His•Tag®
GST•Tag™
Purification and Detection Tools

Advancing your life science discoveries™
Introduction

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

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

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

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

Part 1 Contents

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Protein Extraction Reagents Overview

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

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

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

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

The Insect PopCulture Reagent allows for centrifugation-free protein extraction from total cultures of baculovirus-infected insect cells in suspension or adherent cells on tissue culture plates. The improved method increases processing efficiency and target protein yields and is amenable to automated expression screening and affinity purification methods.
BugBuster® Protein Extraction Reagents
Simple extraction of soluble protein from E. coli without sonication

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

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

BugBuster® Protein Extraction Reagent

- **Product**: BugBuster® Protein Extraction Reagent
- **Size**: 100 ml
- **Cat. No.**: 70584-3

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

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**Product listing continued on next page**
BugBuster® HT Protein Extraction Reagent

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

BugBuster 10X Protein Extraction Reagent

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

BugBuster (primary amine-free) Protein Extraction Reagent

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

### Nucleic acid digestion by Benzonase® Nuclease

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

### Viscosity reduction by Benzonase® Nuclease

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

### Table: BugBuster® Protein Extraction Reagents

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<tr>
<td></td>
<td>500 ml</td>
<td>70923-4</td>
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</table>
PopCulture™ Reagent
Protein extraction from E. coli cultures directly in the growth medium

PopCulture™ Reagent is a detergent-based concentrate that can be added directly to cultures of E. coli to effectively extract proteins without the need for cell harvest. Recombinant proteins can be directly screened in the crude extract, or purified by adding an affinity matrix, washing the matrix-target protein complex to remove spent culture medium and cellular contaminants, and eluting the purified protein from the matrix. The entire culturing, extraction, and purification process can be performed in the original culture tube or multiwell plate. This “in-media” protein screening or purification procedure may be adapted to high-throughput robotic processing of samples for proteomics research and any application that would benefit from the increased speed and convenience it provides. Successful purification of intact fusion proteins from total culture extracts has been demonstrated using His•Mag™ and GST•Mag™ Resins (1, 2). Use of His•Mag™ or GST•Mag™ Agarose Beads enables the entire procedure to be carried out in a single tube without the need for columns or centrifugation (3). Addition of rLysozyme™ Solution or the use of pLysS hosts increases the efficiency of protein extraction with the procedure. Benzonase® Nuclease may also be added to reduce the viscosity of the extract.

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

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

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.

REFERENCES
2. InNovations 15, 14–19.
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-acetylglucosamine linkages in bacterial cell walls. The specific activity of rLysozyme (1,700 KU/mg) for *E. coli* lysis is 250 times greater than that of chicken egg white lysozyme. rLysozyme is optimally active at physiological pH. Very small amounts of rLysozyme (3–5,000 U/gram cell paste) enhance the efficiency of protein extraction with BugBuster®, BugBuster HT and PopCulture™ Reagents. In the absence of protein extraction reagents, direct lysis of *E. coli* can be achieved by treatment of 1.0 gram cell paste with 45–60 KU rLysozyme. The product is supplied as a ready-to-use solution at a concentration of 30 KU/µl in 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 NaCl, 0.1 mM EDTA, 1 mM DTT, and 0.1% Triton X-100. rLysozyme Solution is stable at –20ºC.

Unit Definition: one unit of rLysozyme is defined as the amount of enzyme necessary to cause a decrease of 0.025 A450 units per minute at 25ºC 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.

Bacterial Cell Lysis

Protein Extraction Reagents

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

The enzyme consists of two subunits of 30 kDa each. It is functional between pH 6 and 10 and from 0ºC to 42ºC and requires 1–2 mM Mg²⁺ 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 x 10^6 units/mg protein. The > 99% purity grade is tested for endotoxins and contains < 0.25 EU/1,000 units. The product is supplied as a 0.2 µm filtered solution in 50% glycerol. Store at –20ºC.

Unit definition: one unit is defined as the amount of enzyme that causes a ∆A260 of 1.0 in 30 minutes, which corresponds to complete digestion of 37 µg DNA.

Comparison of chicken egg white lysozyme and rLysozyme activities

Activities were measured in a standard activity assay.

Nucleic Acid Removal

Benzonase® Nuclease

Effective viscosity reduction and removal of nucleic acids from protein solutions

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

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Unit definition: one unit is defined as the amount of enzyme that causes a ∆A260 of 1.0 in 30 minutes, which corresponds to complete digestion of 37 µg DNA.

