ Reagents; SDS; subcellular fractions; translation reactions; or purified proteins. In most applications, there is negligible interference from endogenous RNases.

The S•Tag Rapid Assay Kit includes a set of matched reagents designed to perform 100 standard assays.

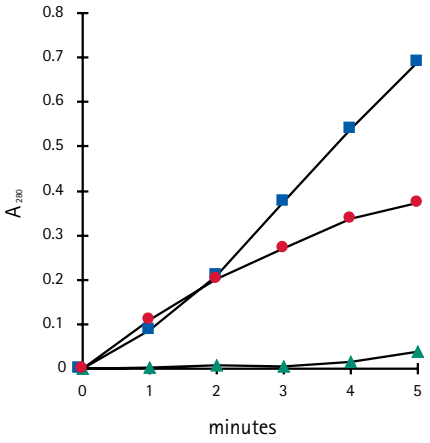
Product	Size	Cat. No.	Price
S•Tag™ Rapid Assay Kit	100 assays	69212-3	

Components:
- 1 ml S•Tag Grade S-protein
- 4 ml 10X S•Tag Assay Buffer
- 2.5 pmol S•Tag Standard



S•Tag Rapid Assay

Substrate	Poly(C)
Endpoint	A_{280}
Elapsed time	20 min
Detection limit	20 fmol



S•Tag Rapid Assay time course
Reactions with the indicated samples were performed according to the standard protocol.

- Rabbit reticulocyte lysate *in vitro* translation of S•Tag β–galactosidase
- Crude *E. coli* extract of a pET construct expressing S•Tag β–galactosidase
- Blank (*in vitro* translation reaction with no template)

Strep-Tactin® Resins and Purification Kits

Rapid one-step purification of Strep-Tag® II fusion proteins

Strep•Tag® technology is based on the strong and specific interaction between biotin and streptavidin. To take advantage of this interaction for recombinant protein affinity purification, an 8-amino acid Strep•Tag II peptide that is capable of binding to the biotin pocket of streptavidin was engineered. Likewise, the Strep•Tactin® protein, a streptavidin derivative, was developed for optimal Strep•Tag II peptide binding. The binding affinity of Strep•Tag II peptide for Strep•Tactin protein is nearly 100 times higher than for streptavidin. The Strep•Tactin family of products offers a wide variety of supports for rapid one-step affinity purification of proteins containing the Strep•Tag II fusion tag. The Strep•Tag II sequence binds to the Strep•Tactin coupled resin, and after unbound proteins are washed away, the purified target protein is competitively eluted with 2.5 mM desthiobiotin, a reversible analog of biotin. The purification steps may be performed under physiological conditions in PBS or other physiological buffers preserving the bioactivity of the target protein.

Product	Size	Cat. No.	Price
Strep•Tactin® Superflow™ Agarose	2 ml 10 ml	71592-3 71592-4	
Strep•Tactin® Superflow™ Columns, 0.2 ml	5 columns	71594-3	
Strep•Tactin® Superflow™ Columns, 1 ml	5 columns	71593-3	
Strep•Tactin® Superflow™ HP Cartridges, 1 ml	5 cartridges	71766-3	
Strep•Tactin® Superflow™ HP Cartridges, 5 ml	1 cartridges 5 cartridges	71767-3 71767-4	
Strep•Tactin® MacroPrep® Resin	2 ml 10 ml	71597-3 71597-4	
Strep•Tactin® MacroPrep Cartridges, 1 ml	5 cartridges	71598-3	
Strep•Tactin SpinPrep™ Kit	25 reactions	71608-3	
D-Desthiobiotin	1 g	71610-3	

Strep•Tactin® Superflow™ Agarose

Strep•Tactin Superflow Agarose is a cross-linked agarose derivatized with Strep•Tactin protein. The binding capacity is 50-100 nmol/ml settled resin, or up to 3 mg of 30-kDa protein per ml settled resin. It can be used for gravity flow as well as for low pressure and FPLC chromatography. Strep•Tactin Superflow Agarose is optimized for column affinity chromatography as opposed to batch purification. It is supplied as a 50% slurry.

Strep•Tactin® Superflow™ Columns

Strep•Tactin Superflow Columns are ready-to-use prepacked chromatography columns designed for gravity flow. Columns are available in two sizes, 0.2 ml and 1 ml, with binding capacities of 10-20 nmol/column and 50-100 nmol/column, respectively.

Strep•Tactin® Superflow™ HP Cartridges

Strep•Tactin® Superflow HP Cartridges provide the ultimate convenience for affinity purification by chromatography workstations with 10-32 connections, such as HPLC and Äkta. The improved cartridge packaging allows for use at higher pressures, up to 20 bar. Adaptors are available separately for use with other liquid chromatography systems, FPLC workstations, and syringes. The cartridges are available in two sizes, 1 ml and 5 ml, with binding capacities of 50-100 nmol/cartridge and 250-500 nmol/cartridge, respectively. For higher purification capacities, the cartridges can be connected in a series using the coupling adaptor (available separately).

Strep•Tactin® MacroPrep® Resin

Strep•Tactin MacroPrep® Resin is a polymethacrylate resin suitable for gravity flow and all low pressure chromatography applications. This resin exhibits non-specific binding properties that differ from the Strep•Tactin Superflow Agarose. The binding capacity is 50-100 nmol/ml settled resin or up to 3 mg of 30-kDa protein per ml settled resin. Strep•Tactin MacroPrep Resin is optimized for column affinity chromatography and not recommended for batch purification. It is supplied as a 50% slurry.

Strep•Tactin® MacroPrep® Cartridges

Strep•Tactin MacroPrep Cartridges provide the ultimate convenience for affinity purification by low pressure chromatography. The luer lock fittings fasten quickly to a syringe or connect several cartridges in a series to allow for higher purification capacities. Adaptors are available separately for use with liquid chromatography systems or FPLC workstations. Each 1-ml cartridge has a binding capacity of 50-100 nmol.

Strep•Tactin® SpinPrep™ Kit

The Strep•Tactin SpinPrep™ Kit is a fast and easy-to-handle method of purifying Strep•Tag II fusion proteins using small spin columns containing Step•Tactin resin. Each column purifies up to 150 µg of Strep•Tag II fusion protein. The kit is supplied with 25 Strep•Tactin SpinPrep Columns, receiver tubes, and optimized wash and elute buffers.

Strep•Tactin® HT96™ Purification Kit

The Strep•Tactin® HT96™ Purification Kit is designed for Strep•Tag® II fusion protein purification in a 96-well format. The Strep•Tactin HT96 Plate contains predispensed Strep•Tactin resin that simply has to be rehydrated and equilibrated before use. The kit will purify up to 100 µg/well or 9.6 mg of Strep•Tag fusion protein per plate. It is compatible with robotic liquid handling systems as well as with standard vacuum manifolds for manual sample processing. The kit contains one each Strep•Tactin HT96 Purification Plate, Prefilter Plate, Wash Plate, Receiver Plate, and buffers.

Product	Size	Cat. No.	Price
Strep•Tactin® HT96™ Purification Kit	1 plate	71605-3	
Strep•Tactin® Buffer Kit	1 kit	71613-3	

Strep•Tactin® Buffer Kit

The Strep•Tactin Buffer Kit is a set of pre-tested buffers designed for use with Strep•Tactin resins for convenient, rapid one-step purification of the Strep•Tag fusion proteins. The kit includes Strep•Tactin Wash, Elution, and Regeneration buffers.

Strep•Tactin® Matrix Selection Guide

Product	Form	Capacity	Features	Applications
Strep•Tactin® Superflow™ Agarose*	Superflow 6 agarose	50-100 nmol/ml	Reusable 3-5 times; Compatible with 1 M urea or guanidine, 2% Triton® X-100, 0.1% SDS, and 50 mM DTT or 2-ME	Small to production scale; FPLC or gravity flow column
Strep•Tactin Superflow Column*, 0.2 ml	Superflow 6 agarose, pre-packed column	10-20 nmol	Reusable 3-5 times; Compatible with 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME	Convenient purification; Gravity flow column
Strep•Tactin Superflow Column*, 1 ml	Superflow 6 agarose, pre-packed column	50-100 nmol	Reusable 3-5 times; Luer fitting on one end; Compatible with 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME	Convenient purification; Gravity flow column
Strep•Tactin Superflow Cartridge*, 1 ml	Superflow 6 agarose, pre-packed cartridge	50-100 nmol	Reusable 3-5 times; Compatible with Luer adaptors; Compatible with 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME	Syringe-driven processing; Vacuum manifold processing; FPLC workstation purification; Rapid purification
Strep•Tactin Superflow Cartridge*, 5 ml	Superflow 6 agarose, pre-packed cartridge	250-500 nmol	Reusable 3-5 times; Compatible with Luer adaptors; Compatible with 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME	Syringe-driven processing; Vacuum manifold processing; FPLC workstation; Rapid purification
Strep•Tactin MacroPrep® Resin*	Polymethacrylate	50-100 nmol/ml	Reusable 3-5 times; 50 um particle size; High flow rates and pressures; Compatible with 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME	Small to production scale; FPLC or gravity flow column
Strep•Tactin MacroPrep Cartridge*	Polymethacrylate, pre-packed cartridge	50-100 nmol	Reusable 3-5 times; Compatible with Luer adaptors; 50-µm particle size; High flow rates and pressures; Compatible with 1 M urea or guanidine, and 50 mM DTT or 2-ME.	Syringe-driven processing; Vacuum manifold processing; FPLC/HPLC workstation; Rapid purification
Strep•Tactin HT96™ Purification Plate*	Superflow 6 agarose	100 µg/well	Compatible with 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME	High-throughput robotic processing; Vacuum manifold; Parallel processing
Strep•Tactin SpinPrep™ Column*	Agarose, pre-packed column	150 µg	Compatible with 1 M urea or guanidine, 2% Triton X-100, 0.1% SDS, and 50 mM DTT or 2-ME	Rapid convenient purification; Parallel processing; Ready-to-use spin column

Note: The Strep•Tag/Strep•Tactin purification system is designed for column purification and it is not recommended to be used in batch purification.
Abbreviations: 2-ME: 2-mercaptoethanol; DTT: dithiothreitol
* manufactured by IBA GmbH

Strep•Tag® II Fusion Protein Detection

Sensitive, versatile detection of Strep•Tag II® fusion proteins

Strep•Tag® Monoclonal Antibody

The Strep•Tag® II Monoclonal Antibody* is a mouse monoclonal antibody (IgG_1) that has high specificity and affinity for the 8-amino acid Strep•Tag II peptide. The purified antibody produces a strong signal at a 1:1000 dilution for Western blots and dot blots. The Strep•Tag II Monoclonal Antibody can detect as little as 5 ng Strep•Tag II fusion protein and displays negligible cross-reactivity with bacterial, mammalian, and insect lysates. The antibody is supplied lyophilized and should be reconstituted in PBS at a concentration of 0.2 mg/ml.

* manufactured by IBA GmbH

Product	Size	Cat. No.	Price
Strep•Tag® II Monoclonal Antibody	100 µg	71590-3	
Strep•Tag® II Antibody HRP Conjugate	75 µl	71591-3	

Strep•Tag® II Antibody HRP Conjugate

The Strep•Tag II Antibody HRP Conjugate* is a peroxidase conjugated Strep•Tag II Monoclonal Antibody that is useful as a single detection reagent thus eliminating cross-reactivity associated with secondary reagents. The purified antibody conjugate produces a strong signal at a 1:4000 dilution for Western blots and dot blots. The Strep•Tag II Antibody HRP Conjugate can detect as little as 5 ng Strep•Tag II fusion protein.

T7•Tag® Affinity Purification Kit

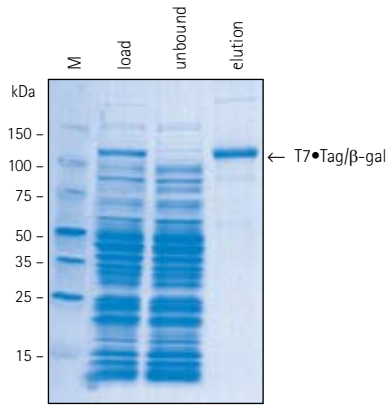
Immunoaffinity purification of T7•Tag® fusion proteins

The T7•Tag® Affinity Purification Kit is designed for rapid immunoaffinity purification of target proteins that carry the T7•Tag sequence (e.g., the 11 amino terminal aa of the T7 gene *10* protein). Purification is based on binding target proteins to T7•Tag Monoclonal Antibody that is covalently coupled to cross-linked agarose beads, washing away unbound proteins, and eluting at pH 2.2. Neutralization Buffer is included to limit protein exposure to low pH. Capacity will vary somewhat between different target proteins, but the beads are standardized to bind a minimum of 300 µg T7•Tag β-galactosidase per milliliter of settled resin. The beads can be used in either batch or column methods and can be regenerated a minimum of five times without loss of binding activity.

