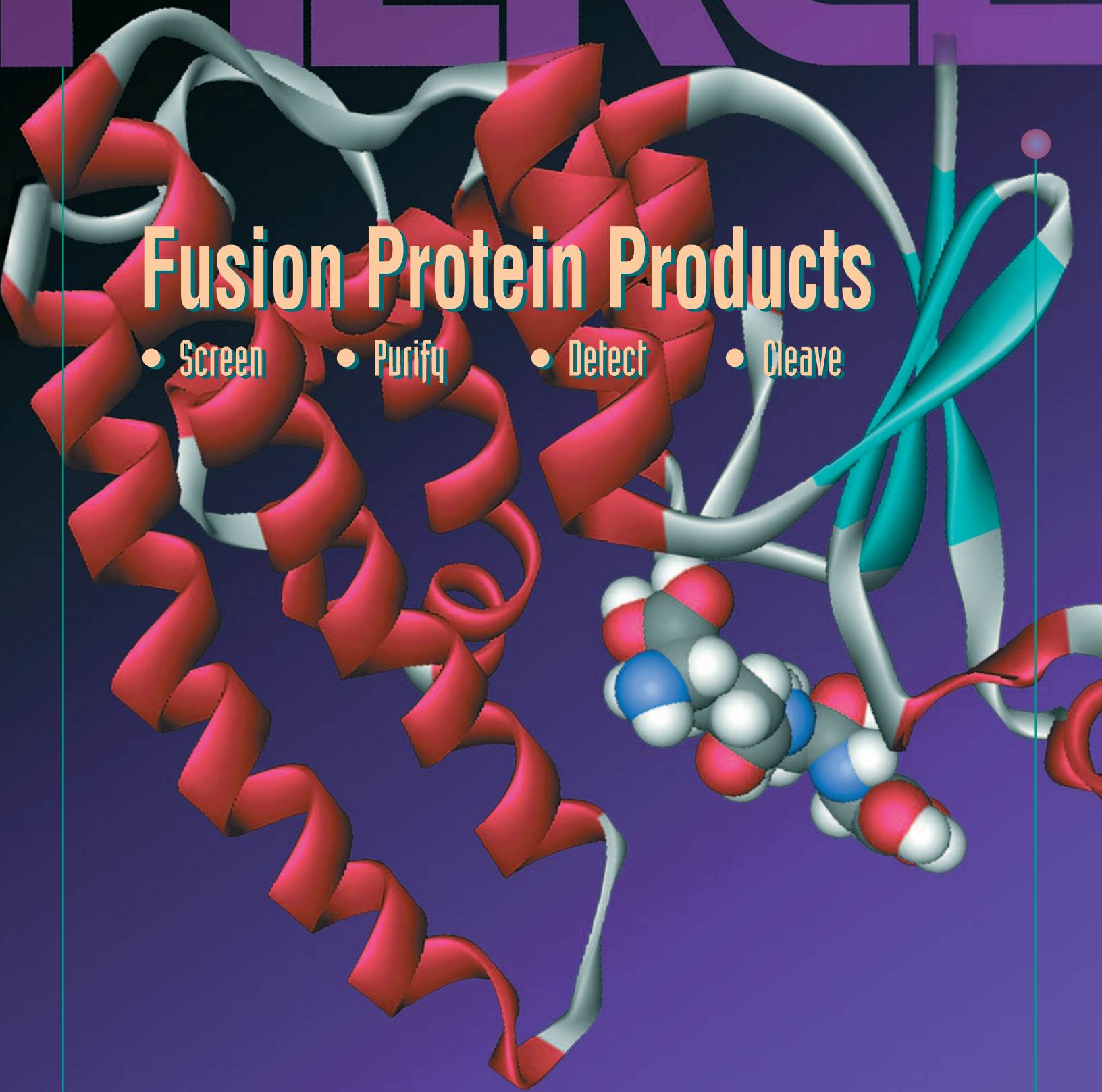
PIERCE



Fusion Protein Products

• Screen • Purify • Detect • Cleave

PIERCE

A fusion protein consists of two gene sequences that are ligated together and transcribed as a single molecule.

One sequence encodes for a "tag," which has a strong affinity for a known ligand.

The other sequence encodes for the target molecule of interest.

The tag is then used as a handle to manipulate the target molecule during screening, purification or interaction studies.

As today's life science researchers look to plasmid vectors to express fusion proteins, they find themselves in need of methods and tools for screening and purifying the resulting populations. Pierce, long recognized as the leading provider of innovative protein studies products, is proud to present its comprehensive line of tools for screening, detecting and purifying today's most frequently used tagged proteins — glutathione S-transferase (GST), maltose binding protein (MBP) and polyhistidine. A complete line of products for biotinylated proteins is also included for those fusion proteins that may have an inaccessible tag.

Pierce offers a host of essential tools for fusion protein purification, screening, detection and protein:protein interaction applications including:

- Reacti-Bind™ Coated Plates (precoated with the target ligand of interest):
  - Glutathione for GST
  - Dextrin for MBP
  - Ni^{+2} or Co^{+2} chelated for 6xHis tag
  - NeutrAvidin™ Biotin-Binding Protein, Avidin and Streptavidin for Biotin
- Pre-dispensed SwellGel® Resin in filterplates for higher capacity high-throughput purification
- Poppers™ Cell Lysis and Extraction Reagents and Kits

After the presence of a desired target molecule is verified, B-PER® Bacterial Protein Extraction Reagent and Y-PER® Yeast Protein Extraction Reagent Kits provide an easy means of efficient purification. These kits include everything needed to purify the target molecule, from lysis buffers to affinity columns to elution buffers. B-PER® Reagent is a patented, mild and nonionic detergent that facilitates simple and fast extraction of targets in both soluble and insoluble forms. Y-PER® Reagent is a proprietary, mild nonionic detergent that allows 100% more extraction from yeast cells than glass beads. B-PER® and Y-PER® Kits may be used for both soluble recombinant protein extractions and insoluble inclusion body protein purification.

In addition to tools for fusion protein screening and purification, Pierce offers numerous complete application kits for the study of protein:protein interactions utilizing fusion proteins:

- ProFound™ Pull-Down Assay Kits
- GTPase Activity Assay Kits

To help complete the process, Pierce offers a full line of primary and secondary antibodies, as well as other detection reagents.

Pierce offers these and thousands of other high-quality products as well as the best customer and technical support available.

Table of Contents

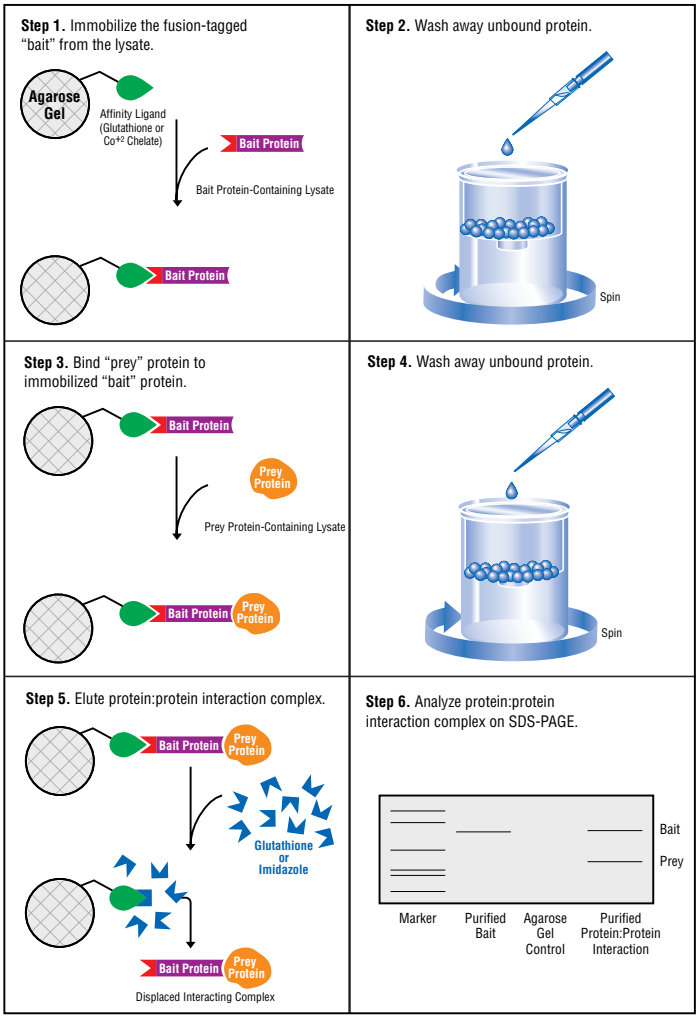
Fusion Protein Applications

Discover a new protein:protein interaction with your GST, 6xHis-tagged or biotin bait protein

Identifying and characterizing the interactions of a given protein has emerged as the most valuable information that can be developed in the post-genomic era. The Pierce Protein:Protein Interaction Kits contain the essential components necessary to enable the capture of an interacting prey protein using the popular pull-down method. All you need to provide is an appropriately tagged and purified target protein as the "bait." The Pierce ProFound™ Pull-Down Protein:Protein Interaction Kits are designed to teach the method to the first-time user and to shorten the time to a result for those more experienced in the method.