Information and Ordering: www.novagen.com
YeastBuster™ Protein Extraction Reagent

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

YeastBuster™ Protein Extraction Reagent is formulated for a fast, efficient and gentle extraction of soluble active proteins from *Saccharomyces cerevisiae* and *Pichia pastoris* cells. The reagent avoids harsh conditions of vigorous mechanical treatment that often result in heat and oxidative degradation of target proteins. The proprietary formulation utilizes a mix of mild detergent, protein stabilization buffer, and tris(hydroxypropyl)phosphine (THP) reducing agent (THP concentrate provided separately). This powerful combination eliminates the inconsistencies associated with tedious mechanical disruption of yeast cells with glass bead abrasives, ultrasonication and pressure disruption, or enzymatic digestion with β-1,3-glucanase lytic enzymes. In practice, cells are harvested by centrifugation and suspended in YeastBuster. Following a brief incubation, insoluble cell debris is removed by centrifugation, and the clarified extract is ready to use. In addition to greater total protein yields in crude extracts and recovery of enzymatically active protein, the extracts are fully compatible with GST•Bind™ and Ni-NTA His•Bind® immobilized metal affinity chromatography (IMAC) purification methods. The reagent is available in 100 and 500 ml sizes.

**Features**

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

**A. SDS-PAGE**

<table>
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<tr>
<th>Lane</th>
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<th>Glass Beads</th>
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<tr>
<td>M</td>
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<td>1</td>
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<td>6</td>
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**B. Protein and reporter assays**

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<tr>
<td>GST (∆ A₄₅₀/min)</td>
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<td>β-gal (∆ A₄₅₀/min)</td>
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**Figure 1. Performance comparison of YeastBuster™ Protein Extraction Reagent, another commercial reagent, and the glass bead method**

Panel A. SDS-PAGE analysis (4–20% gradient gel) and Coomassie blue staining of extracted proteins. *S. cerevisiae* cells containing a recombinant plasmid expressing a 35.6 kDa GST•Tag™/His•Tag® fusion protein were grown at 30°C, induced for expression, and harvested at OD₆₀₀ of 1.2. Cells were collected by centrifugation at 3,000 × g and resuspended in ice cold sterile water. Equal volumes of cells were dispensed into microcentrifuge tubes, and pelleted at 3,000 × g. Cell pellets (~65 mg wet weight) were resuspended in 330 µl of the respective extraction reagents supplemented with 0.5 mM AERSF 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 (NH₄)₂SO₄, 1 mM DTT, and 2% glycerol, adding approximately 50 µl acid-washed glass beads (100–150 µm diameter), and vortexing the sample on high for 4 min with intermittent chilling on ice. All samples were centrifuged at 16,000 × g for 5 min prior to SDS-PAGE analysis.

Panel B. Analysis of total protein and reporter activities. Total protein extracted by the three methods was determined using Non-Interfering Protein Assay™ Kit. GST activity was determined using GST•Tag Assay Kit. β-gal activity was determined using the host expressing *LacZ*. Cells were grown and processed as described for Panel A. Samples of the extracts were assayed using Novagen’s BetaRed™ β-Gal Assay Kit. Data reflect the average of duplicate assays.

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

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

Mammalian and Insect Cell Lysis

CytoBuster™ Protein Extraction Reagent

Simple extraction of soluble protein from mammalian and insect cells

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

![Coomassie blue stained gel and Western blot](image)

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

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

Insect PopCulture™ Reagent

Protein extraction directly from insect cell cultures

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

**Features**

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

**REFERENCES**


Information and Ordering: www.novagen.com

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# Protein Purification and Detection

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His•Tag® Fusion Proteins

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 (Ni²⁺) immobilized on NTA- and IDA-based His•Bind and His•Mag™ resins. After unbound proteins are washed away, the target protein is recovered by elution with either imidazole or slight reduction in pH. This versatile system enables proteins to be purified under gentle, non-denaturing conditions, or in the presence of either 6 M guanidine or urea.

The various His•Bind supports cover many applications for fusion protein purification (see Affinity Resins and Buffers beginning on page 18). Choices include small scale cellulose-based columns and cartridges for convenient handling of multiple samples, bulk easy-to-handle agaroses for batch and gravity flow columns, His•Mag Agarose Beads for rapid purification of multiple samples with minimum handling time, and high flow rate Superflow™ and Fractogel® resins suitable for production scale purification. Supports are provided either uncharged or pre-charged with Ni²⁺, and both NTA and IDA chemistries are available.

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

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

PopCulture™ His•Mag Purification Kit

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

RoboPop™ Ni-NTA His•Bind® Purification Kit

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

RoboPop His•Mag Purification Kit

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

The following table provides an overview of the various products and their specifications:

<table>
<thead>
<tr>
<th>Product</th>
<th>Culture scale</th>
<th>Processing method</th>
<th>Capacitya</th>
<th>Throughput level</th>
</tr>
</thead>
<tbody>
<tr>
<td>BugBuster® Ni-NTA</td>
<td>Any</td>
<td>Gravity flow column chromatography</td>
<td>5–10 mg/ml of resin</td>
<td>Low</td>
</tr>
<tr>
<td>His•Bind® Purification Kit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BugBuster His•Bind Purification Kit</td>
<td>Any</td>
<td>Gravity flow column chromatography</td>
<td>5–10 mg/ml of resin</td>
<td>Low</td>
</tr>
<tr>
<td>PopCulture™ His•Mag™ Purification Kit</td>
<td>3 ml</td>
<td>Magnetic</td>
<td>375 µg/culture</td>
<td>Low</td>
</tr>
<tr>
<td>RoboPop™ Ni-NTA His•Bind</td>
<td>96 × 5 ml</td>
<td>Filtration</td>
<td>1 mg/culture</td>
<td>High</td>
</tr>
<tr>
<td>Purification Kit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RoboPop His•Mag Purification Kit</td>
<td>96 × 1 ml</td>
<td>Magnetic</td>
<td>125 µg/culture</td>
<td>High</td>
</tr>
</tbody>
</table>

* 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.

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

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

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

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

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

The Culture Plate is compatible with Novagen’s Magnetight™ HT96™ Separation Stand (see page 28), which is recommended for efficient processing of magnetic affinity supports in deep well plates. The 96-well Deep Well Culture Plate with Sealers is available separately.

The RoboPop His•Mag Purification Kit will purify up to 12 mg of His•Tag fusion proteins per plate (up to 125 µg/well). Stated yields are based on 1 ml cultures and binding capacities of the beads, and will vary with the folding properties, expression levels, and solubility of individual fusion proteins. The RoboPop His•Mag Purification Kit has been validated for robotic sample processing with the MultiPROBE® II liquid handling workstation from PerkinElmer Life Sciences.

**RoboPop™ His•Mag™ Purification Kit**

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

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

PopCulture™ His•Mag™ Purification Kit

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

PopCulture Reagent is a detergent-based concentrate that can be added directly to cultures of E. coli to effectively extract proteins without the need for centrifugation. Use of His•Mag Agarose Beads enables the entire procedure to be carried out in a single tube without using columns or centrifugation.

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

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

<table>
<thead>
<tr>
<th>Protein Method</th>
<th>Yield/µg</th>
<th>Purity/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard His•Bind®</td>
<td>74</td>
<td>83</td>
</tr>
<tr>
<td>PopCulture™ His•Bind</td>
<td>111</td>
<td>89</td>
</tr>
<tr>
<td>PopCulture Ni-NTA His•Bind</td>
<td>170</td>
<td>85</td>
</tr>
<tr>
<td>PopCulture His•Mag™</td>
<td>128</td>
<td>94</td>
</tr>
</tbody>
</table>

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 Details

<table>
<thead>
<tr>
<th>Product</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>PopCulture™ His•Mag™ Purification Kit</td>
<td>15 ml PopCulture Reagent</td>
</tr>
<tr>
<td></td>
<td>3 × 1 ml His•Mag Agarose Beads</td>
</tr>
<tr>
<td></td>
<td>80 ml 8X Binding Buffer</td>
</tr>
<tr>
<td></td>
<td>2 × 25 ml 8X Wash Buffer</td>
</tr>
<tr>
<td></td>
<td>50 ml 4X Elute Buffer</td>
</tr>
<tr>
<td></td>
<td>300 KU rLysozyme™ Solution</td>
</tr>
<tr>
<td></td>
<td>1 ml rLysozyme Dilution Buffer</td>
</tr>
</tbody>
</table>

### SDS-PAGE Analysis

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

**Target protein**

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.

### Table

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

**PopCulture™ His•Mag™ purification**

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

2. **Target protein**

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.
**Protein Purification and Detection**

**His•Tag® Fusion Proteins**

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

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

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

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

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

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

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

**BugBuster His•Bind Purification Kit** 70793-3

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

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**His•Bind® purification of a poorly expressed protein**

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

**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 Ni²⁺ or Cu²⁺). The metal is held by chelation with reactive groups covalently attached to a solid support. The most commonly used chelators include nitriloacetic acid (NTA⁺*) and iminodiacetic acid (IDA), which have four and three sites available for interaction with metal ions, respectively. The two chemistries confer different properties to the affinity support and conditions used for binding, washing and elution of target proteins for both native and denaturing conditions. In practice, the additional chelation site available with NTA minimizes leaching of the metal during the purification and is compatible with up to 20 mM β-mercaptoethanol for reduction of disulfide bonds. The higher metal leaching rates of IDA-based resins in the presence of other chelating or reducing components can produce poor purification results when these products are present in the buffer. However, IDA supports can be recycled many hundreds of times with no loss in performance. For both types of support the conditions can be modified to optimize the purification of individual target proteins expressed in specific systems. Most often, the imidazole concentrations of the wash and elution buffers under native conditions are adjusted to minimize co-purification of non-specifically bound proteins.