Product	Cat. No.	Price
T7•Tag® Affinity Purification Kit	69025-3	

Components:

- 1 ml T7•Tag Antibody Agarose
- 20 ml 10X T7•Tag Bind/Wash Buffer
- 20 ml 10X T7•Tag Elution Buffer
- 20 ml T7•Tag Neutralization Buffer
- 1 Chromatography Column



T7•Tag affinity purification
A pET recombinant expressing a T7•Tag/β-gal fusion protein was grown and induced with IPTG. Cells were lysed using a French pressure cell and insoluble material removed by centrifugation. The soluble extract was diluted 4-fold with an extract from a non-recombinant to intentionally reduce the relative target protein concentration. The target protein was purified on T7•Tag Antibody Agarose using the kit protocol and the indicated samples were analyzed by SDS-PAGE (4–20% gradient gel) and Coomassie blue staining. Lane M: Perfect Protein™ Markers, 15–150 kDa.

T7•Tag LumiBlot™ Kits

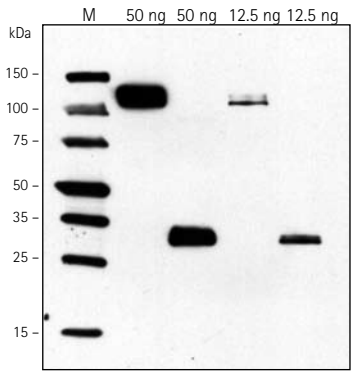
Chemiluminescent Western blot detection of T7•Tag® fusion proteins

The T7•Tag AP and HRP LumiBlot Kits combine sets of optimized reagents for sensitive, convenient chemiluminescent detection of T7•Tag proteins on Western and dot blots. A choice of alkaline phosphatase (AP) or horseradish peroxidase (HRP) based detection chemistries is provided to suit particular applications and preferences. Each kit detects subnanogram levels of target protein on Western blots with development times of 10 minutes or less. The kits provide enough reagents for 25 blots.

Product	Size	Cat. No.	Price
T7•Tag® AP LumiBlot™ Kit	25 blots	70237-3	
T7•Tag® HRP LumiBlot™ Kit	25 blots	70238-3	

Components:

- 50 µl T7•Tag AP Conjugate
  or
  100 µl T7•Tag HRP Conjugate
- 250 µl T7•Tag Positive Control
- 4 × 50 ml 10X TBST Wash Buffer
- 25 g Blocking Reagent
- 50 ml SuperSignal® HRP Substrate
  or
  40 ml CDP-*Star*® AP Substrate
  (includes Nitro-Block-II™ signal enhancer)
- pkg/25 gLOCATOR™ Luminescent Labels
- pkg/25 Development Folders



LumiBlot detection of T7•Tag proteins
Induced cell extracts of T7•Tag fusion proteins were blotted to nitrocellulose and detected with the T7•Tag HRP LumiBlot Kit. The Perfect Protein Western Markers in lane M were detected simultaneously with S-protein HRP Conjugate. The exposure time was 12 minutes. Amounts of total protein loaded are indicated.

T7•Tag® Monoclonal Antibody and Conjugates

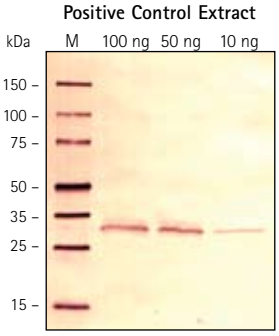
Sensitive, versatile detection of T7•Tag® fusion proteins

The T7•Tag® Antibody is a mouse monoclonal antibody (IgG_{2b}) directed against the 11–amino acid gene *10* leader peptide expressed by many of the pET translation vectors as well as pSCREEN™ and pRSET vectors. Because the peptide is the natural amino-terminal end of the T7 major capsid protein, the antibody also recognizes T7 bacteriophage (e.g., the T7Select® Vectors). The 50-µg package size provides enough purified antibody to perform 50 Western blots (10 cm × 10 cm) or 50 immunoprecipitation assays.

For increased versatility and sensitivity, this antibody is also available labeled with biotin and as a direct conjugate with alkaline phosphatase or horseradish peroxidase. When used with the Novagen Streptavidin AP Conjugate, the biotinylated antibody enables a 5-fold increase in detection sensitivity of target proteins on Western blots compared to an anti-IgG conjugate strategy. This form of the antibody also enables specific detection in the presence of other mouse IgGs. The package size of the Biotinylated T7•Tag Antibody enables processing of 125 Western blots.

The AP or HRP conjugates are useful as single detection reagents and eliminate the need for secondary antibodies or streptavidin conjugates. The enzyme-conjugated antibody enables detection with only one binding step and eliminates cross-reactivity associated with secondary reagents. The package size provides enough conjugate for 50 Western blots. Please see the Western Blot Kits and Reagents section for complete kits for chemiluminescent Western detection of the T7•Tag Monoclonal Antibody.

Product	Size	Cat. No.	Price
T7•Tag®Monoclonal Antibody	50 µg 250 µg	69522-3 69522-4	
Biotinylated T7•Tag® Monoclonal Antibody	125 µl	69968-3	
T7•Tag® Antibody AP Conjugate	50 µl	69999-3	
T7•Tag® Antibody HRP Conjugate	100 µl	69048-3	



T7•Tag Western Blot
The T7•Tag positive control total protein extract was run on an SDS gel and transferred onto nitrocellulose. The blot was incubated with a 1:10,000 dilution of T7•Tag antibody followed by a secondary antibody and development with NBT/BCIP substrates.

Specificity	T7•Tag peptide MetAlaSerMetThrGlyGlyGlnGlnMetGly; N-terminal, C-terminal or internal
Species/Isotype	Mouse monoclonal IgG_{2b}
Cross-reactivity	Negligible with bacterial, insect or mammalian cell lysates
Sensitivity	1–10 ng (Western blot developed with chromogenic substrates) < 1 ng (AP or HRP conjugate developed with chemiluminescent substrates, or biotin conjugate developed with Streptavidin AP and chromogenic substrates). Also suitable for immunoprecipitation and immunohistochemistry (1–3)
Form	Stabilized solution of antibody or antibody conjugate in 50% glycerol; includes Positive Control Extract containing 31.1 kDa target protein
Working dilution	1:5,000–1:10,000

1. Krek, W., Ewen, M. E., Shirodkar, S., Arany, Z., Kaelin, W. G., Jr., and Livingston, D. M. (1994) *Cell* **78**, 161–172.
2. Roberts, S. G. E. and Green, M. (1994) *Nature* **371**, 717–720.
3. Joneson, T., McDonough, M., Bar-Sag, D., and Van Aelst, L. (1996) *Science* **274**, 1374–1376.

HSV•Tag® Monoclonal Antibody

Sensitive, highly specific detection of HSV•Tag® fusion proteins

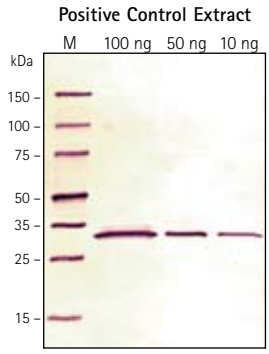
The HSV•Tag® Antibody is a mouse monoclonal antibody (IgG_1) that has high specificity and affinity for an 11–amino acid peptide derived from Herpes Simplex Virus (HSV) glycoprotein D. The peptide is encoded by the HSV•Tag sequence in appropriate expression vectors and by the Positive Control 36mer in the Novagen NovaTope® System. The purified antibody produces a strong signal at a 1:10,000 dilution on Western blots (1), dot blots, and colony lifts when used with the Novagen Anti-Mouse IgG AP Conjugate. See the Western Blot Kits and Reagents section on page 69 for additional information on conjugates and substrates compatible with detection of HSV•Tag and other Novagen monoclonal antibodies.

References

1. Sakai, J., Duncan, E. A., Rawson, R. B., Hua, X., Brown, M. S., and Goldstein, J. L. (1996) *Cell* **85**, 1037–1046.

Product	Size	Cat. No.	Price
HSV•Tag® Monoclonal Antibody	40 µg	69171-3	
	200 µg	69171-4	

Specificity	HSV•Tag peptide GlnProGluLeuAlaProGluAspProGluAsp; C-terminal or internal
Species/Isotype	Mouse monoclonal IgG_1
Cross-reactivity	Negligible with bacterial, insect, or mammalian cell lysates
Sensitivity	2 ng (Western blot developed with chromogenic substrates)
Form	Stabilized solution of 1 mg/ml pure antibody in 50% glycerol; includes Positive Control Extract containing 31.1kDa target protein
Working dilution	1:5,000–1:10,000



HSV•Tag Western Blot
HSV•Tag Positive Control Extract was detected by incubation with HSV•Tag Antibody (1:10,000 in TBST), followed by incubation with goat anti-mouse AP conjugate and color development with NBT/BCIP. S-protein AP conjugate was included in the secondary antibody incubation for detection of the Novagen Perfect Protein™ Western Markers (M).

Nus•Tag™ Monoclonal Antibody

Sensitive, specific detection of Nus•Tag™ fusion proteins

The Nus•Tag™ Monoclonal Antibody is a mouse monoclonal antibody (IgG_1) with high affinity for the 54.8-kDa NusA protein from *E. coli* (1, 2). This purified antibody specifically detects fusion proteins containing the Nus•Tag sequence expressed with the pET-43.1, pET-44, and pET-506 vector series. Expression of polypeptides fused with the 495-aa NusA (Nus•Tag) can markedly enhance the solubility of recombinant proteins expressed in *E. coli*.

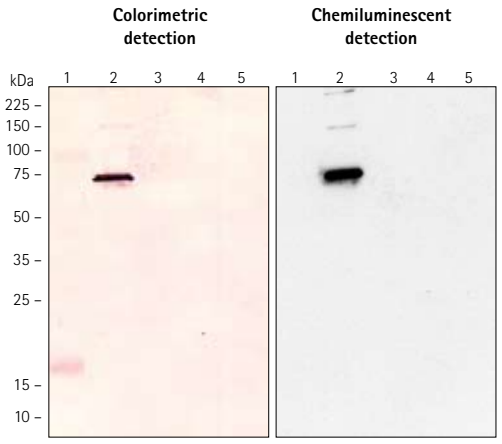
The 50-µg package size provides enough purified antibody to perform 50 Western blots (10 cm × 10 cm).

References

1. Harrison, R. G. (2000) *inNovations* **11**, 4–7.
2. Davis, G. D., Elisee, C., Newham, D. M., and Harrison, R. G. (1998) *Biotechnol. Bioeng.* **65**, 382–388.

Product	Size	Cat. No.	Price
Nus•Tag™ Monoclonal Antibody	50 µg	71127-3	
	250 µg	71127-4	

Specificity	495-aa NusA 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 ng (Western blot developed with chromogenic substrates) < 1 ng (AP or HRP conjugate developed with chemiluminescent substrates)
Applications	Western blot
Form	Stabilized solution (1 mg/ml) in 50% glycerol
Working dilution	1:10,000 for Western blotting



Lane	Sample
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 Nus•Tag fusion protein
Two parallel blots were incubated with Nus•Tag Monoclonal Antibody, and then incubated with Anti-Mouse IgG AP or HRP Conjugate and processed by colorimetric (left panel) or chemiluminescent (right panel) detection. Lanes are indicated.

Trx•Tag™ Monoclonal Antibody

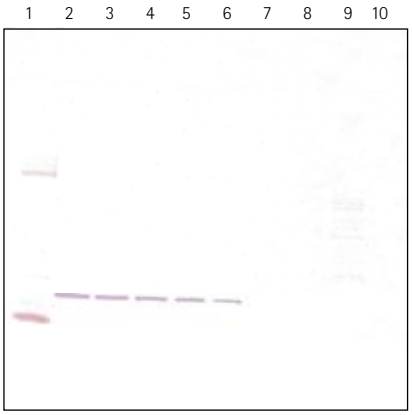
Specific detection of Trx•Tag™ fusion proteins

The Trx•Tag™ Monoclonal Antibody is a mouse monoclonal antibody (IgG_{2b}) directed against the 109-amino acid a Trx•Tag sequence encoded by the Novagen pET-48 expression vectors. This protein G purified antibody is suitable for immunoblot detection or immunoprecipitation of Trx•Tag fusion proteins.

The Trx•Tag Monoclonal Antibody detects as little as 5 ng of Trx•Tag fusion proteins with negligible cross-reactivity with bacterial, insect, or mammalian lysates.