How it works

The pull-down assay is an *in vitro* technique that consists of a purified and tagged "bait" protein for which a binding partner (the "prey") is being sought. The bait protein is bound to the appropriate support. In a typical pull-down experiment, bound bait protein is washed and introduced to a protein pool derived from a cell lysate.



= Fusion Tag (GST or polyHis)

Generalized scheme for use of a ProFound™ Pull-Down GST or PolyHis Protein:Protein Interaction Kit.

After the prescribed incubation and washing steps, the "interactor" or prey protein(s) can be eluted under the appropriate conditions. The prey protein "pulled-down" by the method is detected in-gel or on a Western blot.

- Provides a complete, affordable and easy-to-use strategy for discovery of protein:protein interactions
- Uses common laboratory equipment and reagents (e.g., microcentrifuges, mini-gels, protein stains)
- Adapts to single- or multiple-sample demands
- Features a flexible pull-down format
- Assays for and detects interactors in one day
- Includes cell lysis buffer in each kit
- Aids in the capture of weak or transient interactions

Applications

- Discover a new protein:protein interaction
- Confirm a putative interaction with a prey protein captured from a cell lysate or with a previously purified prey protein
- Extract protein:protein interaction information from *in vitro* transcription/translation lysates

PRODUCT #	DESCRIPTION	PKG. SIZE
21516	**ProFound™ Pull-Down GST Protein:Protein Interaction Kit** *Sufficient materials for conducting 25 pull-down assays using a GST-tagged protein as the bait.*	Kit
	Includes: Immobilized Glutathione	750 µl settled gel
	ProFound™ Lysis Buffer	250 ml
	Glutathione	1 gm
	BupH™ Tris Buffered Saline	1 pack (makes 500 ml)
	Handee™ Spin Cup Columns Accessory Pack	27 columns
	Collection Tubes and Caps Accessory Pack	100 tubes
21277	**ProFound™ Pull-Down PolyHis Protein:Protein Interaction Kit** *Sufficient materials for conducting 25 pull-down assays using a polyhistidyl-tagged protein as the bait.*	Kit
	Includes: Immobilized Glutathione	750 µl settled gel
	ProFound™ Lysis Buffer	250 ml
	4 M Imidazole Stock Solution	5 ml
	BupH™ Tris Buffered Saline	1 pack (makes 500 ml)
	Handee™ Spin Cup Columns Accessory Pack	27 columns
	Collection Tubes and Caps Accessory Pack	100 tubes
21115	**ProFound™ Pull-Down Biotinylated-Protein:Protein Interaction Kit** *Sufficient materials for conducting 25 pull-down assays using a purified and biotinylated protein as the bait.*	Kit
	Includes: Immobilized Streptavidin	1.5 ml settled gel
	BupH™ Tris Buffered Saline	1 pack (500 ml)
	Biotin Blocking Buffer	15 ml
	Wash Buffer (Acetate, pH 5.0)	100 ml
	Elution Buffer (pH 2.8)	50 ml
	Handee™ Spin Cup Columns Accessory Pack	27 columns
	Collection Tubes and Caps Accessory Pack	200 x 2 ml tubes

See ProFound™ HA and c-Myc IP/Co-IP kits on page 11.

Measure active small GTPases with Pierce's powerful – yet simple – new assay kits

Monomeric p21 GTP-binding proteins (small GTPases) serve as molecular switches in regulating a wide range of essential biochemical pathways in eukaryotic cells. The families of Ras and Rho (including Cdc42, Rac1 and Rho) are of special interest as they influence the cell's response to the changing environment. Research results have indicated that these proteins regulate numerous cell functions such as proliferation, differentiation, transformation, apoptosis, migration, actin cytoskeleton reorganization, and cell cycle progression. Like other G-proteins, small GTPases cycle between an inactive, GDP-bound state and an active, GTP-bound state.

- High sensitivity – SwellGel® Technology combined with enhanced SuperSignal® Technology allows the use of less sample
- Convenience – no need to express and purify GST-PBD or RBD fusion proteins
- Speed – simultaneous incubation of cell lysate with GST-PBD or RBD and the pre-measured SwellGel® Immobilized Glutathione Disc directly in a spin column
- Ease-of-use – pull-down conditions have been optimized for immediate success, even for first-time users
- Efficiency – the spin column and receiver tubes allow efficient separation of liquid and resin, preventing sample loss

The new Pierce EZ-Detect™ Rac1, Cdc42, Ras and Rho Activation Kits measure the activation of small GTPases by isolating them via their specific downstream effectors. The respective binding domain of the downstream effector for each small GTPase is expressed as a GST-fusion protein which, when immobilized on a resin, is used to pull down the active or GTP-bound GTPase (Figure 1). GST-RBD (Ras binding domain) of Raf1 can pull down active Ras, GST-PBD (p21 binding domain) of Pak1 can pull down active Rac1 and Cdc42, and GST-Rhotekin-RBD can pull down active Rho. The pulled-down active GTPase is then detected by a Western blot using a specific antibody.

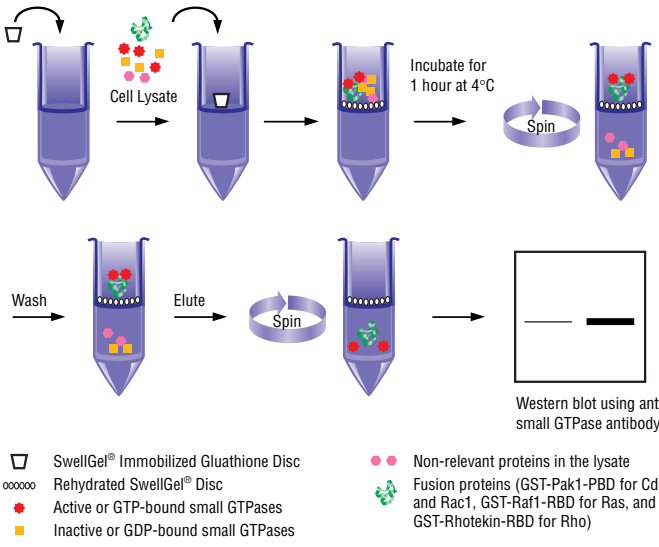


Figure 1. Schematic protocol for EZ-Detect™ Ras, Cdc42, Rac1 and Rho Activation Kits.

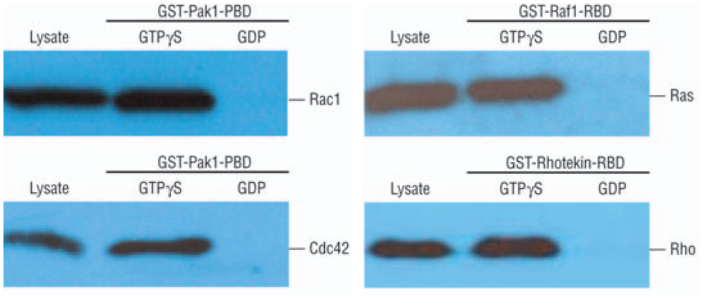


Figure 2. Detection of the active Ras, Cdc42, Rac1 and Rho using EZ-Detect™ Ras, Cdc42, Rac1 and Rho Activation Kits. NIH3T3 cell lysate treated with GTPγS or GDP was incubated with GST-Raf1-RBD, GST-Pak1-PBD or GST-Rhotekin-RBD and SwellGel® Immobilized Glutathione. Half of the eluted pull-down samples (25 µl) and 20 µg of lysate were analyzed by Western blot using anti-Rac1, anti-Cdc42, anti-Pan-Ras or anti-Rho antibody. There were no active GTPases detected (see GDP lanes).