### His•Bind and His•Mag Matrix Selection Guide

<table>
<thead>
<tr>
<th>Product</th>
<th>Form</th>
<th>Capacity</th>
<th>Features</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni-NTA His•Bind® Resin</td>
<td>Ni-charged NTA® agarose</td>
<td>5–10 mg/ml</td>
<td>Minimal Ni⁺⁺ leaching</td>
<td>Small to medium scale gravity flow column</td>
</tr>
<tr>
<td>Ni-NTA His•Bind® Superflow</td>
<td>Ni-charged NTA Superflow agarose</td>
<td>5–10 mg/ml</td>
<td>Minimal Ni⁺⁺ leaching</td>
<td>Small to production scale FPLC or gravity flow column</td>
</tr>
<tr>
<td>His•Bind® Resin</td>
<td>Uncharged IDA agarose</td>
<td>8 mg/ml</td>
<td>Reusable many times</td>
<td>Small to medium scale gravity flow column</td>
</tr>
<tr>
<td>His•Bind® Column</td>
<td>Ni-charged IDA agarose, prepacked column</td>
<td>10 mg</td>
<td>Pre-packed column</td>
<td>Convenient purification Gravity flow column</td>
</tr>
<tr>
<td>His•Bind® Fractogel® (S)</td>
<td>Uncharged Tentacle IDA methacrylate</td>
<td>&gt; 10 mg/ml</td>
<td>20–40 µm particle size</td>
<td>Small to production scale FPLC or gravity flow column</td>
</tr>
<tr>
<td>His•Bind® Fractogel® (M)</td>
<td>Uncharged Tentacle IDA methacrylate</td>
<td>&gt; 10 mg/ml</td>
<td>40–80 µm particle size</td>
<td>Small to production scale FPLC or gravity flow column</td>
</tr>
<tr>
<td>His•Bind® Quick 300 Cartridge</td>
<td>Ni-charged IDA cellulose packed cartridge</td>
<td>0.5 mg</td>
<td>Luer fitting on both ends</td>
<td>Syringe-driven processing Vacuum Manifold processing Rapid purification</td>
</tr>
<tr>
<td>His•Bind® Quick 900 Cartridge</td>
<td>Ni-charged IDA cellulose packed cartridge</td>
<td>2 mg</td>
<td>Luer fitting on both ends</td>
<td>Syringe-driven processing Vacuum Manifold processing Rapid purification</td>
</tr>
<tr>
<td>His•Bind® Quick Column</td>
<td>Ni-charged IDA cellulose packed cartridge</td>
<td>5 mg</td>
<td>Luer fitting on one end</td>
<td>Vacuum Manifold processing Rapid purification of multiple samples</td>
</tr>
<tr>
<td>His•Mag® Agarose Beads</td>
<td>Ni-charged IDA magnetic agarose</td>
<td>5 mg/ml</td>
<td>3 µm magnetic agarose beads</td>
<td>Rapid small scale purification Magnetic separation HT compatible</td>
</tr>
</tbody>
</table>

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

* manufactured by QIAGEN

### Product Listing

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni-NTA His•Bind® Resin</td>
<td>10 ml</td>
<td>70666-3</td>
</tr>
<tr>
<td></td>
<td>25 ml</td>
<td>70666-4</td>
</tr>
<tr>
<td>Ni-NTA His•Bind® Superflow</td>
<td>40–90 µm</td>
<td>100 ml</td>
</tr>
<tr>
<td>His•Bind® Resin</td>
<td>10 ml</td>
<td>69670-3</td>
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<td>50 ml</td>
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<td>100 ml</td>
<td>69670-5</td>
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<tr>
<td>His•Bind® Columns</td>
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<td>70971-3</td>
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<tr>
<td></td>
<td>pkg/25</td>
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<tr>
<td>His•Bind® Fractogel® (S)</td>
<td>25 ml</td>
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<td></td>
<td>50 ml</td>
<td>70693-3</td>
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<tr>
<td>His•Bind® Quick 300 Cartridge</td>
<td>100 ml</td>
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</tr>
<tr>
<td></td>
<td>200 ml</td>
<td>70155-4</td>
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<tr>
<td>His•Bind® Quick 900 Cartridge</td>
<td>500 ml</td>
<td>70156-3</td>
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<td></td>
<td>1000 ml</td>
<td>70156-4</td>
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<tr>
<td>His•Mag® 2 ml</td>
<td>71002-3</td>
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</tr>
<tr>
<td>Agarose Beads</td>
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</table>

Available separately:

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatography Columns</td>
<td>pkg/4</td>
<td>69673-3</td>
</tr>
</tbody>
</table>

Product listing continued on next page
Protein Purification and Detection

His•Tag® Fusion Proteins

His•Tag® Affinity Resins and Buffer Kits continued

His•Bind® Columns

Designed for convenience, the single-use His•Bind Columns are pre-packed with 1.25 ml bed volume of Ni²⁺-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 Ni²⁺ charging, binding, washing and elution of up to ten 2.5 ml columns. The His•Bind Quick Buffer Kit contains the same components except that the 8X Charge Buffer is not included (the resin is provided pre-charged with Ni²⁺).