Product	Size	Cat. No.	Price
Trx•Tag™ Monoclonal Antibody	50 µg	71542-3	

Specificity	109-amino acid TrxA protein; precise epitope not determined
Species/Isotype	Mouse monoclonal IgG_{2b}
Cross-reactivity	Negligible with bacterial, insect or mammalian cell lysates
Sensitivity	10 ng (immunoblot developed with chromogenic substrates) 5 ng (immunoblot developed with HRP conjugate and chemiluminescent substrates. Also suitable for immunoprecipitation.
Form	Liquid with 50% glycerol in 1X PBS.
Working dilution	1:5000 for immunoblotting



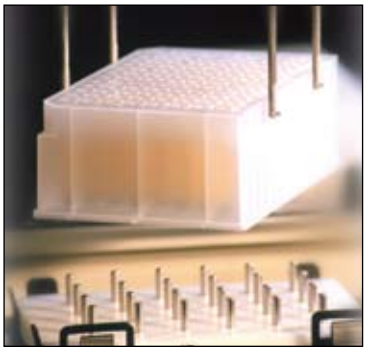
Lane	Sample
1	Trail Mix™ Western Markers (5 µl + 5 µl 2-mercaptoethanol solution)
2	IC-J purified protein, 25 ng
3	IC-J protein, 20 ng
4	IC-J protein, 15 ng
5	IC-J protein, 10 ng
6	IC-J protein, 5 ng
7	Origami™ negative control 1 µg
8	Insect negative control 15 µg
9	Mammalian negative control 20 µg
10	Blank

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™ (see page 22) and GST•Mag™ Purification Kits (see page 30).

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



Magnetight Separation Stand and Multitube Rack

Powerful magnetic separation for 1.5-ml, 15-ml, and 50-ml tube

The versatile Magnetight Separation Stand allows efficient magnetic separations using 1.5-ml, 15-ml, and 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.	Price
Magnetight™ Separation Stand	69964-3	
Magnetight™ Multitube Rack	70747-3	

Separation Stand

Multitube Rack

Vacuum Manifold

Convenient simultaneous processing of up to 12 samples

The Novagen 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 the Novagen His•Bind® Quick Cartridges (see page 24).

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 mercury. 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.	Price
Vacuum Manifold	70147-3	

Part 3
Protease and Cleavage Capture Kits

Site-specific Proteases and Cleavage Capture Kits

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. The Novagen preparation is tested for activity using the same assay as for unmodified thrombin, and for greater than 99% binding to Streptavidin Agarose.

Thrombin Cleavage Capture Kit

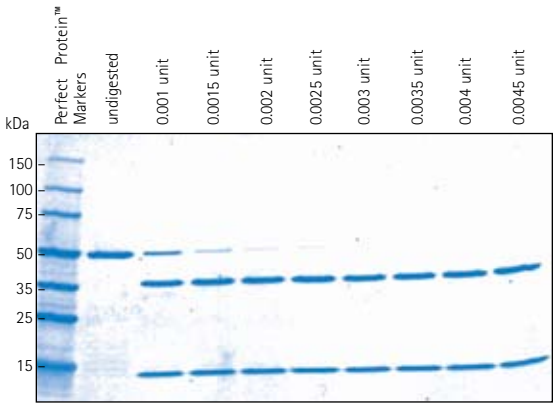
The Thrombin Cleavage Capture Kit is designed for cleavage of fusion proteins followed by convenient and quantitative removal of thrombin protease. The method is based on the use of Biotinylated Thrombin for digestion and its subsequent removal with Streptavidin Agarose. The kit is suitable for use with 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 two 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	Size	Cat. No.	Price
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	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



Biotinylated Thrombin cleavage
The indicated amounts of Biotinylated Thrombin were used to cleave 2 µg of Cleavage Control Protein in an overnight digestion. Samples were analyzed by SDS-PAGE (4–20% gradient gel) followed by staining with Coomassie blue. The 0.0045-unit lane represents a 2.25-fold 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. The Novagen 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 , 100 mM NaCl, and 5 mM $CaCl_2$, pH 8.0.

Product	Size	Cat. No.	Price
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

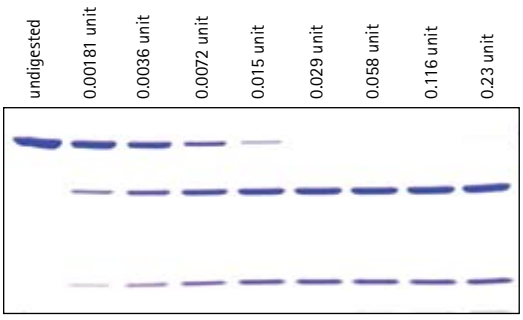
Factor Xa Cleavage Capture Kit

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.	Price
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



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-unit lane corresponds to 0.25 unit 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). The Novagen 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, 50 mM NaCl, and 2 mM $CaCl_2$, pH 7.4.

Product	Size	Cat. No.	Price
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

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.

Tag•off™ High Activity rEK

Increased cleavage rate of fusion proteins

Tag•off™ High Activity rEK is a highly purified preparation of the catalytic subunit of human enterokinase that recognizes the identical cleavage site as the native enzyme, AspAspAspAspLys↓(1). The Tag•off High Activity rEK substrate binding site (ArgArgArgLys) differs from the bovine enterokinase site (LysArgArgLys) by a single amino acid, which is believed to account for its higher substrate affinity and the resulting higher specific activity. The preparation has been functionally tested for activity with thioredoxin/human epidermal growth factor and thioredoxin/human IL-13 fusion proteins (1). The Tag•off High Activity rEK as a highly purified (99% by Coomassie staining) enzyme solution and includes rEK 1X Dilution/Storage Buffer, 10X Cleavage Buffer, and a Cleavage Control Protein.

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

Product	Size	Cat. No.	Price
Tag•off™ High Activity rEK	50 U	71537-3	

Components:

- 50 U Tag•off High Activity rEK
- 2 ml 1X rEK Dilution/Storage Buffer
- 1 ml 10X rEK Cleavage Buffer
- 10 µg Cleavage Control Protein

References

1. Gasparian, M.E., Ostapchenko, V. G., Schulga, A. A., Dolgikh, D. A., and Kirpichnikov, M. P. (2003) *Protein Expr. and Purif.* **31**, 133-139.

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.

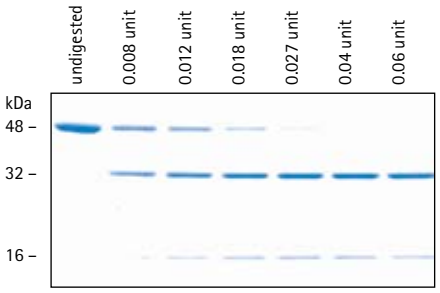
Following cleavage of the target protein, rEK is removed with > 99% efficiency from the reaction by affinity capture on EKapture™ Agarose. Following capture of rEK, the EKapture Agarose is removed by spin filtration. Because the same buffer conditions are used for both cleavage and capture, no buffer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



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-unit lane corresponds to 1 enzyme unit per 50 µg target protein, which exhibits > 95% cleavage.

Tag•off™ rEK Cleavage Capture Kit

Increased cleavage rate of fusion proteins

Tag•off™ rEK 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, greater than 99% of Tag•off rEK can be quickly removed from the reaction by affinity capture on EKapture agarose available in the Tag•off rEK Cleavage Capture Kit. Just add EKapture Agarose to the rEK digest, incubate at room temperature for 5 minutes and remove the resin by spin filtration. Because the same buffer conditions are used for both cleavage and capture, no buffer change is necessary. The Tag•off rEK Cleavage Capture Kit includes the EKapture Agarose and Spin Filters in addition to the same components available with the Tag•off High Activity rEK (enzyme, dilution buffer, cleavage buffer and control protein).

Product	Cat. No.	Price
Tag•off™ rEK Cleavage Capture Kit	71540-3	

Components:

- 50 U Tag•off High Activity rEK
- 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

HRV 3C Protease

Highly efficient fusion tag removal at low temperature

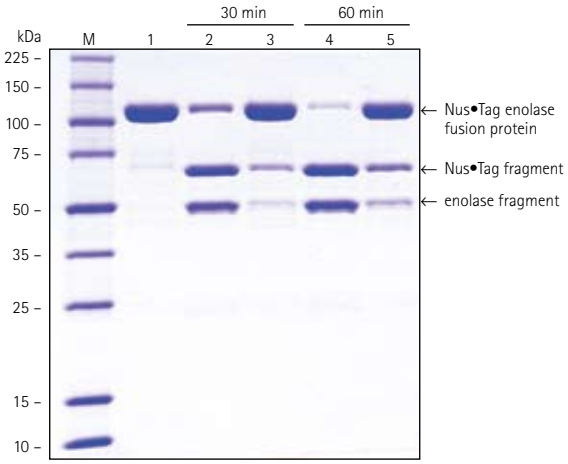
Recombinant type 14 3C protease from human rhinovirus (HRV 3C) is a highly purified recombinant 6XHis-fusion protein, which recognizes the same cleavage site as the native enzyme: LeuGluVal-LeuPheGln↓GlyPro. The small, 22-kDa size of the protease, optimal activity at 4°C, high specificity, and His•Tag® fusion make HRV 3C protease an ideal choice for rapid removal of fusion tags. The pET expression vectors pET-47b(+) through pET-50b(+) incorporate the HRV 3C protease cleavage site in combinations with His•Tag, S•Tag™, thioredoxin (Trx•Tag™), glutathione-S-transferase (GST•Tag™), and NusA (Nus•Tag™) sequences. The combination of pET-47b(+) to pET-50b(+) vectors for expression, HRV 3C protease for fusion tag cleavage, and Ni-NTA His•Bind® metal affinity chromatography for protein purification as well as protease and fusion tag removal allows production of recombinant proteins free of vector-encoded sequences. Our newest pET expression vector pET-52b(+), InsectDirect™ expression vector pIEx™-9, and multisystem expression vectors pIEx/Bac™-3,5, and pTriEx™-6 incorporate the HRV 3C protease cleavage site in combination with Strep•Tag® II and His•Tag sequences. Supplied as: 2000 U/ml in 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.5 mM THP, and 50% glycerol, pH 8.0.

Unit Activity: One unit will cleave >95% of 100 µg His•Tag fusion control protein in 50 mM Tris-HCl, 150 mM NaCl, pH 7.5 at 4°C for 16 h.

Product	Size	Cat. No.	Price
HRV 3C Protease	500 U	71493-3	

Components:

- 500 U HRV 3C Protease
- 10 µg HRV 3C Cleavage Control Protein
- 10 ml 10X HRV 3C Cleavage Buffer



Lane	Sample
M	Perfect Protein™ Markers, 10–225 kDa
1	3 µg purified Nus•Tag enolase fusion protein
2	3 µg of 30-min HRV 3C Protease reaction
3	3 µg of 30-min competitor's protease reaction
4	3 µg of 60-min HRV 3C Protease reaction
5	3 µg of 60-min competitor's protease reaction

Cleavage efficiency comparison of HRV 3C Protease with a competitor's protease
Using a 1:100 (w/w) ratio of protease:target protein, 500 µg of purified Nus•Tag enolase fusion protein was incubated in a 500-µl reaction volume at 4°C under the same cleavage reaction conditions as the experiment described in Figure 3. The reaction was quenched by adding equal volumes to 4X SDS Sample Buffer and then immediately placing the samples into a water bath at 75°C for 5 min.

Part 4
Accessory Products

iFOLD™ Protein Refolding Systems

96-well screens for optimal refolding conditions

Protein functional and structural studies often require a large amount of pure, correctly folded protein, which is commonly produced in *Escherichia coli* (*E. coli*) expression systems. However, production of foreign proteins in *E. coli* can result in the formation of inclusion bodies (IB) – dense, insoluble, aggregates of misfolded protein. IB also have useful attributes – they are easily purified, resistant to proteolysis, and can be solubilized with chaotropic agents. Defining conditions that promote refolding of a chemically denatured protein into its native conformation is empirical and often time consuming. Simultaneous and systematic evaluation of a large number of refolding conditions increases chances of identifying an optimal refolding condition for a given protein.

We have taken the tedium and guesswork out of finding the optimal refolding condition for your target protein! Our iFOLD™ Protein Refolding Systems provide comprehensive refolding screens in a 96-well plate format and are based on an extensive literature review of successful refolding experiments and information contained in the REFOLD database (http://refold. med.monash.edu.au). The systems differ in the chemistry used to denature the inclusion bodies and in the refolding additives included in the 96-well plate. In addition to a 96-well plate containing 92 (System 1) or 94 (System 2) unique refolding additive combinations, the systems include the reagents needed to purify inclusion bodies and solubilize the component proteins.