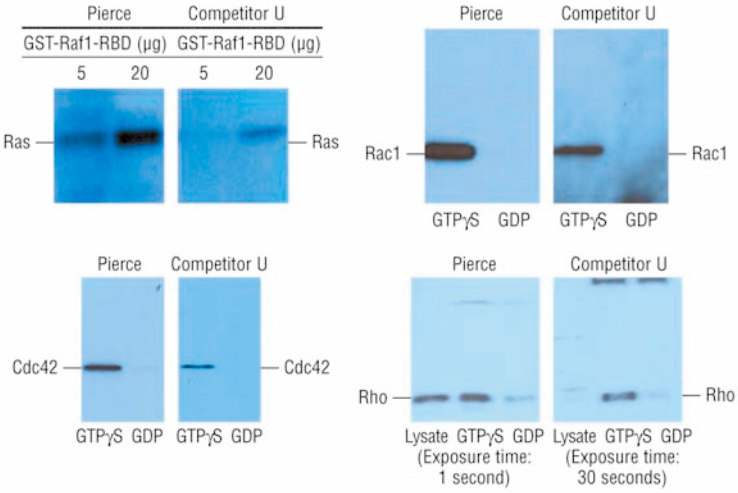


Figure 3. Performance comparison of EZ-Detect™ Ras, Rac1, Cdc42 and Rho Activation Kits with competitor kits. Affinity precipitation of Rac1, Cdc42, Ras and Rho from NIH3T3 cell lysates (500 µg) treated with GTPγS or GDP was carried out using Pierce's and competitor U's kits. Half of eluted pulled-down samples (25 µl) were analyzed by Western blot (SuperSignal® West Pico Chemiluminescent Substrate, Product # 34080) using anti-Rac1*, anti-Pan-Ras, anti-Cdc42 or anti-Rho antibody. Analyses were conducted according to the manufacturer's instructions.

*Anti-Rac antibody was used for competitor U's assay.

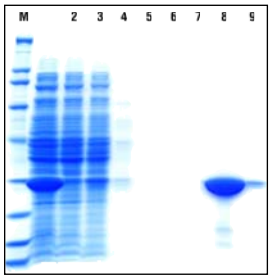
PRODUCT #	DESCRIPTION	PKG. SIZE
89854	EZ-Detect™ Rho Activation Kit	Kit
89855	EZ-Detect™ Ras Activation Kit	Kit
89856	EZ-Detect™ Rac1 Activation Kit	Kit
89857	EZ-Detect™ Cdc42 Activation Kit	Kit

Visit our web site for kit component information.

Tools for Polyhistidine-Tagged Targets

Poppers™ 6xHis Fusion Protein Purification Kits

- Optimized system provides the highest purity (up to 95%) in the least amount of time (Figure)
- Greater convenience – ready to use with no sonicator required; complete lysis achieved with B-PER® Bacterial Protein Extraction Reagent. Y-PER® Yeast Protein Extraction Reagent eliminates the need for glass beads
- Increased capacity – 10 mg of tagged protein bound per column with the B-PER® Fusion Protein Column Purification Kit
  - Approximately 1 mg with the B-PER® Fusion Protein Spin Purification Kit
  - 100% more fusion protein recovered with Y-PER® Reagent versus glass beads with over 10 mg/column capacity



SDS-PAGE analysis of the purification of 6xHistidine-tagged GFP from *E. coli* using the B-PER® 6xHis Fusion Protein Purification Kit. Fractions were collected from each of the purification steps and assayed using gradient 4-20% SDS-PAGE. The gel was stained with GelCode™ Blue Stain Reagent (Product # 24592). **Lane 1**: crude lysate extracted from *E. coli* expressing 6xHistidine-tagged GFP using B-PER® Reagent, **Lane 2**: flow through of the lysate after loading onto a Ni-chelated column, **Lanes 3-4**: two washes with 3 ml of 6xHis Wash Buffer 1, **Lanes 5-7**: three washes with 3 ml of 6xHis Wash Buffer 2, and **Lanes 8-9**: 6xHistidine-tagged GFP eluted from the column with 6xHis Elution Buffer.

PRODUCT #	DESCRIPTION	PKG. SIZE
78100	**B-PER® 6xHis Fusion Protein Purification Kit**	Kit
	Includes: B-PER® Bacterial Protein Extraction Reagent	165 ml
	6xHis Wash Buffer 1	45 ml
	6xHis Wash Buffer 2	60 ml
	Elution Buffer	45 ml
	Immobilized Nickel Chelated Column	5 x 1 ml
78300	**B-PER® 6xHis Fusion Protein Spin Purification Kit**	Kit
	Includes: B-PER® Bacterial Protein Extraction Reagent	165 ml
	6xHis Wash Buffer	40 ml
	Elution Buffer	45 ml
	Nickel Chelated Agarose	8 ml
78994	**Y-PER® 6xHis Fusion Protein Column Purification Kit**	Kit
	Sufficient reagents for five 6xHis fusion protein purifications from S. cerevisiae, S. pombe, B. subtilis *or* E. coli *(up to 6 gm of wet cell paste/purification).*	
	Includes: Y-PER® Yeast Protein Extraction Reagent	200 ml
	Nickel Chelated Columns	5 x 1 ml
	Wash Buffer 1	60 ml
	Wash Buffer 2	60 ml
	Elution Buffer	45 ml

For B-PER® and Y-PER® Reagents alone see page 6.

SwellGel® Nickel Chelated Discs

The SwellGel® 96-well System bridges the gap between high-throughput microscale and spin column purification. It is a highly stable nickel-chelated agarose for performing high-throughput fusion protein purification with the popular 6xHis tag.

- 96-well filterplate format
- High-performance, high-capacity system sets a new standard for 6xHis-tagged fusion protein purification
  - Higher throughput format binds more than 1 mg of 6xHis-tagged green fluorescent protein (GFP) per well
- Pre-charged, pre-equilibrated, pre-dispensed discs provide convenience and consistency
- Easy to use – just peel off the seal and purify!
  - Add your protein sample and the disc rehydrates to a nickel-chelated agarose gel
- Excellent stability and room temperature storage

SwellGel® Cobalt Chelated Discs

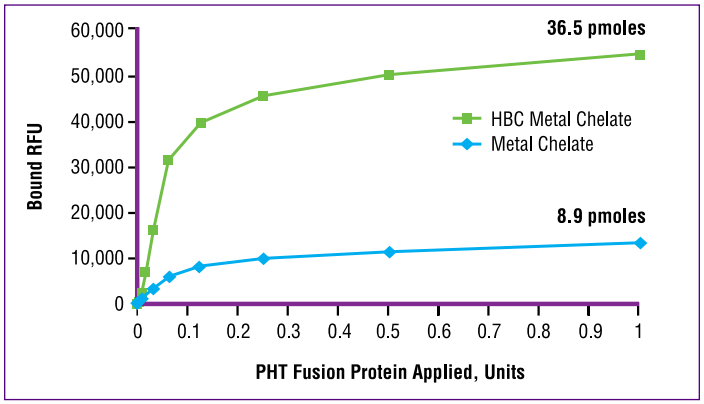
All the great features and benefits of SwellGel® Discs in a convenient, easy-to-dispense bottle.

- Binds 2-5 mg (depending on size of protein)
- Rehydrates in 20 seconds
- Composed of dehydrated beaded agarose

PRODUCT #	DESCRIPTION	PKG. SIZE
20147	**SwellGel® Nickel Chelated Discs***	Kit
	Each disc = 100 µl of bed volume.	
	Includes: 96-Well Purification Filterplate	1
	SwellGel® Nickel Chelated Discs	96
	Collection Plate	1
	Start Buffer	20 ml
	4 M Imidazole Stock Solution	12 ml
75824	**SwellGel® Nickel Chelated Discs**	Each
	Each disc = 100 µl of bed volume.	
	Includes: 96-Well Purification Filterplate	1
	SwellGel® Nickel Chelated Discs	96
	Collection Plate	1
89827	**SwellGel® Nickel Chelated Discs**	96 Discs
	Each disc = 200 µl of bed volume.	
89838	**SwellGel® Cobalt Chelated Discs**	96 Discs
	Each disc = 200 µl of bed volume.	

HisGrab™ Metal Chelate High Binding Capacity (HBC) Coated Plates

- Quantitate previously undetectable polyhistidine-tagged proteins
- Wider dynamic range with four-fold greater capacity than regular nickel chelate-coated plates
- Special blocking solution (Product # 37547) improves signal:noise ratio for enhanced assay sensitivity



A comparison of the binding of a relative concentration of a polyhistidine-tagged fluorescent fusion protein to both standard HisGrab™ Metal Chelate and the HisGrab™ Metal Chelate High Binding Capacity (HBC) Plates. HisGrab™ Metal Chelate HBC Plates exhibit a four-fold greater capacity for binding purified polyhistidine-tagged protein when assayed using a 100 µl volume. Incubation time was two hours for the binding of polyhistidine-tagged fusion protein.