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.

Information and Ordering: www.novagen.com

Product Cat. No.
His•Bind® Purification Kit 70239-3
Components:
• 10 ml His•Bind Resin
• 1 His•Bind Buffer Kit
• pkg/4 Chromatography Columns

Product Cat. No.
His•Bind Buffer Kit 69755-3
Components:
• 2 x 80 ml 8X Binding Buffer
• 25 ml 8X Wash Buffer
• 50 ml 4X Elute Buffer
• 50 ml 4X Strip Buffer
• 20 ml 8X Charge Buffer

Available separately:

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

Product Cat. No.
His•Bind Quick Buffer Kit 70665-3
Components are the same as 69755-3 except that only one bottle of 8X Binding Buffer is included and 8X Charge Buffer is omitted (His•Bind Quick resins are pre-charged).

Product Cat. No.
Ni-NTA Buffer Kit 70899-3
Components:
• 2 x 125 ml 4X Ni-NTA Bind Buffer
• 125 ml 4X Ni-NTA Wash Buffer
• 50 ml 4X Ni-NTA Elute Buffer
The His•Tag® Monoclonal Antibody is a mouse monoclonal antibody (IgG1) directed against the His•Tag sequence encoded by many of Novagen’s expression vectors as well as many other commercially available vectors. The antibody recognizes five consecutive histidine residues regardless of the surrounding amino acid context. The high affinity ($K_d = 5 \times 10^{-8} - 1 \times 10^{-9} \text{ M}$) enables sensitive, specific detection of His•Tag fusion proteins at antibody concentrations of 0.1 to 0.2 µg/ml. The His•Tag Monoclonal Antibody binds to N-terminal, C-terminal and internal His•Tag sequences. This antibody also detects the recombinant marker bands in the Perfect Protein™ and Trail Mix™ Western Markers for convenient visualization of accurate internal standards on Western blots.

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

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

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

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

### Specificity
- HisHisHisHisHis; N-terminal, C-terminal or internal

### Species/Isotype
- Mouse monoclonal IgG1

### Cross-reactivity
- Negligible with bacterial, yeast, insect, or mammalian cell lysates

### Sensitivity
- 2 ng (Western blot developed with chromogenic substrates)

### Applications
- Western blot, immunoprecipitation, and immunolocalization

### Form
- Lyophilized, BSA-free

### Working dilution
- 1:1,000–1:2,000 of antibody working solution [lyophilized antibody should be dissolved in 15 µl (3 µg) or 500 µl (100 µg) sterile water prior to dilution]

### Stability
- Lyophilized: 1 year at 2–8°C; in solution: 3 months at 2–8°C, 6 months at –20°C

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

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

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

His•Tag® Western and LumiBlot™ Reagents
Sensitive detection of His•Tag fusion proteins

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

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

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

His•Tag® Antibody Plate
For reliable and specific immobilization of His•Tag fusion proteins

The His•Tag® Antibody Plate is a 96-well ELISA-compatible plate containing immobilized His•Tag Monoclonal Antibody. The antibody is covalently immobilized to the surface using a method that retains maximal binding activity. The antibody specifically recognizes five consecutive histidines, and so will bind with high affinity ($K_d = 5 \times 10^{-8} – 1 \times 10^{-9} \text{ M}$) 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.

Information and Ordering: www.novagen.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 condition (10 mM reduced glutathione) avoids target protein denaturation.

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

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

BugBuster® GST•Bind Purification Kit

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

PopCulture™ GST•Mag Purification Kit

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

RoboPop GST•Bind Purification Kit

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

RoboPop™ GST•Mag Purification Kit

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Culture scale</th>
<th>Processing method</th>
<th>Capacitya</th>
<th>Throughput level</th>
</tr>
</thead>
<tbody>
<tr>
<td>BugBuster® GST•Bind™ Purification Kit</td>
<td>Any</td>
<td>Gravity flow column chromatography</td>
<td>5–8 mg/ml of resin</td>
<td>Low</td>
</tr>
<tr>
<td>PopCulture™ GST•Mag™ Purification Kit</td>
<td>3 ml</td>
<td>Magnetic</td>
<td>150 µg/culture</td>
<td>Low</td>
</tr>
<tr>
<td>RoboPop GMT•Bind Purification Kit</td>
<td>96 × 5 ml</td>
<td>Filtration</td>
<td>0.8 mg/culture</td>
<td>High</td>
</tr>
<tr>
<td>RoboPop GST•Mag Purification Kit</td>
<td>96 × 1 ml</td>
<td>Magnetic</td>
<td>50 µg/culture</td>
<td>High</td>
</tr>
</tbody>
</table>

a Capacities are based on 1 or 5 ml cultures and binding capacities of the resins. Yields will vary with the expression levels, folding properties, and solubility of individual fusion proteins.
RoboPop™ GST•Bind™ Purification Kit

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

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

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

REFERENCES


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

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

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

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

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

The Culture Plate is compatible with Novagen’s Magnetight™ HT96™ Separation Stand (see page 28), which is recommended for efficient processing of magnetic affinity supports in deep well plates. The 96-well Deep Well Culture Plate with Sealers is available separately (at right).