Protocol at-a-glance

- Express target protein
- Harvest cells
- Lyse cells by sonication plus Lysonase™ Bioprocessing Agent
- Centrifuge to pellet inclusion bodies
- Wash inclusion bodies with TRITON® X-100 (System 1) or NDSB-201 (System 2)
- Denature inclusion bodies with N-lauroylsarcosine (System 1) or guanidine-HCl or urea (System 2)
- Refold target protein by rapid dilution into iFOLD™ Protein Refolding System 1 or 2 buffer matrix
- Assay* for correctly folded and active target protein

**Assay is determined by end user.*

iFOLD™ Protein Refolding System 1

iFOLD™ Protein Refolding System 1 is designed to determine optimal protein refolding conditions by a systematic evaluation of 92 different buffers covering a range of pH and different salt, cyclodextrin, redox agent, and refolding additive concentrations. Following cell lysis, membrane components and contaminating proteins trapped within inclusion body pellets are removed by a series of detergent and buffer washes. The purified inclusion bodies are denatured by addition of TCEP [Tris(2-carboxyethyl)phosphine] and N-Lauroylsarcosine and refolded by rapid dilution into the iFOLD 96-well buffer matrix.

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

Components:

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

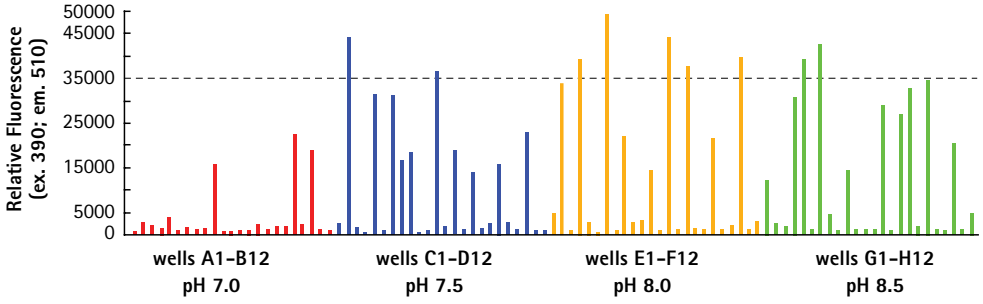
Features

- All reagents for inclusion body purification and pre-dispensed 96-well plate-based protein refolding matrix
- Uses N-lauroylsarcosine, a chaotropic anionic detergent, to denature the purified inclusion bodies
- 92 unique buffer and refolding additive combinations for simultaneous and systematic evaluation of protein refolding conditions
- pH range 7.0 – 8.5
- Refolding additives include salts, cyclodextrin, redox agents, chaotropes, glycols
- Refolding conditions based on extensive literature review and REFOLD database (http://refold.med.monash.edu.au)

Trx-GFP refolding and purification yields

Stage	Sample mass	RFU/mg protein
IB pellet	1.72 g	n/a
Denatured and dialyzed IB pellet	504 mg	25
After refolding	500 mg	250,000
After concentration and buffer exchange	269 mg	5,500,000

Large-scale refolding of trx-GFP using a refolding buffer identified in the iFOLD System 1 screen. Inclusion bodies were prepared according to part A. Approximately 500 mg of denatured inclusion bodies were rapidly diluted into 5.0 L of iFOLD System 1 refolding buffer E7 (50 mM Tris-Cl, pH 8.0, containing 250 mM NaCl, 12.5 mM methyl-β-D-cyclodextrin, and 1.0 mM Tris(2-carboxyethyl)phosphine. After an overnight incubation at 22±2°C, the sample was concentrated to 500 mL, precipitant removed by centrifugation, and dialyzed into a storage buffer containing 10% (v/v) glycerol and 0.03% BRIJ-35. Approximately 270 mg of trx-GFP with a relative fluorescence intensity (390 nm ex, 510 nm em) of 5,500,000 units per mg of protein was recovered.



Refolding screen for trx-GFP

iFOLD Protein Refolding System 1 refolding screen for α thioredoxin-green fluorescent protein fusion (trx-GFP). The trx-GFP fusion protein was expressed as inclusion bodies using Overnight Express™ System 1 media (71300). Inclusion bodies were harvested, washed, and denatured according the iFOLD System 1 technical bulletin. Denatured inclusion bodies were added to all wells of the iFOLD System 1 refolding plate to a final protein concentration of 0.1 mg/mL. The iFOLD System 1 plate was incubated at room temperature (22±2°C) with shaking for approximately 24 h. The degree of refolding for each of the 96 refolding reactions was quantified by measuring the relative fluorescent intensity (390nm ex, 510nm em) of a 1:4 dilution of the refolding reaction.

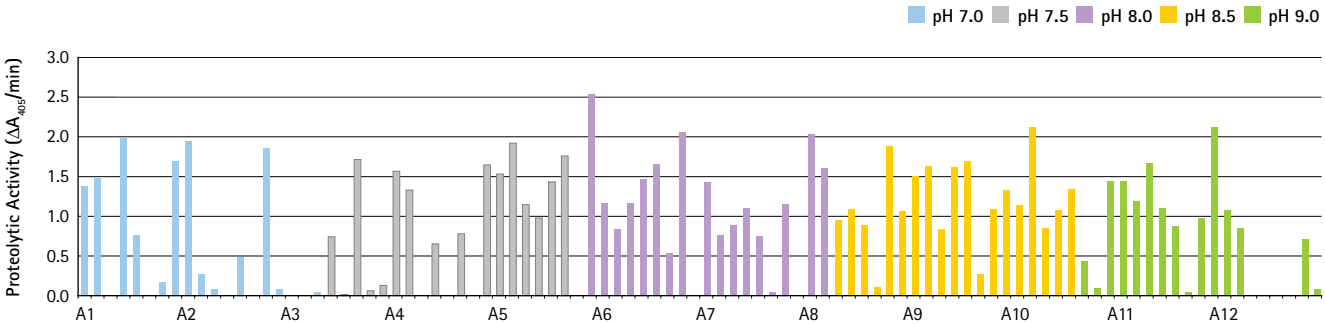
NEW iFOLD™ Protein Refolding System 2

iFOLD™ Protein Refolding System 2 is a detergent-free kit that provides a systematic evaluation of 94 conditions representing different buffering systems, pH values, and combinations of salt, redox agents, and refolding additives. Following cell lysis, membrane components and contaminating proteins trapped within inclusion body pellets are removed by a series of washes with a non-detergent sulfobetaine (NDSB). The purified inclusion bodies are reduced and denatured by addition of Tris(2-carboxyethyl) phosphine (TCEP) and guanidine hydrochloride and refolded by rapid dilution into the iFOLD System 2 detergent-free 96-well buffer matrix.

Product	Cat. No.	Price
iFOLD™ Protein Refolding System 2	71719-3	
Components: • 30 mL 10X IB•Prep™ Buffer • 100 µL Lysonase™ BioProcessing Reagent • 0.5 mL 1.0 M TCEP • 10 mL 1.5 M NDSB-201 • 10 mL iFOLD System 2 Denaturation Buffer (50 mM Tris-HCl, 0.2 M NaCl, 2.0 mM EDTA, 7.0 M GuHCl, pH 8.0) • 1 iFOLD Protein Refolding Plate 2 • 2 Aluminum plate sealers		

Features

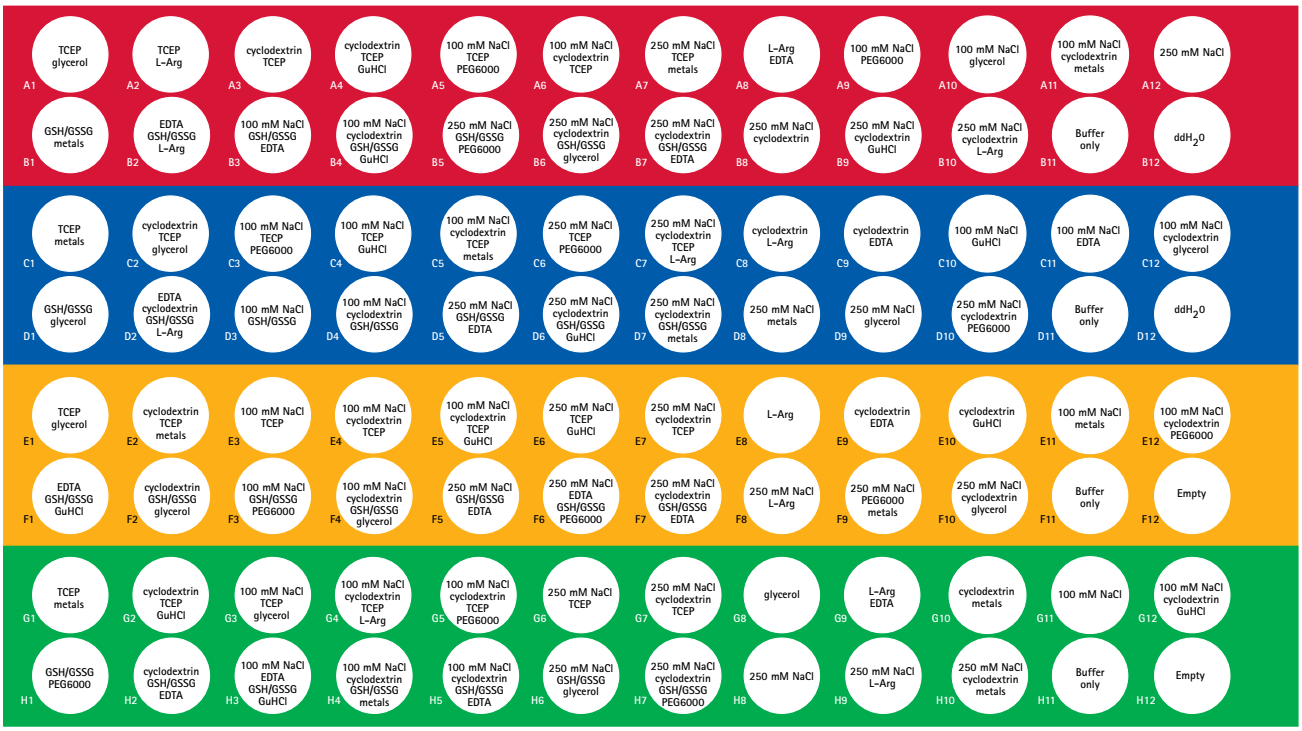
- Entirely detergent-free System 2 contains inclusion body purification reagents and pre-dispensed 96-well plate-based protein refolding matrix
- Uses guanidine hydrochloride or urea (not included) to denature the purified inclusion bodies
- 95 unique buffer and refolding additive combinations for simultaneous and systematic evaluation of protein refolding conditions
- pH range 7.0 – 9.0
- Refolding additives include salts, redox agents, cyclodextrin, chaotropes, glycols, nondetergent sulfobetains (NDSBs)
- Refolding conditions based on extensive literature review and REFOLD database (http://refold.med.monash.edu.au)
- High-throughput compatible



Refolded HRV 3C protease activity from IB washed with NDSB-201
HRV 3C IB were washed with NDSB-201, using the iFOLD System 2 protocol. Washed inclusion bodies were denatured with 7.0 M GuHCl and diluted into the iFOLD Protein Refolding Plate 2. After refolding for 20 h at 22°C, reactions were dialyzed in D-Tube96™ Dialyzers against 2 x 4.0 L buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5.0 mM DTT, and 0.03% BRIJ®-35 Detergent overnight at 10°C. Enzymatic activity of the refolded and dialyzed proteins was quantified by measuring cleavage of a peptide substrate (Glu-Ala-Leu-Phe-Gln-pNa: Bachem L-2050).