PRODUCT #	DESCRIPTION	PKG. SIZE
15143	**HisGrab™ Metal Chelate, High Binding Capacity (HBC) Clear 96-Well Plates**	5 plates
15146	**HisGrab™ Metal Chelate, High Binding Capacity (HBC) Clear 8-Well Strip Plates**	5 plates
15147	**HisGrab™ Metal Chelate, High Binding Capacity (HBC) White Opaque 96-Well Plates**	5 plates
15148	**HisGrab™ Metal Chelate, High Binding Capacity (HBC) Black Opaque 96-Well Plates**	5 plates
37547	**Blocker™ Metal Chelate Compatible Formulation**	20 ml

His-Grab™ Metal Chelate Plates

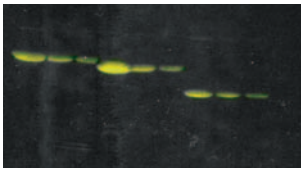
- Selectively bind recombinantly expressed fusion proteins containing 6xHis-tags
- Pre-chelated with Ni^{2+}
- Lower detection limit of 1 ng of polyhistidine fusion protein
- Detergents used to lyse cells don't interfere with protein binding
- Pre-blocked to reduce nonspecific interactions

PRODUCT #	DESCRIPTION	PKG. SIZE
15142	**HisGrab™ Metal Chelate Plates** 96-Well Plate in an 8-Well Strip Plate Format	5 plates
15242	**HisGrab™ White Opaque Metal Chelate Plates** 96-Well Plate (for chemiluminescent detection)	5 plates
15342	**HisGrab™ Black Opaque Metal Chelate Plates** 96-Well Plate (for fluorescent detection)	5 plates

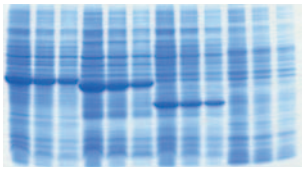
GelCode® 6xHis Protein Tag Staining Kit

Detect 6xHistidine-tagged protein directly on the gel!

6xHis Protein-Tagged Preparations Stained with GelCode® 6xHis Protein Tag Staining Kit



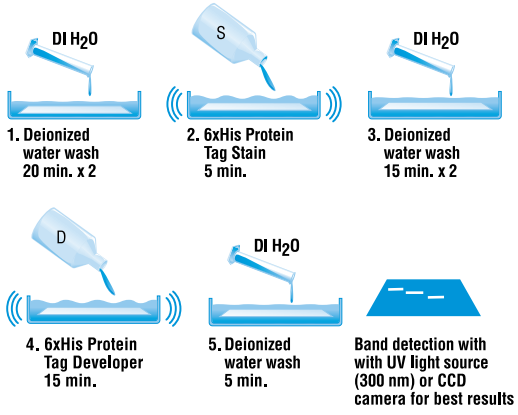
Lysates of three *Escherichia coli* preparations expressing different 6xHis-tagged proteins and one control *E. coli* lysate. The fluorescent bands seen are 6xHis-tagged proteins stained with the Pierce 6xHis Protein Tag Staining Kit.



When these same lysates were stained with GelCode™ Blue Stain Reagent, all of the protein bands in the preparation could be seen.

- Works two- to three-times faster than Western blotting
- Detects directly on the gel – can eliminate the need for a membrane transfer step and Western blot detection with as little as 0.2 µg (5.7 pmol) of 6xHis-tagged protein
- Ready-to-use, two-reagent formula
- Fluorescent detection is designed to be specific for 6xHis-tagged proteins only – see only what you want to see; CCD camera detection suggested for low abundance situations. Transilluminator for UV detection works best for high abundance tagged protein preparations
- Compatible with Pierce GelCode™ Blue Stain Reagent – stain for 6xHis-tagged protein specifically and follow with GelCode™ Blue Stain Reagent for a total protein profile determination

GelCode™ 6xHis Protein Tag Staining Protocol



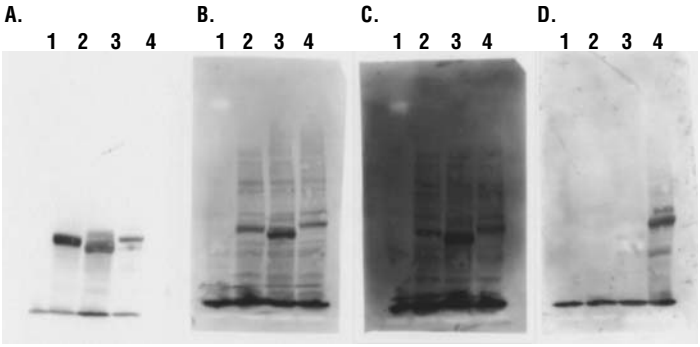
PRODUCT #	DESCRIPTION	PKG. SIZE
24575	**GelCode™ 6xHis Protein Tag Staining Kit** *Sufficient reagent to stain 10 SDS-PAGE mini gels.* Includes: Product # 24570, GelCode™ 6xHis Protein Tag Stain: Reagent Set Product # 24572, 6xHis Protein Control Set	Kit
24570	**GelCode™ 6xHis Protein Tag Stain: Reagent Set** *Sufficient reagent to stain 10 SDS-PAGE mini gels.* Includes: 6xHis Protein Tag Stain 6xHis Protein Tag Developer	Kit 500 ml 500 ml
24572	**6xHis Protein Control Set*** For use with the GelCode™ 6xHis Protein Tag Stain: Reagent Set. *Sufficient material to control 50-100 SDS-PAGE runs.*	Kit

**Also available for use with Product # 24570 and Product # 15165 (INDIA™ HisProbe™-HRP)*

Tools for Polyhistidine-Tagged Targets

SuperSignal® West Pico HisProbe™ Kit

- Complete – contains everything needed for the detection of histidine-tagged fusion proteins
- Fast – one-step probe incubation eliminates the need to run a lengthy two-step primary/secondary antibody sequential reaction protocol
- Sensitive – when used in combination with SuperSignal® West Pico Chemiluminescent Substrate, kit allows the detection of even low-expression histidine-tagged clones
- High specificity – more specific for the detection of polyhistidine-tagged fusion proteins of interest
- More versatile than anti-poly-His antibody-based systems – detects polyhistidine fusion proteins usually undetectable when using some monoclonal anti-poly-His antibodies



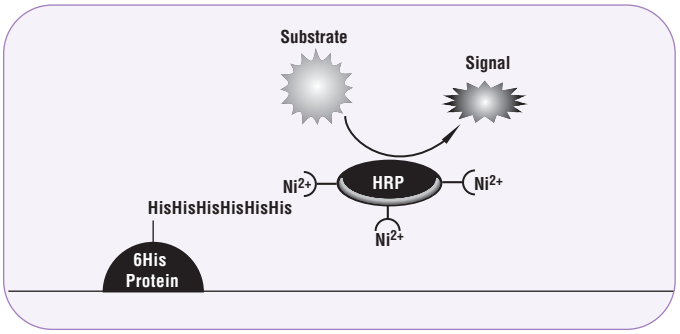
Comparison of polyhistidine-tagged (PHT) fusion protein detection methods. Procedure: Four different crude lysates were electrophoresed and transferred to nitrocellulose membranes. All samples contained equal protein concentrations. **Lane 1:** crude lysate from noninfected sf9 cells (negative control), **Lane 2:** crude lysate from infected sf9 cells expressing PHT fusion protein pA, **Lane 3:** crude lysate from infected sf9 cells expressing PHT fusion proteins pB, **Lane 4:** crude lysate from infected sf9 cells expressing PHT fusion protein pC. **Panel A:** blot probed with INDIA™ HisProbe™-HRP, **Panel B:** blot probed with nickel-activated tetradentate HRP at 0.5X concentration, **Panel C:** blot probed with nickel-activated tetradentate HRP at 1.0X concentration, **Panel D:** blot probed with anti-His antibody. All probes were detected using SuperSignal® West Pico Chemiluminescent Substrate. Results: Panel A using INDIA™ HisProbe™-HRP shows high specific binding and low background. Panels B and C using tetradentate chelation method both show nonspecific binding and high background. Panel D using anti-poly-His failed to recognize two of the three fusion proteins.

PRODUCT #	DESCRIPTION	PKG. SIZE
15168	**SuperSignal® West HisProbe™ Kit** *Sufficient reagents for fifty 7.5 x 10 cm blots* Includes: INDIA™ HisProbe™-HRP SuperSignal® West Pico Chemiluminescent Substrate Blocker™ BSA in TBS (10X) BupH™ Tris Buffered Saline Surfact-Amps™ 20 (10%) ampules	Kit 2 mg 500 ml 1 x 125 ml 10 x 500 ml 6 x 10 ml

INDIA™ HisProbe™-HRP

INDIA™-HRP and its activated derivatives are used for the detection of molecules that have an affinity for specific metals. INDIA™ HisProbe™-HRP is a nickel (Ni^{2+})-activated derivative of horseradish peroxidase (HRP). This product is optimized for direct detection of recombinant histidine-tagged proteins and other histidine-rich proteins.