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

---

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

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 x 3 ml cultures with yields up to 150 µg GST•Tag fusion protein per 3 ml culture, based on bead binding capacity. The kit is compatible with Novagen's Magnetically Separation Stand. (See page 28 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 tube or well

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**Product**

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PopCulture™ GST•Mag™ Purification Kit</td>
<td>71113-3</td>
</tr>
</tbody>
</table>

**Components:**

- 15 ml PopCulture Reagent
- 3 x 1 ml GST•Mag Agarose Beads
- 2 x 100 ml 10X GST Bind/Wash Buffer
- 40 ml 10X Glutathione Reconstitution Buffer
- 1 g Glutathione, Reduced
- 300 KIU rLysozyme™ Solution
- 1 ml rLysozyme Dilution Buffer

---

**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**

<table>
<thead>
<tr>
<th>Product</th>
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</tr>
</thead>
<tbody>
<tr>
<td>BugBuster® GST•Bind™ Purification Kit</td>
<td>70794-3</td>
</tr>
</tbody>
</table>

**Components:**

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

---

**GST•Mag**

![Image](image_url)

**PopCulture™ GST•Mag™ purification**

Induced cultures of E. coli strain BL21(DE3) containing pET-41D(+), 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.

---

**Table:**

<table>
<thead>
<tr>
<th>Product</th>
<th>Average bead size</th>
<th>Binding capacity</th>
<th>Beads/ml culture</th>
<th>Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST•Mag Agarose Beads</td>
<td>3 µm</td>
<td>up to 2 µg/µl</td>
<td>25 µl settled beads (50 µl 50% w/v suspension)</td>
<td>Glutathione-derivatised magnetic agarose</td>
</tr>
</tbody>
</table>
The GST•Bind™ and GST•Mag™ purification systems are based on the widely recognized affinity of glutathione-S-transferase (GST•Tag™) fusion proteins for immobilized glutathione. Proteins are quickly and easily purified to near homogeneity in a single chromatographic step. Glutathione resin-based purifications require that the GST domain is soluble and properly folded. The gentle elution condition (10 mM reduced glutathione) avoids target protein denaturation.

**GST•Bind Resin**

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

**GST•Mag Agarose Beads**

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

**GST•Bind Buffer Kit**

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

**GST•Tag™ Assay Kit**

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.

**REFERENCE**

The GST•Tag™ Monoclonal Antibody is a mouse monoclonal antibody (IgG1) with high affinity for the 26 kDa glutathione-S-transferase (GST) domain from *Schistosoma japonicum*. This highly purified antibody is superior for detecting fusion proteins containing the GST•Tag expressed with the pET-41 or pET-42 vector series or other GST-encoding vectors.

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

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

### REFERENCES


### GST•Tag™ Monoclonal Antibody

**Sensitive, specific detection of GST•Tag fusion proteins**

- **Specificity**: 220 aa GST protein; precise epitope not determined
- **Species/Isotype**: Mouse monoclonal IgG1
- **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

---

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

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

---

### Immunohistochemical detection of GST expressed in transfected COS-1 cells

A pTriEx™ vector expressing GST was transiently transfected into COS-1 cells with GeneJuice™ Transfection Reagent. Twenty-four hours after transfection, cells were fixed, blocked with BSA and horse serum, and then exposed to GST•Tag™ Monoclonal Antibody (1:10,000 dilution) followed by a Cy3 conjugated Goat Anti-Mouse IgG. Hoechst 33258 was used for visualization of cell nuclei. A, Immunofluorescent staining of GST; B, Hoechst staining of the same field as in A showing both transfected and non-transfected cells.
The versatile Magnetight™ Separation Stand allows efficient magnetic separations using 1.5 ml, 15 ml or 50 ml centrifuge tubes. The stand uses permanent, extremely strong, rare earth magnets embedded in the body and protected by a nylon polymer housing. The configuration of holes allows tube walls to come in the closest possible proximity to the magnets, which enables efficient separations in a minimum amount of time. With its compact design, the stand and inserted tube can be easily held in one hand while pipetting solutions away from magnetized pellets with the other. The stand contains four places for 1.5 ml tubes and one place each for 15 ml and 50 ml tubes.