iFOLD™ Systems Plate Layouts

System 1



System 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	MOPS cyclodextrin EDTA GSH + GSSG (1) NaCl + KCl (1)	MOPS L-Arg EDTA GSH + GSSG (2) NaCl + KCl (2)	MOPS NDSB 256 (1) metals TCEP NaCl + KCl (2)	HEPES trehalose EDTA GSH + GSSG (1) NaCl + KCl (1)	HEPES GSH + GSSG (2) NaCl + KCl (2)	EPPS NDSB 256 (1) GSH + GSSG (1)	EPPS NDSB 201 (1) EDTA GSH + GSSG (2)	EPPS cyclodextrin TCEP NaCl + KCl (2)	TAPS GSH + GSSG (1)	TAPS sorbitol GSH + GSSG (2) NaCl + KCl (2)	CHES sorbitol GSH + GSSG (1) NaCl + KCl (1)	CHES sorbitol EDTA GSH + GSSG (2) NaCl + KCl (1)
B	MOPS L-Arg GSH + GSSG (1) NaCl + KCl (1)	MOPS NDSB 256 (1) EDTA GSH + GSSG (2) NaCl + KCl (1)	MOPS NDSB 201(2) metals TCEP	HEPES NDSB 201 (1) GSH + GSSG (1) NaCl + KCl (2)	HEPES EDTA GSH + GSSG (2)	EPPS sorbitol EDTA GSH + GSSG (1)	EPPS NDSB 256 (2) GSH + GSSG (2) NaCl + KCl (1)	EPPS metals TCEP NaCl + KCl (1)	TAPS NDSB 201 (2) EDTA GSH + GSSG (1)	TAPS PEG 3350 EDTA GSH + GSSG (2) NaCl + KCl (2)	CHES NDSB 256 (2) EDTA GSH + GSSG (1) NaCl + KCl (2)	CHES L-Arg EDTA GSH + GSSG (2) NaCl + KCl (2)
C	MOPS PEG 3350 EDTA GSH + GSSG (1)	MOPS cyclodextrin EDTA GSH + GSSG (2) NaCl + KCl (2)	MOPS NDSB 256 (2) metals TCEP NaCl + KCl (1)	HEPES NDSB 256 (2) EDTA GSH + GSSG (1) NaCl + KCl (2)	HEPES NDSB 256 (1) EDTA GSH + GSSG (2)	EPPS cyclodextrin GSH + GSSG (1) NaCl + KCl (2)	EPPS trehalose EDTA GSH + GSSG (2)	TAPS L-Arg metals TCEP NaCl + KCl (1)	TAPS NDSB 201 (2) GSH + GSSG (1) NaCl + KCl (2)	TAPS NDSB 256 (2) EDTA GSH + GSSG (2) NaCl + KCl (1)	CHES NDSB 201 (1) EDTA GSH + GSSG (1) NaCl + KCl (2)	CHES cyclodextrin GSH + GSSG (2) NaCl + KCl (1)
D	MOPS EDTA GSH + GSSG (1) NaCl + KCl (1)	MOPS NDSB 201 (1) GSH + GSSG (2)	HEPES sorbitol TCEP NaCl + KCl (1)	HEPES NDSB 256 (2) GSH + GSSG (1)	HEPES PEG 3350 GSH + GSSG (2)	EPPS NDSB 256 (1) EDTA GSH + GSSG (1)	EPPS NDSB 201 (2) EDTA GSH + GSSG (2) NaCl + KCl (2)	TAPS trehalose TCEP	TAPS L-Arg EDTA GSH + GSSG (1) NaCl + KCl (2)	TAPS NDSB 256 (1) GSH + GSSG (2) NaCl + KCl (2)	CHES NDSB 256 (1) GSH + GSSG (1) NaCl + KCl (1)	CHES cyclodextrin GSH + GSSG (2) NaCl + KCl (1)
E	MOPS trehalose GSH + GSSG (1)	MOPS NDSB 201 (2) GSH + GSSG (2)	HEPES NDSB 201 (1) metals TCEP NaCl + KCl (1)	HEPES L-Arg GSH + GSSG (1) NaCl + KCl (1)	HEPES L-Arg	EPPS PEG 3350 GSH + GSSG (1) NaCl + KCl (1)	EPPS trehalose GSH + GSSG (2) NaCl + KCl (1)	TAPS sorbitol TCEP NaCl + KCl (1)	TAPS cyclodextrin metals NaCl + KCl (2)	TAPS NDSB 201 (1) GSH + GSSG (2)	CHES NDSB 256 (1)	CHES PEG 3350 GSH + GSSG (2) NaCl + KCl (1)
F	MOPS sorbitol metals	MOPS NDSB 256 (2) metals NaCl + KCl (2)	HEPES cyclodextrin TCEP	HEPES NDSB 201 (2) NaCl + KCl (1)	HEPES	EPPS NDSB 256 (2)	EPPS sorbitol metals TCEP NaCl + KCl (2)	TAPS cyclodextrin metals TCEP	TAPS trehalose metals NaCl + KCl (1)	CHES sorbitol NaCl + KCl (1)	CHES NDSB 201 (1) metals TCEP NaCl + KCl (1)	CHES NDSB 256 (2) TCEP NaCl + KCl (1)
G	MOPS trehalose NaCl + KCl (2)	MOPS NaCl + KCl (2)	HEPES trehalose metals TCEP NaCl + KCl (2)	HEPES NDSB 201(2) metals NaCl + KCl (2)	EPPS NDSB 201 (2) metals NaCl + KCl (1)	EPPS PEG 3350 NaCl + KCl (2)	EPPS L-Arg TCEP NaCl + KCl (2)	TAPS PEG 3350 TCEP	TAPS NDSB 256 (1) NaCl + KCl (1)	CHES trehalose metals NaCl + KCl (2)	CHES NDSB 201 (2) TCEP NaCl + KCl (1)	CHES L-Arg metals TCEP
H	MOPS	MOPS NDSB 201 (1) metals NaCl + KCl (1)	HEPES PEG 3350 metals NaCl + KCl (2)	HEPES cyclodextrin	EPPS	EPPS metals NaCl + KCl (2)	ddH2O	TAPS NDSB 201 (1) NaCl + KCl (2)	TAPS	CHES	CHES TCEP NaCl + KCl (1)	CHES PEG 3350 metals TCEP

NDSB Refolding Agents

NDSB-195

NDSB-195 (Dimethylethylammonium Propane Sulfonate) has been useful as a mild solubilizing and stabilizing agent. Reduces aggregation and significantly improves protein renaturation. It is zwitterionic over a wide pH range and does not absorb significantly in the near-UV range. Purity is ≥ 95% by TLC. Supplied as a white solid. M.W. 195.3.

NDSB-201

NDSB-201 [3-(1-Pyridino)-1-propane Sulfonate] is useful for solubilizing membrane and nuclear proteins during purification. It does not interfere with colorimetric assays using *p*-nitrophenylphosphate and *o*-nitrophenyl-β-D-galactopyranoside as substrates. It reduces aggregation and significantly improves protein renaturation. It is zwitterionic over a wide pH range. Purity is ≥ 97% by elemental analysis (nitrogen). Supplied as a white solid. CAS 15471-17-7, M.W. 201.2.

NDSB-211

NDSB-211 (Dimethylhydroxyethylammonium Propane Sulfonate) is useful in improving the efficiency of solubilization of membrane proteins, in isolating nuclear proteins, in chromatographic purification of halophilic proteins in low ionic strength buffers, and in protein crystallization. Purity is ≥ 95% by TLC. Supplied as a white solid. M.W. 211.3.

NDSB-221

NDSB-221 [3-(1-Methylpiperidinium)-1-propane Sulfonate] is zwitterionic over a wide pH range. NDSB-221 at 1.8 M yields about 35% native hen egg white lysozyme, which is about 10-fold higher than in the absence of NDSB-221. At about 1.5 M, NDSB-221 efficiently increases the renaturation of β-galactosidase. Purity is ≥ 95% by TLC. Supplied as a white solid. M.W. 221.3.

NDSB-256

NDSB-256 (Dimethylbenzylammonium Propane Sulfonate) has been useful as a solubilizing and stabilizing agent for chicken egg white lysozyme and *E. coli* β-galactosidase. At 1 M, NDSB-256 will restore 30% of the enzymatic activity of denatured egg white lysozyme. At 800 mM it will restore 16% of the enzymatic activity of denatured β-galactosidase. Purity is ≥ 99% by TLC. Supplied as a white solid. M.W. 257.4.

NDSB-Set

The NDSB Set contains the following non-detergent sulfobetaines: 5 g NDSB-195, 25 g NDSB-201, and 5 g NDSB-256.

Product	Size	Cat. No.	Price
NDSB-195	5 g 25 g	480001	
NDSB-201	25 g 250 g	480005	
NDSB-211	1 g 5 g	480013	
NDSB-221	5 g 25 g	480014	
NDSB-256	5 g 25 g	480010	
NDSB Set	1 set	480012	

D-Tube™ Dialyzers

Dialysis and electroelution from polyacrylamide or agarose gels

The D-Tube™ Dialyzers can be used for dialysis and electroelution of proteins, RNA, DNA, and oligonucleotides from polyacrylamide or agarose gels. The disposable, single-use tubes require no syringes, microcentrifuge, or laborious steps to manipulate small sample volumes. The sample is added and removed using a standard laboratory pipette. Available with molecular weight cutoffs (MWCO) from 3.5 to 14 kDa, the D-Tube Dialyzers are designed in three volume capacities: mini (10–250 µl), midi (50–800 µl), and maxi (500–3000 µl). The membrane is ultra-clean, EDTA-treated regenerated cellulose, sulfur- and heavy metal-free. Each kit contains 10 D-Tube Dialyzers and one floating rack that can hold up to four D-Tube Dialyzers in the exchange buffer.

Features:

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

Product	Size	Cat. No.	Price
D-Tube™ Dialyzer Mini, MWCO 6–8 kDa	1 kit	71504-3	
D-Tube™ Dialyzer Mini, MWCO 12–14 kDa	1 kit	71505-3	
D-Tube™ Dialyzer Midi, MWCO 3.5 kDa	1 kit	71506-3	
D-Tube™ Dialyzer Midi, MWCO 6–8 kDa	1 kit	71507-3	
D-Tube™ Dialyzer Maxi, MWCO 3.5 KDa	1 kit	71508-3	
D-Tube™ Dialyzer Maxi, MWCO 6–8 KDa	1 kit	71509-3	
D-Tube™ Dialyzer Maxi, MWCO 12–14 KDa	1 kit	71510-3	
Components: • 10 D-Tubes • 1 Floating Rack			
Floating Rack, Mini	10 racks	71512-3	
Floating Rack, Mini	10 racks	71513-3	
Floating Rack, Mini	10 racks	71514-3	

D-Tube Dialyzer Size	Volume	MW Cutoff
Mini	10 to 250 µl	6-8 kDa
	10 to 250 µl	12-14 kDa
Midi	50 to 800 µl	3.5 kDa
	50 to 800 µl	6-8 kDa
Maxi	500 to 3000 µl	3.5 kDa
	500 to 3000 µl	6-8 kDa
	500 to 3000 µl	12-14 KDa

D-Tube™ Electroelution Accessory Kit

Optimized reagents for protein and nucleic acid precipitation following electroelution

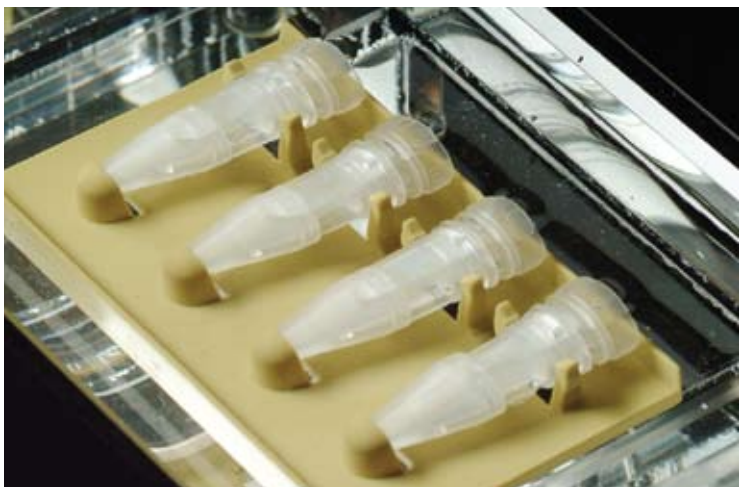
The combination of D-Tube™ Dialyzers and the D-Tube Electroelution Accessory Kit provides a unique tool for extraction of any protein, protein-protein complex, or protein-DNA complex from non-denaturing and denaturing (SDS) polyacrylamide gels with 60% recovery yield in less than 2 hours. Extracted proteins are compatible with most downstream applications such as MALDI-MS, animal immunization for antibody production, HPLC, peptide mapping, and functional assays. In addition, D-Tube Dialyzers can be used for oligonucleotides, RNA, and DNA extraction from both polyacrylamide and agarose gels. Efficient extraction (> 90%) is achieved for 15-nt oligos and for DNA fragments of up to 80 kbp. The D-Tube Electroelution Accessory Kit provides one D-Tube support tray which is compatible with most commercially available horizontal electrophoresis units and optimized reagents for protein and nucleic acid precipitation following electroelution.

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

Components:
- 1 ml MS Precipitation Buffer
- 10 ml TCA, 20%
- 2 × 1 ml 3 M NaAc, pH 5.2
- 3 Supporting Trays, Mini, Midi, Maxi

Features:

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



NEW D-Tube™ Dialyzers Mega

Dialysis of larger volume samples

The D-Tube™ Dialyzers Mega are easy-to-handle dialyzers in a screw capped tube format with dialysis membrane windows for buffer exchange and removal of solutes. The disposable, single-use tubes require no syringes or laborious steps to manipulate samples. The sample is added and removed using standard laboratory pipet. Available in different plastic colors with molecular weight cut-offs 3.5 KDa (Blue) and 6-8 KDa (Pink), the D-Tube Dialyzers Mega are designed in three volume capacities: 10 ml, 15 ml, and 20 ml. The membrane is ultra-clean, EDTA-treated regenerated cellulose, sulfur- and heavy metal-free. Each kit contains 10 or 50 tubes and one or 5 floating racks to hold one D-Tube Mega in the exchange buffer.