- Fast — one-step probe eliminates the need to run a lengthy two-step primary/secondary reaction protocol
- High specificity — more specific for the detection of polyhistidine-tagged proteins than many antibody-based systems
- Sensitive — when used in conjunction with SuperSignal® Chemiluminescent Substrate (Product # 34080), detection of even low-expression histidine-tagged clones is possible
- Also available in a kit for Western blotting (SuperSignal® HisProbe™ Kit)



PRODUCT #	DESCRIPTION	PKG. SIZE
15165	**INDIA™ HisProbe™-HRP**	2 mg

Cell Lysis Solutions

Poppers™ Cell Lysis Reagents

Poppers™ Liquid Cell Lysis Reagents are pre-mixed and ready to use. They're the new wave of cell lysis!
No hit-or-miss homemade recipes, no glass beads, no sonicators, no French presses, no freezing and no thawing! Just pour and explore!

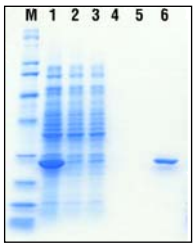
Popper (Product #)	Organisms/Samples	Dialyze[1]	Compatibility	Notes
B-PER® Reagent 78248 500 ml 78243 165 ml	Gram(-) bacteria, *S. aureus H. pylori, E. coli* strains BL21(D3)> JM109> DH5α>M15, Archaebacteria, nematodes, *Acinetobacter* sp., Insect cells	Yes	Reporter assays, IPs,[2] Western blot, GST- and His-tag purification	Protease inhibitors[3] may be added to prevent protein degradation. Salts, chelating agents, reducing agents can be added for more efficient lysis. Do not exceed 0.5 M NaCl. Better lysis if cells are frozen in B-PER® Reagent.
B-PER® II Reagent 78260 250 ml (For smaller volume samples)	Gram(-) bacteria, *S. aureus H. pylori, E. coli* strains BL21(D3)> JM109> DH5α>M15, Archaebacteria, nematodes, *Acinetobacter* sp., Insect cells	Yes	Reporter assays, IPs,[2] Western blot, GST- and His-tag purification	Protease inhibitors[3] may be added to prevent protein degradation. Salts, chelating agents, reducing agents can be added for more efficient lysis. Better lysis if cells are frozen in B-PER® Reagent.
B-PER® PBS Reagent 78266 500 ml	Gram(-) bacteria, *S. aureus H. pylori, E. coli* strains BL21(D3)> JM109> DH5α>M15, Archaebacteria, nematodes, *Acinetobacter* sp., Insect cells	Yes	Reporter assays, IPs,[2] Western blot, GST- and His-tag purification	Protease inhibitors[3] may be added to prevent protein degradation. Salts, chelating agents, reducing agents can be added for more efficient lysis. Better lysis if cells are frozen in B-PER® Reagent.
Y-PER® Reagent 78990 500 ml 78991 200 ml 75768 25 ml	*S. cerevisiae, Schizosaccharomyces pombe, C. albicans B. subtilis, E. coli, P. pastoris, Strep. avidinii, Acinetobacter* sp.	No	IPs,[2] Western blot, β-Gal enzyme assays, IEF after dialysis, GST- and His-tag purification	Protease inhibitors[3] may be added to prevent protein degradation. Use at room temperature. Double incubation time for use at 4°C. Use log phase cells. For stationary phase cells, add 0.1 M DTT or 20-50 mM TCEP. Will work with 1 mM EDTA. Does not lyse spores. Cannot use with ion exchange columns.
Y-PER® Plus Reagent 78998 25 ml 78999 500 ml	Yeast (*S. cerevisiae*) *Acinetobacter* sp.	Yes	GST- and His-tag purification, Western blot	Protease inhibitors[3] may be added to prevent protein degradation. The addition of up to 2 M NaCl may result in increased efficiency of lysis and protein yield.
M-PER® Reagent 78503 25 ml 78501 250 ml 78505 1 L	Cultured mammalian cells. COS7, NIH3T3, Hepa 1-6, 293, CHO, MDA, MB231, FM2 and Insect cells	Yes	Luciferase, β-Gal (low signal), CAT, kinase assays, ELISAs, immobilized glutathione, Western blot	Protease inhibitors[3] may be added to prevent protein degradation. Adding 150 mM NaCl results in increased efficiency of lysis and higher protein yield in some cell lines. A PBS rinse of cells prior to lysis removes contaminants such as phenol red and increases protein yield.
T-PER® Reagent 78510 500 ml	Heart, liver, kidney and brain	Yes	Luciferase, β-Gal, CAT, kinase assays, Western blot, ELISAs, immobilized glutathione	Protease inhibitors[3] may be added to prevent protein degradation. Mechanical disruption of the tissue is still required. Can also be used for cultured cells.
NE-PER® Reagent Kit 78833	Tissue: calf liver Cultured cells: epithelial (HeLa), fibroid (Cos7), kidney (NIH3T3), liver (Hepa 1), brain (C6)	No (CER) Yes (NER)	EMSA (if using <3 µl or 10%, otherwise dialyze first in SAL MINIs),[4] Western blot, Reporter assays, IEF (after dialysis to reduce salt concentration)	Protease inhibitors[3] may be added to prevent protein degradation. Packed cell vol.: 2 x 10^6 HeLa cells =10 µl = 20 mg Tissue Yield (calf liver): 3-4 mg cytoplasmic protein/100 mg tissue; 1-1.5 mg nuclear protein/100 mg tissue Cell Yield (HeLa): 300-400 µg cytoplasmic protein/10^6 cells; 40-60 µg nuclear protein/10^6 cells. Positive controls tested: cytoplasmic (-Gal, PKC, Hsp90); nuclear (Oct-1, p53, DNA polymerase)
Mem-PER® Reagent Kit 89826	Cultured cells: brain (C6), epithelial (HeLa), fibroblasts (NIH3T3), yeast (*S. cerevisiae*)	Yes[5]	Western blot	Protease inhibitors[3] may be added to prevent protein degradation. Can dialyze against another detergent (e.g., CHAPS). Extraction efficiency is generally >50% with the cell lines tested (having proteins with up to two transmembrane segments).
NEW! Optiprep™ Mitochondria Isolation Kit 89874	Mammalian cells	NA	Western blot, 2D Western blots, electrophoresis. Applications include apoptosis, signal transduction, and metabolic studies.	Protease inhibitors[3] may be added to prevent protein degradation (Halt™ Protease Inhibitor, EDTA free, Product # 78415). Douncing will increase isolation efficiency vs. detergent alone; however, multiple samples can be processed simultaneously using the reagent-based method.

1. The detergent can be removed by dialysis
2. Immunoprecipitation
3. Halt ™ Protease Inhibitor Cocktail, Product # 78410 and 78415 (EDTA-free)
4. Slide-A-Lyzer® MINI Dialysis Units.
5. Samples prepared in Mem-PER® Reagent can be dialyzed if the buffer contains detergent (e.g., CHAPS).

Tools for GST-Tagged Targets

Poppers™ GST Fusion Protein Purification Kits

- Faster, more efficient systems for the purification of GST fusion proteins
- Optimized system provides the highest purity in the least amount of time (Figure)
- Greater convenience – ready to use with no sonicator required; complete lysis achieved with B-PER® Bacterial Protein Extraction Reagent. Y-PER® Yeast Protein Extraction Reagent eliminates the need for glass beads
- Increased capacity
  - 10 mg of tagged protein bound per column with the B-PER® Fusion Protein Column Purification Kit
  - Yields approximately 1 mg purified protein per reaction with the B-PER® Fusion Protein Spin Purification Kit
  - 100% more fusion protein recovered with Y-PER® Reagent versus glass beads with over 10 mg/column capacity



SDS-PAGE analysis of GST purification using B-PER® GST Fusion Protein Purification Kit. Fractions from each purification step were subjected to SDS-PAGE analysis using gradient 4%-20% SDS-PAGE stained with GelCode™ Blue Stain Reagent (Product # 24592). **Lane 1**: the crude lysates extracted from *E. coli* with B-PER® Reagent, **Lane 2**: the flow-through from the crude lysates, **Lanes 3-4**: wash fractions of Wash Buffer 1, **Lane 5**: wash fraction of Wash Buffer 2, and **Lane 6**: GST eluted from the column with the elution buffer (50 mM glutathione).