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

Convenient simultaneous processing of up to 12 samples

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

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

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

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

Product  |  Cat. No.
---      |  ---
Vacuum Manifold |  70147-3
Part 3 Contents

Fusion Tag Removal
- Restriction Grade and Biotinylated Thrombin ................. 31
- Thrombin Cleavage Capture Kit .................................. 31
- Restriction Grade Factor Xa ...................................... 32
- Factor Xa Cleavage Capture Kit ............................... 32
- Recombinant Enterokinase ........................................ 33
- Enterokinase Cleavage Capture Kit ......................... 33
**Fusion Tag Removal**

**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 CaCl₂, 50 µg fusion protein and enzyme.

**Biotinylated Thrombin**

Biotinylated Thrombin is identical in activity to Restriction Grade Thrombin, but has covalently attached biotin for easy removal of the enzyme from cleavage reactions using immobilized streptavidin. Novagen’s preparation is tested for activity using the same assay as for unmodified thrombin, and for greater than 99% binding to Streptavidin Agarose (see below).

**Thrombin Cleavage Capture Kit**

*Highly efficient, specific cleavage of fusion proteins*

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

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

---

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

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

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

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

**Available separately:**

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

**Biotinylated Thrombin cleavage**

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

**Restriction Grade Factor Xa**

*Specific cleavage of fusion proteins*

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

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

**Unit definition:** one unit of Restriction Grade Factor Xa cleaves 50 µg Xa Cleavage Control Protein to > 95% completion in 16 hours at 21°C in a buffer containing 50 mM Tris-HCl pH 8.0, 100 mM NaCl, and 5 mM CaCl₂.

**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.

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**Factor Xa cleavage**

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

---

**Product**  
**Size**  
**Cat. No.**

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor Xa, Restriction Grade</td>
<td>400 U</td>
<td>69036-3</td>
</tr>
</tbody>
</table>

**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

**Product**  
**Cat. No.**

<table>
<thead>
<tr>
<th>Product</th>
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<tbody>
<tr>
<td>Factor Xa Cleavage Capture Kit</td>
<td>69037-3</td>
</tr>
</tbody>
</table>

**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
- pkg/10 Spin Filters, 2 ml capacity

**Available separately:**

<table>
<thead>
<tr>
<th>Product</th>
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<tr>
<td>Xa Cleavage Control Protein</td>
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<tr>
<td>Spin Filter, 2 ml</td>
<td>pkg/10</td>
<td>69072-3</td>
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</table>
Fusion Tag Removal

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., Asp-Asp-Asp-Asp-Lys) and has similar enzymatic activity. rEK exhibits superior rates of cleavage of fusion proteins containing the recognition sequence when compared to the native enzyme (1). Novagen’s rEK is purified to near homogeneity and, unlike some preparations of native bovine enterokinase, exhibits no secondary cleavage arising from contaminating proteases. The preparation is also functionally tested for activity with fusion proteins.

**Unit definition:** One unit is defined as the amount of enzyme needed to cleave 50 µg fusion protein in 16 hours at 23°C in a buffer containing 20 mM Tris-HCl pH 7.4, 50 mM NaCl, and 2 mM CaCl₂.

REFERENCES

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.
## Accessory Products

### Part 4 Contents

<table>
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Protease Inhibitor Cocktail Set I (with EDTA)

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

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

Risk and Safety Statements:
R: 22-36/37/38; S: 26-36

Protease Inhibitor Cocktail Set II (with EDTA)

This lyophilized cocktail includes five protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, serine, and metalloproteases, as well as aminopeptidases. It is recommended for use with bacterial cell extracts (except those being used for metal chelation chromatography). Reconstitute each vial with 1 ml DMSO and 4 ml water to obtain 5 ml stock solution. Slight turbidity in the reconstituted solution is normal. When reconstituted, each vial contains 20 mM AEBSF, 1.7 mM Bestatin, 200 µM E-64, 85 mM EDTA, and 2 mM Pepstatin A. One set contains 1 vial of lyophilized inhibitors plus 1 vial DMSO. Five sets contain 5 vials of lyophilized inhibitors plus 5 vials DMSO, enough for 25 ml total after addition of water. Five milliliters is recommended for the inhibition of proteases extracted from 20 g E. coli. Risk and Safety Statements: R: 36/37/38; S: 26-36

Protease Inhibitor Cocktail Set III (without EDTA)

This liquid cocktail includes six protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, and serine proteases, as well as aminopeptidases. It is recommended for use with bacterial cell extracts being used for metal chelation chromatography, mammalian cell and tissue extracts. Each 1 ml vial contains 100 mM AEBSF, 80 µM Aprotinin, 5 mM Bestatin, 1.5 mM E-64, 2 mM Leupeptin, and 1 mM Pepstatin A as a solution in DMSO. Contains no metal chelators. One milliliter is recommended for the inhibition of proteases extracted from 20 g of bovine liver or 20 g E. coli. Risk and Safety Statements: R: 36/37/38; S: 26-36

Protease Inhibitor Cocktail Set IV (without EDTA)