Product	MWCO	Cat. No.	Size	Price
D-Tube™ Dialyzers Mega, 10 ml	3.5 kDa	71739-3	10 D-Tubes	
		71739-4	50 D-Tubes	
	6-8 kDa	71740-3	10 D-Tubes	
		71740-4	50 D-Tubes	
D-Tube™ Dialyzers Mega, 15 ml	3.5 kDa	71742-3	10 D-Tubes	
		71742-4	50 D-Tubes	
	6-8 kDa	71743-3	10 D-Tubes	
		71743-4	50 D-Tubes	
D-Tube™ Dialyzers Mega, 20 ml	3.5 kDa	71745-3	10 D-Tubes	
		71745-4	50 D-Tubes	
	6-8 kDa	71746-3	10 D-Tubes	
		71746-4	50 D-Tubes	
Floating Rack, Mega		71748-3	10 racks	

Features

- Efficient dialysis of larger volume samples – 10, 15 and 20 mL
- Large dual membrane surface with MWCO of 3-5 or 6-8 kDa
- Convenient sample addition – no syringes necessary
- Ideally suited for buffer exchange of protein samples into a physiologically relevant buffer prior to refolding optimization screening with the iFOLD™ Protein Refolding System 1

NEW D-Tube96™ Dialyzers

High throughput dialysis

D-Tube96™ Dialyzers allow convenient, high throughput dialysis of 96 samples simultaneously. The device features the advantages of the D-Tube™ Dialyzer Mini in a 96-tube format. D-Tube Dialyzers are easy to handle tubes with dual membranes providing a large surface area for fast, efficient dialysis. The membrane is ultra clean, EDTA-treated, regenerated cellulose that is sulfur- and heavy metal-free. After screening for optimal refolding conditions with the iFOLD™ Protein Refolding Systems, D-Tube96 Dialyzers provide convenient buffer exchange for the 96 protein samples into a physiologically relevant buffer.

Product	Size	Cat. No.	Price
D-Tube96™ Dialyzer, MWCO 6–8 kDa	1 kit	71712-3	
D-Tube96™ Dialyzer, MWCO 12–14 kDa	1 kit	71713-3	

Components:
- 1 D-Tube96 Dialyzer, 6-8 kDA or 12-14 kDa
- 1 Aluminum Plate Sealer
- 96 D-Tube Dialyzer caps

Features

- 96-tube format for high throughput applications
- Sample addition by multi-channel pipet, or robotic dispensing
- Kit includes floatable rack, 96 D-Tube Dializers and 96 caps
- Unused tubes can be removed for future use

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 IMAC resins. For these applications we recommend Protease Inhibitor Cocktail Set III.

Protease Inhibitor Cocktail Set I, with Animal–Free Aprotinin

The animal-free formulation includes the same five protease inhibitors as the original version but contains a different grade of Aprotinin: a recombinant bovine Aprotinin instead of Aprotinin preparation obtained from bovine lung.

Protease Inhibitor Cocktail Set II (with EDTA)

This lyophilized cocktail includes five protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, serine, and metalloproteases, as well as aminopeptidases. It is recommended for use with bacterial cell extracts (except those being used for metal chelation chromatography). Reconstitute each vial with 1 ml DMSO and 4 ml water to obtain 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. 5 ml is recommended for the inhibition of proteases extracted from 20 g *E. coli*.

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*.

Protease Inhibitor Cocktail Set III, with Animal–Free Aprotinin

The animal-free formulation includes the same six protease inhibitors as the original version but contains a different grade of Aprotinin: a recombinant bovine Aprotinin instead of Aprotinin preparation isolated from bovine lung.

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.

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 H2O 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.

Protease Inhibitor Cocktail Set V, with Animal–Free Aprotinin

The animal-free formulation includes the same four protease inhibitors as the original version but contains a different grade of Aprotinin: a recombinant bovine Aprotinin instead of Aprotinin preparation isolated from bovine lung.

Protease Inhibitor Cocktail Set VII

This cocktail is recommended for purification of proteins containing His•Tag® sequences. Cocktail contains five protease inhibitors (in 1 ml DMSO) with broad specificity for the inhibition of cysteine, serine, aspartic, and thermolysin-like proteases and aminopeptidases. Each vial contains the following amount of inhibitors: 100 mM AEBSF, 5 mM Bestatin, 1.5 mM E-64, 2mM Pepstatin A, and 200 µM Phosphoramidone. One ml is recommended for the inhibition of proteases in 10 g cells.

Protease Inhibitors continued

Protease Inhibitors

Protease	MW	Target Protease Class and Mechanism of Action	Solubility	Suggested Concentration
AEBSF, Hydrochloride	239.5	Water-soluble, non-toxic alternative to PMSF. Irreversible inhibitor of serine proteases. Reacts covalently with a component of the active site. Inhibits chymotrypsin, kallikrein, plasmin, trypsin, and related thrombolytic enzymes.	H_2O	0.1–1 mM
Aprotinin	6512	A competitive and reversible inhibitor of proteolytic and esterolytic activity. A serine protease inhibitor. In cell culture, it extends the life of cells.	H_2O	0.6–2.0 µg/ml
Aprotinin, Recombinant	6512	A competitive and reversible inhibitor of proteolytic and esterolytic activity. A serine protease inhibitor. In cell cultures, extends the life of cells and prevents proteolytic damages of intact cells.	H_2O	0.6–2.0 µg/ml
Benzamidine, Hydrochloride	156.6	Inhibitor of trypsin and trypsin-like enzymes.	H_2O, ethanol	0.5–4.0 mM
Bestatin	308.4	Binds to cell surfaces and reversibly inhibits aminopeptidase B and leucine aminopeptidase.	DMSO, methanol	1–10 µM
E-64	357.4	An irreversible cysteine protease inhibitor that has no effect on cysteine residues in other proteins. Specific active site titrant.	H_2O, DMSO	1–10 µM
EDTA, Disodium	380.2	A reversible metalloprotease inhibitor. A chelator that may interfere with other metal ion–dependent processes.	H_2O	1–10 mM
Leupeptin	493.6	A reversible inhibitor of trypsin-like proteases and cysteine proteases.	H_2O	10–100 µM
Pepstatin A	685.9	An inhibitor of aspartic proteases. Reversibly inhibits cathepsin D, cathepsin G, pepsin, and renin.	DMSO, methanol	1 µM

AEBSF, Hydrochloride

AEBSF, Hydrochloride [4-(2-Aminoethyl)benzenesulfonylfluoride, HCl] is a specific irreversible inhibitor of serine proteases. This compound inhibits chymotrypsin, kallikrein, plasmin, thrombin, trypsin, and related thrombolytic enzymes and is a stable, nontoxic alternative to PMSF and DFP. Use at the same molar concentrations (0.1–1.0 mM) as PMSF for most applications. Off-white solid. Hygroscopic. Purity: > 97% by HPLC. Solubility: 100 mM in water. Stability: only slight hydrolysis at pH 8–9. CAS 30827-99-7, MW 239.5.

Benzamidine, Hydrochloride

Benzamidine, Hydrochloride is an inhibitor of trypsin and trypsin-like enzymes. It inhibits Factor VII autoactivation. White solid. Purity: ≥ 97% by titration, Soluble in water. RTECS CV6260000, CAS 1670-14-0, MW 156.6.

Product	Size	Cat. No.	Price
Protease Inhibitor Cocktail Set I (with EDTA)	1 vial 10 vials	539131	
Protease Inhibitor Cocktail Set I, (with Animal-Free Aprotinin)	1 ml 10 × 1 ml	535142	
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 III (with Animal-Free Aprotinin)	1 ml 5 × 1 ml	535140	
Protease Inhibitor Cocktail Set IV (without EDTA)	1 ml 1 set	539136	
Protease Inhibitor Cocktail Set V, EDTA-Free	1 ml 10 vials	539137	
Protease Inhibitor Cocktail Set V, (with Animal-Free Aprotinin)	1 ml 10 × 1 ml	535141	
Protease Inhibitor Cocktail Set VII	1 ml 1 set	539138	
AEBSF, Hydrochloride	50 mg 100 mg 500 mg 1 g	101500	
Benzamidine, Hydrochloride	5 g 25 g	199001	

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.	Price
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: 1:5000–1:10,000 (up to 1:50,000 for ELISA) Goat Anti-Mouse IgG HRP Conjugate: 1:5000–1:10,000 (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. The Novagen 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.	Price
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	

Trail Mix™ Western Markers and Blot Kits

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

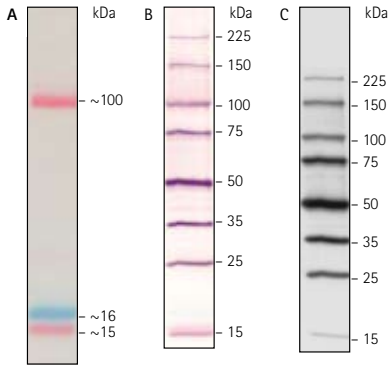
Trail Mix™ Western Markers consist of the Perfect Protein™ Western Markers supplemented with a group of three prestained indicator proteins to allow direct visualization of protein mobility during electrophoresis. Trail Mix Western Markers and Western Blot Kits have all of the features of Perfect Protein 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.

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

Components for AP and HRP Kits:
- 25 lanes Trail Mix Western Markers
- 50 µl S-protein AP *or* HRP Conjugate

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.



A Unstained gel and Western transfer

B AP Western blot (S-protein AP conjugate) colorimetric detection

C AP Western blot (S-protein AP conjugate) chemiluminescentdetection

Perfect Protein™ Western Markers and Blot Kits

Accurate size markers detectable on any Western blot

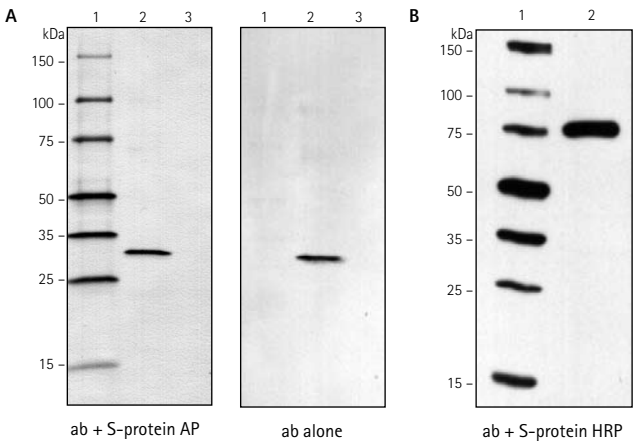
The Perfect Protein™ Western Markers consist of seven recombinant His•Tag®/S•Tag™ fusion proteins that can be detected on any Western blot using the His•Tag Monoclonal Antibody with AP conjugated secondary antibody or S-protein AP or HRP Conjugates. These markers serve as precise size standards that 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.

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

Components for AP and HRP Kits:
- 25 lanes Perfect Protein Western Markers
- 50 µl S-protein AP *or* HRP Conjugate

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



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.

Perfect Protein™ Markers

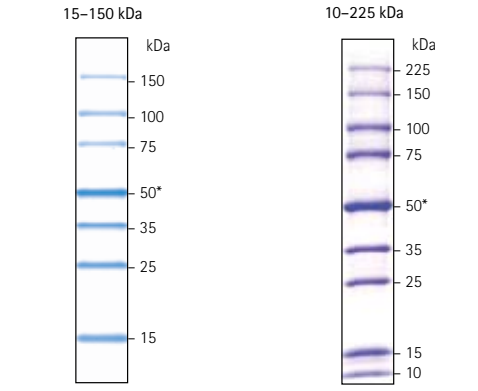
Precisely sized, conveniently spaced for accurate protein size determination

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 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.). Each protein marker carries the His•Tag® sequence which can be visualized by 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. When 5 µl of Perfect Protein Markers are run on a gel this corresponds to 1 mg of the 50 kDa band and 0.5 mg of all other bands).

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; when 5 ul of Perfect Protein Markers are run on a gel this corresponds to 1 µg of the 50 kDa band and 0.5 µg of all other bands). The markers are supplied with 4X Sample Buffer. The 4X SDS Sample Buffer is a standard formulation commonly used for SDS-PAGE analysis of proteins. The solution includes DTT for complete denaturation of disulfide bonds. The buffer can be used at a final concentration of 2X for most applications.