Glutathione Immobilized on Agarose

- Binds recombinantly expressed fusion proteins containing GST for affinity purification in batch or column formats
- Capacity is 8 mg horse liver GST/ml gel
- GST fusion proteins are eluted with a gentle, competitive glutathione buffer
- Eluted fractions containing the purified GST fusion protein are easily detected using Reacti-Bind™ Glutathione Coated Plates
- Reusable

Immobilized GST

- One-step removal of GST-reactive antibodies produced as a result of using the entire GST fusion protein for antibody production
- Loading is 2 mg GST/ml gel
- Reusable

ImmunoPure® Anti-Glutathione S-Transferase

- A monoclonal antibody for the detection of GST fusion proteins

GST Orientation Kit

- Covalently attaches purified GST fusion protein to an affinity matrix
- The resulting glutathione support can be used for the purification of antibody/proteins that have an affinity for the specific fusion protein
- The purified antibody isolated with the use of this immobilized antigen support can be used directly for Western blotting applications, ELISAs and dot blots

GST Cross-linked to Glutathione

PRODUCT #	DESCRIPTION	PKG. SIZE
78200	**B-PER® GST Fusion Protein Column Purification Kit**	Kit
	Includes: B-PER® Bacterial Protein Extraction Reagent	165 ml
	Immobilized Glutathione Columns	5 x 1 ml
	Wash Buffer 1	60 ml
	Wash Buffer 2	85 ml
	Glutathione	1 g
78400	**B-PER® GST Fusion Protein Spin Purification Kit**	Kit
	Includes: B-PER® Bacterial Protein Extraction Reagent	165 ml
	Immobilized Glutathione Agarose	8 ml
	Wash Buffer	85 ml
	Glutathione	15 x 16 mg
78997	**Y-PER® GST Fusion Protein Column Purification Kit**	Kit
	Sufficient reagents for five GST fusion protein purifications from S. cerevisiae, S. pombe, B. subtilis *or* E. coli *(up to 6 g of wet cell paste/purification).*	
	Includes: Y-PER® Yeast Protein Extraction Reagent	200 ml
	Immobilized Glutathione Columns	5 x 1 ml
	Wash Buffer 1	60 ml
	Wash Buffer 2	85 ml
	Glutathione (reduced)	5 x 184 mg
78201	**GST Orientation Kit**	Kit
	Includes: Glutathione (reduced)	5 x 184 mg
	Immobilized Glutathione	2 x 2 ml
	BupH™ Modified Dulbecco's PBS Buffered Saline Pack (Wash Buffer I)	500 ml
	ImmunoPure® Elution Buffer	2 x 15 ml
	ImmunoPure® Blocking Buffer	6 ml
	Neutralization Buffer	5 ml
	ImmunoPure® Gentle Ag/Ab Elution Buffer	100 ml
	Disuccinimidyl Suberate (DSS)	2 x 13.2 mg
	Serum Separators	2
	Porous Discs	5
	Column Extenders	2
	Tris BupH™ Pack (Wash Buffer II)	1
20205	**Immobilized GST**	2 x 2 ml
20211	**Immobilized GST**	5 ml gel
15160	**Immobilized Glutathione – Gel**	10 ml
30001	**ImmunoPure® Anti-Glutathione S-Transferase**	0.1 mg
20237	**GST**	1 mg
20239	**GST**	10 x 1 mg

SwellGel® Immobilized Glutathione Discs

- High-throughput and high-capacity GST-tagged fusion protein purification
  - Capacity to bind up to 1 mg per well compared to the 10 ng per well possible with coated microplates
  - Unlike coated microplates, the SwellGel® System allows elution of the protein easily from the affinity chromatography media
- Pre-dispensed immobilized ligands on discs that rehydrate easily into a gel slurry
  - Convenience and consistency – all of the setup work is already done, saving you time and money
  - Avoid the hassles of dispensing media into 96-well plates
- Applications include tag-free protein purification directly in the SwellGel® Plate
  - Provides a powerful tool for the rapid assay of thousands of protein or peptide samples in a single experiment
- Excellent stability allows for storage at room temperature
  - Stability studies show improved shelf life over stored wet resins
  - Unlike alternative products or homemade options, SwellGel® Discs do not require refrigeration

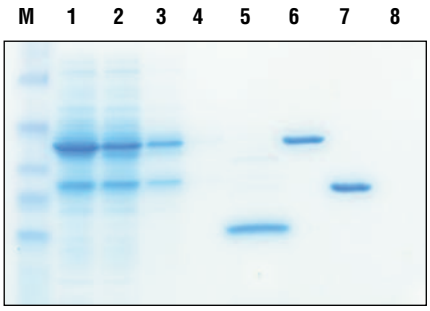


Figure 1. Thrombin cleavage of fusion protein bound to SwellGel® Immobilized Glutathione. The GST-ISG15 fusion protein was bound on SwellGel® Immobilized Glutathione followed by standard procedure of GST fusion protein purification. Thrombin was added directly on the SwellGel® Glutathione and incubated with fusion protein. The tag-free ISG15 was collected in the flow-through. **Lane M:** molecular weight marker, **Lane 1:** total cell lysate, **Lane 2:** flow-through, **Lane 3-4:** washes, **Lane 5:** flow-through following thrombin digestion of GST-ISG15 fusion protein bound to SwellGel® Immobilized Glutathione, **Lane 6:** purified GST-ISG15, and **Lane 7:** purified GST.

PRODUCT #	DESCRIPTION	PKG. SIZE
89816	**SwellGel® Immobilized Glutathione Discs***	Kit
	Each disc = 200 µl of bed volume.	
	Includes: 96-Well Purification Filterplate	1
	SwellGel® Immobilized Glutathione Discs	96
	Collection Plate	1
	BupH™ TBS Pack	1 pouch
	BupH™ PBS Pack	1 pouch
89815	**SwellGel® Immobilized Glutathione Discs**	Each
	Each disc = 200 µl of bed volume.	
	Includes: 96-Well Purification Filterplate	1
	SwellGel® Immobilized Glutathione Discs	96
	Collection Plate	1
89817	**SwellGel® Immobilized Glutathione Discs**	96 Discs
	Each disc = 200 µl of bed volume.	

Reacti-Bind™ Glutathione Coated Plates

- Bind recombinantly expressed fusion proteins containing GST, allowing ELISA analysis of the proteins
- Lower detection limit is 1 ng of GST fusion protein
- Easy quantitation of antibodies raised against GST-fused proteins
- Simple format for protein:protein interaction studies
- Detergents used to lyse cells don't inhibit binding to pre-coated plates as they do with plain polystyrene
- Pre-blocked to reduce nonspecific binding

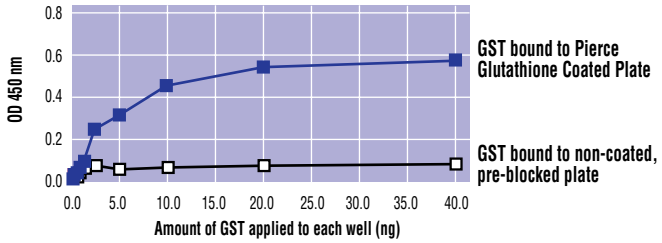


Figure 2. Comparison of binding of glutathione S-transferase on glutathione. Coated plates vs. non-coated, pre-blocked plates. Glutathione S-transferase was incubated with Reacti-Bind™ Glutathione Coated Plates and with non-coated pre-blocked plates. GST was detected with Anti-GST antibody conjugated to HRP with Turbo-TMB as the substrate.

Reacti-Bind™ Anti-GST Coated Plates

- A unique alternative to glutathione-coated plates for binding GST fusion proteins
- Sensitive – detects GST proteins down to 7.5 ng/well
- Flexible – binds native or denatured forms of GST; an alternative to glutathione-coated plates
- Saves time – pre-blocked, so there's no need to coat plates and block
- Compatibility – Poppers™ Cell Lysis Reagents will not interfere with GST fusion protein binding

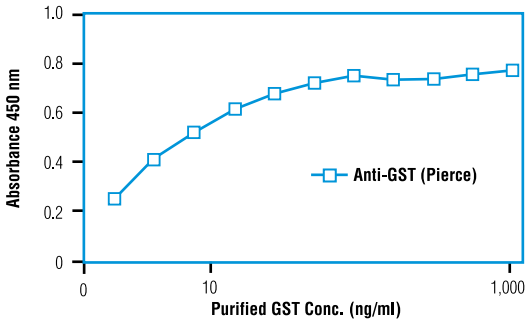


Figure 3. Reacti-Bind™ Anti-GST Coated Plates were tested for their ability to bind and detect GST. The plates were assayed with purified GST that was detected using a rabbit anti-GST antibody (1 µg/ml) followed by donkey anti-rabbit HRP conjugate. The signal was developed with 1-Step™ Turbo TMB•ELISA Substrate (Product # 34022).