This liquid cocktail includes four protease inhibitors with broad specificity for the inhibition of aspartic-, cysteine-, metallo-, and serine-proteases. It is recommended for fungal and yeast cell extracts. Each 1 ml vial contains 100 mM AEBSF, HCl, 1.5 mM E-64, 2 mM Pepstatin A, and 500 mM 1,10-Phenanthroline as a solution in DMSO. Risk and Safety Statements: R: 25-36/37/38-50/53; S: 26-36-45-60-61

Protease Inhibitor Cocktail Set V, EDTA-Free

This cocktail includes four protease inhibitors for the inhibition of serine- and cysteine-proteases, but not metalloproteases. Reconstitute each vial with 1 ml H2O to obtain 1 ml of 100X concentrated stock solution. 1X stock solution contains 500 µM AEBSF, HCl, 150 mM Aprotinin), 1 µM E-64, and 1 µM Leupeptin Hemisulfate. Note: this product is hygroscopic. Risk and Safety Statements: R: 36/37/38; S: 26-36

Information and Ordering: www.novagen.com
Western Blot Reagents

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).

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

Optimal performance and convenience for Western and dot blot applications

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

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

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

**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™ Western Markers and Blot Kits**

**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.
**Perfect Protein™ Markers**

Precisely sized, conveniently spaced for accurate protein size determination

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

The Perfect Protein Markers, 15–150 kDa, include protein sizes of 15, 25, 35, 50, 75, 100 and 150 kDa. Each vial contains 400 µg protein (50 µg per band except for 100 µg of the 50 kDa band as a high-intensity reference).

The Perfect Protein Markers, 10–225 kDa, include the protein sizes listed above and two additional proteins, 10 kDa and 225 kDa, for applications requiring a broader size range. Each vial contains 500 µg protein (50 µg per band except for 100 µg of the 50 kDa band as a high-intensity reference).

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

**Trail Mix™ Protein Markers**

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

Trail Mix™ Protein Markers are a mixture of Novagen’s Perfect Protein™ Markers and three prestained indicator proteins that together allow direct visualization of protein migration during electrophoresis. Unlike other marker sets in which the entire ladder is prestained, Trail Mix uses only three reference bands (at 100, 16 and 15 kDa) to confirm separation and indicate gel orientation. Prestaining can cause band broadening or affect mobility, reducing the precision with which mobility and molecular weight determinations can be made. The prestained bands in Trail Mix do not affect the migration or band sharpness of the Perfect Protein Markers.

When stained with Coomassie blue, 10 bands appear, ranging from 10 kDa to 225 kDa. Besides the prestained bands, the 50 kDa marker serves as a landmark on stained gels, due to its higher concentration in the mixture relative to adjacent bands. The 4X SDS Sample Buffer is a standard formulation commonly used for SDS-PAGE analysis of proteins. The solution includes DTT for complete denaturation of disulfide bonds. The buffer can be used at a final concentration of 2X for most applications. Additionally, each protein marker carries the His•Tag sequence and will be visualized with Western blot analysis when using His•Tag antibody.

---

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

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

**CB-Protein Assay™ Kit**

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

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

![Graph A: Standard protocol](image1)

![Graph B: Micro protocol](image2)

Standard curves generated with the CB-Protein Assay™ Kit

**Non-Interfering Protein Assay™ Kit**

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

The easy-to-use Non-Interfering Protein Assay™ Kit overcomes interference of agents found in protein solutions including detergents, chelating agents, reducing agents, amines, sugars, urea, etc. The Universal Protein Precipitating Agent (UPPA™) is used to precipitate and immobilize the protein in the tube while the interfering reagents are removed. Protein concentration is based on the specific binding of copper to the peptide backbone. As the protein concentration increases, the concentration of unbound copper ions decreases, and the color density is inversely related to the amount of protein present in solution. The kit includes UPPA reagents I and II, Copper Solution I, Color Agents A and B, and BSA standard. Note: one kit is sufficient for 500 protein determinations. **Risk and Safety Statements:** R: 36/37/38; S: 26-36

![Graph](image3)

Standard curve generated using the Non-Interfering Protein Assay™ Kit

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

**Risk and Safety Statements Key:**

- **Risk Statements:**
  - R22 Harmful if swallowed.
  - R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
  - R36/37/38 Irritating to eyes, respiratory system and skin.

- **Safety Statements:**
  - S26 In case of contact with eyes, rinse immediately with plenty of water.
  - S36 Wear suitable protective clothing.
  - S36/37 Wear suitable protective clothing and gloves.

Information and Ordering: www.novagen.com

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<tr>
<td>Non-Interfering Protein Assay™ Kit</td>
<td>1 kit</td>
<td>488250</td>
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United States and Canada
EMD Biosciences, Inc.
441 Charmany Drive
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Fax: 608 238 1388
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web: www.novagen.com
Technical Service:
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e-mail: novatech@novagen.com
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web: www.vwr.com

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Freefax: 0800 62 36 100
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web: www.merckbiosciences.co.uk

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