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



Coomassie blue
4–20% SDS-polyacrylamide gel

Coomassie blue
4–20% SDS-polyacrylamide gel

*High-intensity reference band

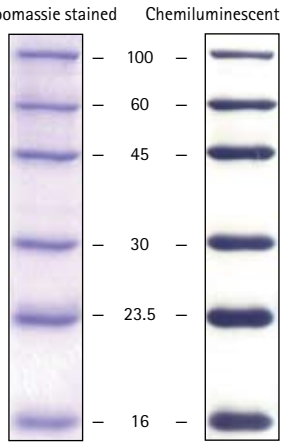
Strep•Tag® II Perfect Protein Markers

Accurate and convenient molecular weight determination via SDS-PAGE and immunoblotting

The Strep•Tag® II Perfect Protein Markers* are a mixture of Strep•Tag II fusion proteins ranging from 16 to 100 kDa. The markers provide precise size references for proteins on SDS-polyacrylamide gels after staining with Coomassie blue. Because each of the proteins contains the Strep•Tag II sequence, the markers can also be detected by the Strep•Tag II Monoclonal Antibody in Western blots. The Strep•Tag II Perfect Protein Markers include protein sizes of 16, 23.5, 30, 45, 60, and 100 kDa that resolve into sharp, evenly stained bands. The markers are provided lyophilized and are supplied with a Strep•Tag II Marker Reconstitution Buffer, which also serves as the loading buffer.

* manufactured by IBA GmbH

Product	Size	Cat. No.	Price
Strep•Tag® Perfect Protein Markers, 16-100 kDa	100 lanes	71614-3	



The Strep•Tag II Perfect Protein Markers (5 µl) were run, in duplicate, on an SDS-PAGE. One lane of the gel was stained by Coomassie blue while other lane was further analyzed by Western blot. The blot was incubated with the Strep•Tag II Monoclonal Anitbody, followed by a Goat Anti-mouse IgG HRP Conjugate. The markers were detected by chemiluninescence.

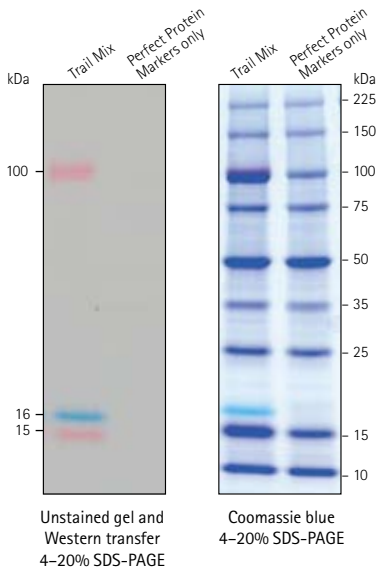
Trail Mix™ Protein Markers

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

Trail Mix™ Protein Markers are a mixture of the Novagen 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 markers are shipped with 4X Sample Buffer. 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.	Price
Trail Mix™ Protein Markers, 10-225 kDa	100 lanes	70980-3	



customer.service@merckbio.com
technical.service@merckbio.com
Visit our website www.merckbio.com

BCA Protein Assay Kit

Simple and reliable protein quantification

The BCA protein assay is based on a biuret reaction, which is the reduction of Cu^{+2} to Cu^{+1} by proteins in an alkaline solution with concentration-dependent detection of the monovalent copper ions. Bicinchoninic acid is a chromogenic reagent that chelates the reduced copper, producing a purple complex with strong absorbance at 562 nm (1, 2). This assay can be used to quantify protein concentration with a wide variety of samples and can be performed in minutes.

The Novagen BCA Protein Assay Kit can be used to determine protein concentration in the range of 20–2000 µg/ml in either a standard assay or microassay configuration. Kit components are sufficient to complete 500 standard-size reactions (50 µl protein sample plus 1 ml reagent) or 2500 micro-scale reactions (25 µl protein sample plus 200 µl reagent) (3). A BSA standard (3 × 1 ml at 2 mg/ml) is provided for convenient preparation of standard curves.

This assay is robust and can be performed in the presence of many chemical compounds. Some reagents, including chelating agents, strong acids or bases, and reducing agents, interfere with the reduction and chelating reactions on which this assay depends (4). The BCA assay is compatible with the following Novagen protein extraction and lysis reagents: BugBuster® Protein Extraction Reagent, PopCulture® Reagent, CytoBuster™ Protein Extraction Reagent, Reportasol™ Extraction Buffer, and Insect PopCulture Reagent. Options for the removal or dilution of interfering substances are described in the kit literature.

Product	Size	Cat. No.	Price
BCA Protein Assay Kit (2500 microplate assays)	500 assays	71285-3	

Components:
- 500 ml BCA Solution
- 15 ml 4% Cupric Sulfate
- 3 × 1 ml BSA Standard, 2 mg/ml

References

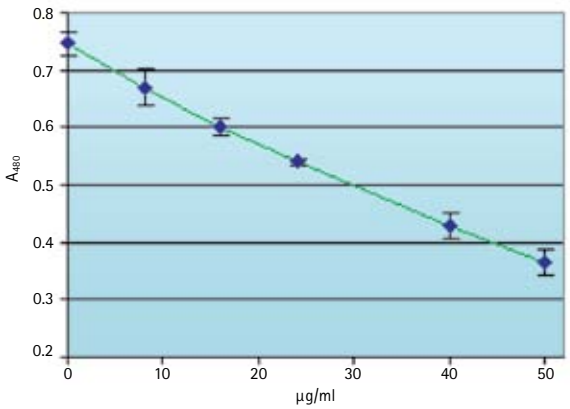
1. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and, Klenk, D. C. (1985) *Anal. Biochem.* **150**, 76–85.
2. Wiechelman, K., Braun, R., and Fitzpatrick, J. (1988) *Anal. Biochem.* **175**, 231–237.
3. Hinson, D. L. and Webber, R. J. (1988) *BioTechniques* **12**, 496–499.
4. Brown, R., Jarvis, K. , Hyland, K. (1989) *Anal. Biochem.* **180**, 136–139.

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.

Product	Size	Cat. No.	Price
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.

Technical Tips for Improving Protein Purification

1. What are some general guidelines to improve yields and purification of recombinant proteins from bacteria?

Careful consideration of expression vector and host strain features, and optimizing induction conditions can increase target protein expression and decrease background protein levels (Fig 1). After cells have been broken open, working quickly helps maintain the integrity of the target protein. BugBuster® Master Mix (Cat. No. 71456) is designed for rapid and complete bacterial cell lysis and nucleic acid digestion. Affinity chromatography, such as immobilized metal affinity chromatography (IMAC), is optimal when the amount of fusion protein being applied to the resin approximates the binding capacity of the resin. Using the appropriate amount of resin reduces nonspecific binding of contaminant proteins.

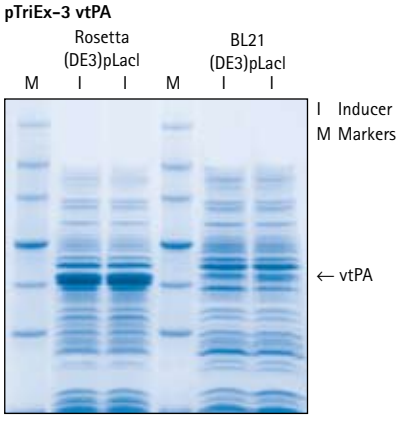


Figure 1. Expression of ΔvtPA in different host strains
DNA fragments encoding vtPA (Δ6-175 deletion mutant of human tissue plasminogen activator) was cloned into pTriEx-3 and transformed into Rosetta™ (DE3)pLacI and BL21 (DE3)pLacI. Cultures were grown at 37°C in LB + 0.5% glucose to an OD_{600} of 0.6-1.0 and samples were induced with 1 mM IPTG for 3 h. Total cell protein samples were prepared and analyzed by SDS-PAGE (4-20% gradient gel) and stained with Coomassie blue. Duplicate induced cultures are shown.

2. Can I use detergents to prepare my cell extract?

Reagents with mild detergents, such as BugBuster Master Mix and PopCulture® Reagent, are compatible with Novagen affinity chromatography resins.

3. Should I use protease inhibitors?

Proteins found in inclusion bodies are usually protected from proteolysis. For soluble proteins, protease inhibitors may (see question 9) or may not be required. Inhibitors are not recommended if they might interfere with target protein activity or prevent tag removal by site-specific proteases.

4. Does the number of histidines influence purification on IMAC?

Early purifications by IMAC took advantage of naturally occurring metal binding affinities in native proteins, without fusion tags (1). Proteins are primarily retained on IMAC resin based on an interaction between the histidine residues and the metal ions, although free cysteines, aromatic residues, and specific metal binding domains also can contribute to binding. Using a vector-encoded metal affinity tag, such as the His•Tag® sequence, provides a universal purification method for all proteins, regardless of their amino acid sequence. With additional histidines in the target protein, higher stringency washes can be used to increase purity. Novagen vectors encoding a His•Tag sequence with 10 histidine residues include: for bacterial expression, pET-16b, pET-19b, pET-51b(+), and pET-52b(+); for expression in insect cells, pIEx™-8, pIEx-9, and pIEx-10; and for expression in mammalian cells, as well as insect cells and bacteria, pTriEx™-5, pTriEx-6, and pTriEx-7. Except for pET-16b and pET-19b, the other vectors listed here also encode the Strep•Tag® II fusion tag sequence for purification with Strep•Tactin® resin, which is useful for dual affinity purification of full-length proteins.

5. How do I determine the optimal amount of resin to use for purification?

Divide the amount of fusion protein by the binding capacity of the resin to determine the optimal amount of resin to use. A number of methods can be used to determine or estimate the amount of target protein in the sample. Some fusion proteins have tags that can be quantitated using an activity assay (e.g., GST•Tag™ or S•Tag™ fusions). If no activity assay is available, first determine the total amount of protein in the extract using BCA (Cat. No. 71285), or another general protein assay. Then separate a small amount of the total protein on SDS-PAGE, visualize, and estimate the percent of target protein in the total protein sample. Multiply the total amount of protein in the extract by the percent of target protein to determine the total amount of target protein.

6. How can I verify whether my protein has the fusion tag?

With C-terminal tags, a reading frame shift due to a cloning problem or to a ribosomal frameshift can result in a protein without a fusion tag. A Western blot using a tag-specific antibody can confirm the presence of the tag.

7. Why doesn't my protein bind to the resin?

If the tag is present on the target protein (see question 6), verify that the chromatography buffers (pH, ionic strength, and concentration of detergents, reducing agents, or chaotropes) are compatible with the resin (Table 1). Under native conditions, the target protein could fold so that the tag is inaccessible. In the case of GST•Tag™ fusions, if the GST domain is not properly folded, the protein cannot bind to glutathione.

8. Can I cleave the fusion tag while the target protein is bound to the affinity purification resin?

To cleave tags from immobilized proteins with site-specific proteases, the cleavage buffer must be compatible with the conditions necessary to retain the tag on the resin. Examples of proteases and affinity resins that are compatible are thrombin and enterokinase with S-protein agarose and recombinant HRV 3C protease with Ni-NTA His-Bind® Resin.

9. Why do I have multiple bands after purification?

When extra bands are seen on a gel after purification on an affinity resin, a Western blot using an antibody against the fusion tag can help answer this question. Proteins smaller than the target may result from premature truncation during translation or from proteolysis. If these smaller proteins include the fusion tag, they will copurify. Truncation may result from frameshift or ribosomal dissociation when translating through rare codons or through secondary structure in the transcript. Bacterial host cells that provide rare tRNAs, such as the Rosetta™2(DE3) cells (Cat. No. 71397) allow full-length translation when rare codons are present (Figure 2).

Before cloning, minimize the introduction of unfavorable secondary structures in the RNA by checking for potential hairpins at cloning junctions or where codon optimization or mutations are planned.

Proteolysis can occur during expression in bacterial cells, but is primarily a problem after cell breakage. Adding protease inhibitor cocktails, keeping extracts cold, and working quickly can help minimize proteolysis after cell breakage. Some target proteins are susceptible to proteolysis within the cell. Coexpressing the target protein with an interacting protein may prevent target protein breakdown (Figure 3). Optimizing expression conditions can also reduce proteolysis during culture. A healthy, vigorous, culture typically has fewer proteolytic products than a culture grown well into stationary phase. Reducing IPTG induction times or using Overnight Express™ Media may result in protein preparations with fewer contaminating degradation products.

Large copurifying proteins may be aggregates of the target protein. Sometimes aggregation can be avoided by changing the tag on the protein. For example, GST is a dimer (2) and GST fusions may aggregate (3).