PRODUCT #	DESCRIPTION	PKG. SIZE
15140	**Reacti-Bind™ Glutathione Coated Plates** 96-Well Plate in an 8-Well Strip Plate Format	5 plates
15240	**Reacti-Bind™ White Opaque Glutathione Coated Plates** 96-Well Plate (for chemiluminescent detection)	5 plates
15340	**Reacti-Bind™ Black Opaque Glutathione Coated Plates** 96-Well Plate (for fluorescent detection)	5 plates
15145	**Reacti-Bind™ Anti-GST Coated Clear Plates, 8-Well Strip**	5 plates

Tools for Biotinylated Targets

Reacti-Bind™ NeutrAvidin™ Coated Plates

Pierce recognizes that the use of fusion proteins is an inexact science. The tag portion of the fusion protein is sometimes inaccessible. This is often the result of the tag being internalized during protein folding. In this case, an alternate method of placing a handle on a fusion protein is post-expression biotinylation. This simple process covalently links biotin to the target protein and allows easy manipulation for screening, purifying or studying protein interaction.

- Less nonspecific binding than either avidin or streptavidin
- Easy quantitation of antibodies raised against biotin-containing fusion proteins
- Simple format for protein:protein interaction studies
- Detergents used to lyse cells don't inhibit binding to pre-coated plates as they do with plain polystyrene
- Pre-blocked to reduce nonspecific binding
- Available in clear, black, white or strip plates

PRODUCT #	DESCRIPTION	PKG. SIZE
15115	**Reacti-Bind™ Biotin Binding Plate Sample Pack** One each of the following plates: Product #'s 15120, 15121, 15127 and 15128	4 plates
15123	**Reacti-Bind™ NeutrAvidin™ Coated 96-Well Plates, Clear Plates with Blocker™ BSA**	5 plates
15127	**Reacti-Bind™ NeutrAvidin™ Coated Strip Plates, Clear Plates with SuperBlock® Blocking Buffer**	5 plates
15128	**Reacti-Bind™ NeutrAvidin™ Coated Polystyrene Strip Plates With Blocker™ BSA**	5 plates
15129	**Reacti-Bind™ NeutrAvidin™ Coated Polystyrene 96-Well Plates With SuperBlock® Blocking Buffer**	5 plates
15116	**Reacti-Bind™ NeutrAvidin™ Coated White Polystyrene Plates With SuperBlock® Blocking Buffer**	5 plates
15117	**Reacti-Bind™ NeutrAvidin™ Coated Black Polystyrene Plates With SuperBlock® Blocking Buffer**	5 plates
15400	**Reacti-Bind™ NeutrAvidin™ Coated Clear 384-Well Plates with SuperBlock® Blocking Buffer**	5 plates
15401	**Reacti-Bind™ NeutrAvidin™ Coated White 384-Well Plates with SuperBlock® Blocking Buffer**	5 plates
15402	**Reacti-Bind™ NeutrAvidin™ Coated Black 384-Well Plates with SuperBlock® Blocking Buffer**	5 plates

Immobilized Monomeric Avidin

- Biotinylated proteins are easily eluted with a gentle, competitive biotin buffer
- Binding capacity is 2 mg biotinylated BSA/ml gel
- Binds biotinylated proteins for affinity purification in batch or column format
- Eluted fractions containing the purified biotin-containing fusion proteins are easily detected using Reacti-Bind™ NeutrAvidin™ Coated Plates
- Reusable

PRODUCT #	DESCRIPTION	PKG. SIZE
20228	**ImmunoPure® Immobilized Monomeric Avidin Gel** *Includes disposable column trial kit.*	5 ml
20227	**ImmunoPure® Immobilized Monomeric Avidin Kit** Includes: Monomeric Avidin Column BupH™ Phosphate Buffered Saline Pack (yields 500 ml) Biotin Blocking and Elution Buffer Regeneration Buffer Column Extender	Kit 2 ml 1 pack 200 ml 250 ml
53146	**UltraLink® Immobilized Monomeric Avidin**	5 ml
29129	**ImmunoPure® D-Biotin**	1 g

Immobilized NeutrAvidin™ Gel

- Near covalent strength binding of biotin-containing fusion proteins for affinity purification of antibodies raised against them
- Less nonspecific binding than either avidin or streptavidin
- Binding capacity of 2 mg biotinylated BSA/ml of gel
- Biotin-containing fusion proteins remain on the column while antibodies raised against them or target ligands can be eluted

PRODUCT #	DESCRIPTION	PKG. SIZE
29200	**Immobilized NeutrAvidin™ Gel**	5 ml
31000	**ImmunoPure® NeutrAvidin™ Biotin-Binding Protein** • pI that has been reduced to a neutral state • Deglycosylated, so lectin binding is reduced to undetectable levels • Can be used as a biotin blocking agent in tissues for histochemistry	10 mg
53150	**UltraLink® Immobilized NeutrAvidin™** *Includes disposable column trial kit.* Capacity: Approximately 9-12 µg of biotin/ml gel	5 ml
53151	**UltraLink® Immobilized NeutrAvidin™ Plus** *Includes disposable column trial kit.* Capacity: ≥17 µg of biotin/ml gel	5 ml
31001	**NeutrAvidin™, Horseradish Peroxidase Conjugated** • Lower nonspecific binding than streptavidin conjugates • Better signal:noise ratio in assay systems	2 mg
31002	**NeutrAvidin™, Alkaline Phosphatase Conjugated** • Lower nonspecific binding than streptavidin conjugates • Better signal:noise ratio in assay systems	2 mg
31006	**NeutrAvidin™, Fluorescein Conjugated** • Fluorescent-labeled NeutrAvidin™ Biotin-Binding Protein • Absorption: 490 nm; Emission 520 nm • ≥ 2 moles fluorescein/mole NeutrAvidin™ Protein	5 mg
31007	**EZ-Link™ Maleimide Activated NeutrAvidin™ Biotin-Binding Protein** • Prepare NeutrAvidin™ conjugates of proteins/peptides • Reacts spontaneously with free sulfhydryls in the pH range of 6.5-7.5 • 4-8 moles maleimide/mole NeutrAvidin™ Protein	5 mg

Immobilized Streptavidin Products

- A superior alternative to immobilized Avidin
- Low nonspecific binding
- High biotin affinity
- "Plus" version has twice the amount of Streptavidin loaded per ml of gel

PRODUCT #	DESCRIPTION	PKG. SIZE
20351	**AffinityPak™ Immobilized Streptavidin Columns**	5 x 1 ml
53113	**UltraLink® Immobilized Streptavidin Gel** Capacity: ≥2 mg of biotinylated BSA/ml of gel	2 ml
53114	**UltraLink® Immobilized Streptavidin Gel**	5 ml
53116	**UltraLink® Immobilized Streptavidin Plus Gel** Capacity: ≥3 mg of biotinylated BSA/ml of gel	2 ml
20347	**ImmunoPure® Immobilized Streptavidin Gel** Support: Cross-linked 6% beaded agarose	2 ml
20349	**ImmunoPure® Immobilized Streptavidin Gel**	5 ml

Reacti-Bind™ Streptavidin Coated Polystyrene Plates

- Easy and gentle immobilization of biotin-containing conjugates
- Low nonspecific binding
- No denaturing of the protein component of a conjugate during binding
- Ideal for binding small biotinylated hydrophilic molecules (e.g., peptides) that typically exhibit poor binding to polystyrene
- Pre-blocked with your choice of Blocker™ BSA or SuperBlock® Blocking Buffer
- Binding capacity for 96-well plates: 5 pmoles of biotinylated fluorescein per well using a 100 µl coat volume and 200 µl blocking volume
- Binding capacity for 384-well plates: 4 pmoles of biotinylated fluorescein per well using a 50 µl coat volume and 100 µl blocking volume

Reacti-Bind™ Biotin Coated Polystyrene Plates

A simple format for testing the efficiency of avidin, streptavidin or NeutrAvidin™ Protein conjugations.

Reacti-Bind™ Biotin Coated Plates can be used in any immunoassay with NeutrAvidin™ Biotin-Binding Protein, streptavidin, avidin or other biotin-binding proteins.