If the contaminating proteins share no identity with the target protein, adjusting buffer conditions may minimize contamination. Ionic interactions can be minimized

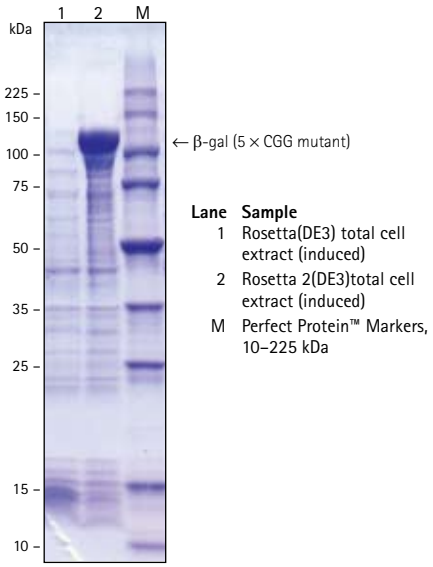


Figure 2. Effect of consecutive CGG rare codons on target protein expression
A pET-15b recombinant plasmid containing five consecutive CGG codons near the 5′-end of the β-gal coding region was transformed into Rosetta(DE3) and Rosetta 2(DE3). Cells were grown in LB broth with carbenicillin and chloramphenicol to an OD600 between 1.0 and 1.2, induced with 1 mM IPTG (3 h at 37°C), and harvested by centrifugation. Cells were resuspended and lysed in SDS sample buffer, followed by sonication to reduce sample viscosity. Proteins were separated on a 4–20% SDS polyacrylamide gel and stained with Coomassie blue.

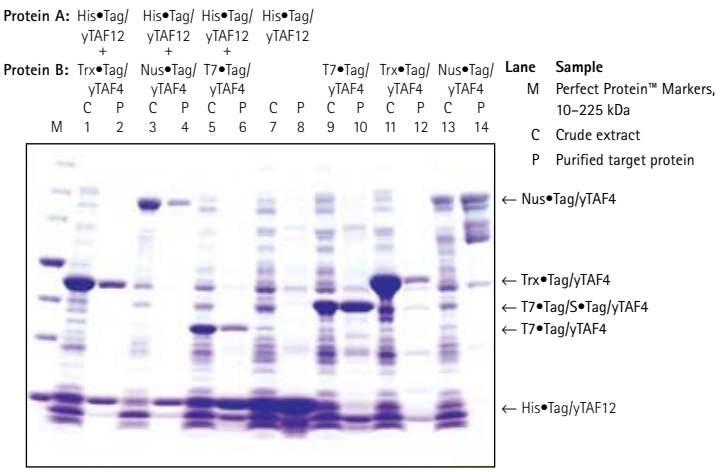


Figure 3. Coexpression and purification of interacting domains of yTAF4 and yTAF12
A yTAF12 fusion to the His•Tag® sequence was expressed alone in pET-30 Ek/LIC or coexpressed in the same vector with yTAF4 using the LIC Duet Trx•Tag™, Nus•Tag™, or T7•Tag® Ek Adaptors. The yTAF4 protein was also expressed alone as a His•Tag, Trx•Tag, or Nus•Tag fusion from the pET-30, pET-32, or pET-43.1 Ek/LIC vectors, respectively. The recombinant plasmids were transformed into Rosetta™(DE3), grown in LB broth, and induced with IPTG at 26°C for 4 h. Cells were harvested by centrifugation and lysed with BugBuster® Protein Extraction Reagent, rLysozyme™ Solution, and Benzonase® Nuclease. Equal volumes were purified by Ni-NTA His•Bind® chromatography under native conditions. Samples representing equal cell mass were analyzed by SDS-PAGE (4–20% gradient) and stained with Coomassie blue.

Table 1. Resin reuse and reagent compatibility

Resin	# of times reused	Detergent Compatible	Reducing Agents[a,b,c]	Denaturing Conditions
His•Bind® Resin	hundreds	yes	1 mM THP, no 2-ME, noDTT	6 M urea 6 M guanidine
Ni-NTA His•Bind	5	yes	1 mM DTT, 1 mM THP, up to 10 mM 2-ME	8 M urea 6 M guanidine
Ni-NTA His•Bind Superflow™	5	yes	1 mM DTT, 1 mM THP, up to 10 mM 2-ME	8 M urea 6 M guanidine
His•Bind Column	single use	yes	1 mM THP, no 2-ME, no DTT	6 M urea 6 M guanidine
His•Bind Fractogel®	up to 10	yes	1 mM THP, no 2-ME, no DTT	6 M urea 6 M guanidine
His•Bind Quick 300 cartridge	single use	yes	1 mM THP, no 2-ME, no DTT	6 M urea 6 M guanidine
His•Bind Quick 900 cartridge	single use	yes	1 mM THP, no 2-ME, no DTT	6 M urea 6 M guanidine
His•Bind Quick Column	single use	yes	1 mM THP, no 2-ME, no DTT	6 M urea 6 M guanidine
His•Mag™ Agarose Beads	single use	yes	1 mM THP, no 2-ME, no DTT	6 M urea 6 M guanidine
His•Bind Resin, Ni-charged	hundreds	yes	1 mM THP, no 2-ME, no DTT	6 M urea 6 M guanidine
GST•Bind™ Resin	several	yes	No	No
GST•Mag™ Agarose Beads	single use	yes	No	No
S-Protein Agarose	several	yes	1 mM 2-ME, 1 mM DTT	2 M urea
T7•Tag® Antibody Agarose	5	yes	1 mM 2-ME, 1 mM DTT	2 M urea
Strep•Tactin® Resins	3-5	yes	50 mM DTT or 2-ME	1 M urea

a= THP, [Tris(hydroxypropyl)phosphine] odorless sulfhydryl reducing agent; b= 2-ME, 2-mercaptoethanol; c= DTT, Dithiothreitol.

References
1. Porath, J. et al. 1975. *Nature* **258**, 598-599.
2. Ji, X. et al. 1992. *Biochemistry* **31**, 10169-10184.
3. Abeliovich, H. and Shlomai, J. 1995. *Anal. Biochem.* **228**, 351-354.

by including salt in the buffers. Reducing agents prevent disulfide bond formation. More stringent wash conditions or different elution conditions (e.g., proteolytic tag removal to release the target protein from the column) can help reduce contaminants.

To prevent proteolysis in cells, use protease deficient bacterial hosts like BL21(DE3). For insect cell expression, the BacVector® 3000 virus is engineered to remove additional genes encoding proteolytic and digestive enzymes.

10. What storage buffer should I use?

Generally, buffer pH should be at least 1 unit above or below the Isoelectric Point (pI) for the protein. When determining the pI of a recombinant protein, the additional amino acids encoded by the vector must be included. Most storage buffers include salt to reduce ionic interactions, and glycerol or sucrose to provide stability at low temperatures. Reducing agents are useful for proteins that are normally found in a reducing environment (e.g., bacterial cytoplasm) and for proteins that contain free sulfhydryl groups. Hydrophobic proteins may be stabilized by the addition of detergents. Protease inhibitors and bacteriostatic agents (sodium azide) are also commonly added to protein storage buffers.

11. How many times can I reuse the resin?

It depends on the resin. Consult the manufacturer's instructions for specific instructions on reuse. Table 1 provides information on the number of times Novagen resins can be reused and their compatibility with detergents, reducing reagents, and denaturants.

Novagen®

INSECT CELL EXPRESSION

Protein expression in insect cells:

faster – easier!

- NEW pIEx/Bac™ Vectors
  One vector, two methods:
  plasmid-based or baculovirus
- InsectDirect™ System
  Plasmid-based protein expression
  in 48 hours
- BacMagic™ DNA
  Baculovirus expression
  without plaque purification

New pIEx/Bac dual-purpose vectors are compatible with the InsectDirect method for rapidly screening clones and with the BacMagic baculovirus method for robust recombinant protein expression.

A higher order of protein expression

InsectDirect™ System

pIEx/Bac™ Dual-Purpose Vectors

BacMagic™ System

For additional information contact

Technical Service
novatech@novagen.com

or visit

www.novagen.com/insect-X

Argentina
Merck Quimica Argentina S.A.I.C.
Tel: +54 11 4546 8100
+54 11 4546 8156
Fax: +54 11 4546 8175
E-mail: mveltri@merck.com.ar
www.merck.com.ar

Australia
Merck Pty. Limited
Tel: +61 3 9728 7600
Fax: +61 3 9728 1351
E-mail: merck@merck.com.au
www.merck.com.au

Brazil
Merck S.A.
Toll Free: 0800 21 9292
Tel: +55 11 3346 8500
Fax: +55 11 3207 5040
E-mail: quimica@merck.com.br
www.merck.com.br

Imprint do Brasil Ltda.
Tel: +55 19 3772 2900
Fax: +55 19 3273 5389
E-mail: imprint@imprint.com.br
www.imprint-corp.com

Caribbean
Merck Centroamericana S.A.
See information under Central America

Central America
Merck, S.A. Guatemala
Tel: +50 2 2410-2300
Fax: +50 2 2434-2954
E-mail: quimicos@merck.com.gt
www.merck.com.gt

Chile
Merck S.A.
Tel: +56 2 3400 000
Fax: +56 2 3400 199
E-mail: mqch@merck.cl
www.merck.cl

China
Merck China
Toll Free: 800 820 8872
Tel: +86 21 3222 4788
Fax: +86 21 6247 9680
E-mail: Bioteam@merck-china.com
www.merckbio.cn

Colombia
Merck Colombia S.A.
Tel: +57 1 425 4770
Fax: +57 1 425 5407
E-mail: mcsa@merck.com.co
www.merck.com.co

Ecuador
Merck C.A.
Tel: +593 2 2981677
Fax: +593 2 2981644
E-mail: sicmerck@merck.com.ec
www.merck.com.ec

Guatemala
Merck Centroamericana S.A.
See information under Central America

Hong Kong
Onwon Trading Limited
Tel: +852 2757 7569
Fax: +852 2757 7211
E-mail: info@onwon.com.hk
www.onwon.com.hk

India
Merck Specialities Private Limited
Tel: +91 22 6660 9184
Tel: +91 22 6660 9000
Fax: +91 22 2495 4590
Fax: +91 22 2495 0307
E-mail: life.science@merck.co.in
www.merck.co.in

Indonesia
Pt. Merck Tbk.
Toll Free: 0800 140 1253
Tel: +62 21 841 3889
Fax: +62 21 841 5537
E-mail: chemicals@merck.co.id

Israel
Mercury Scientific & Industrial
Products Ltd.
Tel: +972 3 9387164
Fax: +972 3 9387174
E-mail: mercury@mercury-ltd.co.il
www.mercury-ltd.co.il

Japan
Merck Ltd.
Tel: +81 0120 189 390
Fax: +81 0120 189 350
E-mail: service@merck.co.jp
www.merck.co.jp

Korea
Merck Limited
Tel: +82 2 2185 3840, 3842
Fax: +82 2 2185 3870
E-mail: service@merck.co.kr
www.merck.co.kr

Malaysia
Merck Sdn Bhd
Tel: +6 03 7494 3688
Fax: +6 03 7491 0850
Fax: +6 03 7491 0860
E-mail: chemlab@merck-de.com.my
www.merck.com.my

New Zealand
Merck Limited
Toll Free: 0800 46 37 25
Tel: +64 06 356 7328
Fax: +64 06 356 7311
E-mail: info@merck.co.nz
www.merck.co.nz

Mexico
Control Técnico Y Representaciones
Monterrey, Nuevo, León
Tel: +52 81 8158 0600
Fax: +52 81 8373 2891
E-mail: ctrscientific@infosel.net.mx

Mexico City
Tel: +52 55 5208 5197
+52 55 5208 5198
+52 55 5208 8116
Fax: +52 55 5203 6229

Pakistan
Merck Marker (Pvt) Ltd.
Tel: +92 21 455 9210
Fax: +92 21 453 5294
E-mail: lab@merck.com.pk
www.merck.com.pk

Peru
Merck Peruana S.A.
Tel: +51 1 6187 500
Fax: +51 1 4372 955
E-mail: merck.peruana@merck.com.pe
www.merck.com.pe

Philippines
Merck Inc.
Tel: +63 2 815 4067
Tel: +63 2 814 5221
Fax: +63 2 815 4883
E-mail: tish.aligada@merck.ph

Singapore
Merck Pte. Ltd.
Customer Hotline: +65 6890 6660
Tel: +65 6890 6638
Fax: +65 6890 6636
E-mail: chem@merck-de.com.sg
www.merck.com.sg

Taiwan
Merck Ltd.
Tel: +886 2 2742 2788
Fax: +886 2 2742 2766
E-mail: bioservice@merck.com.tw
www.merckbio.com.tw

Thailand
Merck Ltd.
Tel: +66 2 667 8333
Fax: +66 2 667 8338
E-mail: customercare@merck.co.th
www.merck.co.th

Venezuela
Merck S.A.
Tel: +58 21 2235 1379
Fax: +58 21 2237 7632
E-mail: mven@merck.com.ve
www.merck.com.ve

Vietnam
Merck Representative Office
Tel: +84 8 932 0187
Fax: +84 8 526 0201
E-mail: merckvnadmin@hcm.vnn.vn

10001427
Protein Purification and Detection
2007 APLA