- Easy to use – simply add the conjugate, rinse and add your detection reagent
- Pre-blocked with Blocker™ BSA to reduce nonspecific binding
- Convenient 8-well strip format

PRODUCT #	DESCRIPTION	PKG. SIZE
15120	Reacti-Bind™ Streptavidin Coated Polystyrene Strip Plates with SuperBlock® Blocking Buffer (Clear)	5 plates
15121	Reacti-Bind™ Streptavidin Coated Polystyrene Strip Plates with Blocker™ BSA (Clear)	5 plates
15122	Reacti-Bind™ Streptavidin Coated Polystyrene Strip Plates with SuperBlock® Blocking Buffer (Clear)	25 plates
15124	Reacti-Bind™ Streptavidin Coated 96-Well Plates with SuperBlock® Blocking Buffer (Clear)	5 plates
15125	Reacti-Bind™ Streptavidin Coated 96-Well Plates with Blocker™ BSA (Clear)	5 plates
15126	Reacti-Bind™ Streptavidin Coated Polystyrene 96-Well Plates with SuperBlock® Blocking Buffer (Clear)	25 plates
15118	Reacti-Bind™ Streptavidin Coated White Polystyrene 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15119	Reacti-Bind™ Streptavidin Coated Black Polystyrene 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15405	Reacti-Bind™ Streptavidin Coated Clear 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15406	Reacti-Bind™ Streptavidin Coated White 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15407	Reacti-Bind™ Streptavidin Coated Black 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15151	Reacti-Bind™ Biotin Coated Polystyrene Plates	5 plates

ImmunoPure® Goat Anti-Biotin

- A polyclonal antibody for the detection of biotin

PRODUCT #	DESCRIPTION	PKG. SIZE
31852	ImmunoPure® Goat Anti-Biotin	1.0 mg

Reacti-Bind™ Streptavidin and NeutrAvidin™ HBC Coated Plates

Take advantage of a new Pierce technology that provides a broader dynamic range.

Figure 1. Fluorescein-labeled oligonucleotide hybridization assay comparison. Comparison of Reacti-Bind™ Streptavidin High Binding Capacity (HBC) Coated Plate with competing High Binding Capacity plate (CHC). Plates were incubated with a biotinylated oligonucleotide, washed and probed with a complementary oligonucleotide labeled with fluorescein at various dilutions. The Y-axis is described as the signal:noise (S/N) ratio.

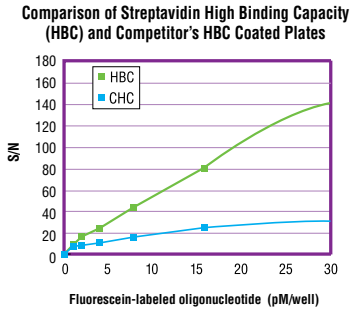
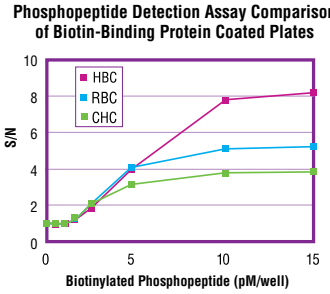


Figure 2. Comparison of NeutrAvidin™ High Binding Capacity (HBC) Coated Plate, NeutrAvidin™ Regular Binding Capacity (RBC) Coated Plates and a competitor's Streptavidin Coated High Binding Capacity Plates (CHC). Plates were incubated with various dilutions of biotinylated, phosphorylated pep-tide. After washing, the plates were incubated with mouse anti-phosphotyrosine antibody (1:1,000) and then detected using an anti-mouse-FITC conjugate (1:666). The Y-axis is described as the signal:noise (S/N) ratio.



Reacti-Bind™ NeutrAvidin™ HBC Coated Plate Characteristics

	96-Well Plate	384-Well Plate
Binding Capacity	60 pmol/well	35 pmol/well
Coat Volume	100 µl/well	50 µl/well
Blocking Volume	200 µl/well	100 µl/well

PRODUCT #	DESCRIPTION	PKG. SIZE
15500	Reacti-Bind™ Streptavidin Coated Plates (HBC), Clear 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15501	Reacti-Bind™ Streptavidin Coated Plates (HBC), Clear 8-Well Strip Plates with SuperBlock® Blocking Buffer	5 plates
15502	Reacti-Bind™ Streptavidin Coated Plates (HBC), White 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15503	Reacti-Bind™ Streptavidin Coated Plates (HBC), Black 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15504	Reacti-Bind™ Streptavidin Coated Plates (HBC), Clear 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15506	Reacti-Bind™ Streptavidin Coated Plates (HBC), Black 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15505	Reacti-Bind™ Streptavidin Coated Plates (HBC), White 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15507	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Clear 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15508	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Clear 8-Well Strip Plates with SuperBlock® Blocking Buffer	5 plates
15509	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), White 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15510	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Black 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15511	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Clear 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15512	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), White 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15513	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Black 384-Well Plates with SuperBlock® Blocking Buffer	5 plates

Applications of HA- or c-Myc-Tagged Targets

ProFound™ HA- or c-Myc-Tagged Protein IP/Co-IP Kits

Complete kit

- Includes all essential components to perform IP and co-IP experiments
- No formulating necessary

High affinity and specificity

- High yield with small quantities of antibody resin
- Nonspecific background minimized or eliminated
- No antibody contamination in sample
- Clean Western blots – easy data interpretation

Simple and flexible

- Useful for IP and co-IP
- Antibody-resin and lysate requirements easily scaled to your needs
- Handee™ Mini-Spin Columns facilitate efficient washing and elution steps

Immunoprecipitation (IP) of epitope-tagged proteins via tag-specific antibodies can be used to determine the protein's cellular localization, to study post-translational modifications or to detect interactions between tagged proteins and other proteins (co-IP). Two common epitope tags for mammalian expression systems are the HA tag (YPYDVPDYA), which is derived from the human hemagglutinin (HA) protein, and the c-Myc tag (EQKLISEEDL), which is derived from the C-terminus of human c-Myc protein.

The most critical requirement for a successful IP or co-IP is the antibody. The new ProFound™ HA or c-Myc Tagged Protein IP/Co-IP Kits include high-affinity, high-specificity anti-HA or anti-c-Myc antibody-coupled agarose that enables IP of HA- or c-Myc-tagged proteins or co-IP of their interacting partners – even when they are in low abundance. The covalent linkage between the antibody and the agarose results in a final IP/co-IP product free of antibody contamination and eliminates nonspecific background that can occur when Protein A or Protein G is used. The kits contain all the necessary reagents, buffers, eluents and a positive control to perform successful IP/co-IP experiments. Spin columns facilitate the handling of small volumes of resin, enabling the entire precipitation assay to be performed in a single device. These columns allow more efficient separation of buffer from the antibody-coupled agarose and prevent resin loss during the wash steps. The ProFound™ Mammalian HA or c-Myc Tagged Protein IP/Co-IP Kits also include M-PER® Mammalian Protein Extraction Reagent, which quickly and gently lyses mammalian cells for easy extract preparation before IP or co-IP.

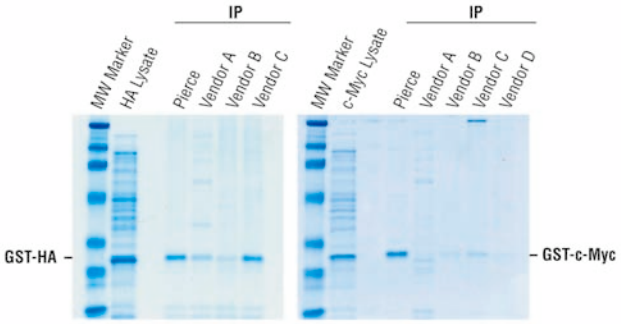


Figure 1. Comparison of the effectiveness of anti-HA- and anti-c-Myc-coupled resins. The coupled resins used in the Pierce ProFound™ IP/Co-IP Kits were compared to those supplied by other vendors in IP experiments. Each IP utilized the same amount of immobilized antibody (10 µg of anti-HA or 5 µg of anti-c-Myc) from each vendor and the same amount of GST-HA or GST-c-Myc lysate (50 µl). IP products were separated by SDS-PAGE and stained with GelCode™ Blue Stain Reagent (Product # 24590).

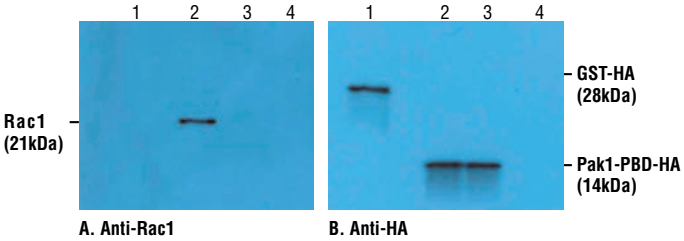


Figure 2. Co-IP of active Rac1 with HA-tagged Pak1-PBD (p21 binding domain). Human 293 cells were transfected with HA-Pak1-PBD alone or co-transfected with constitutively activated Rac1 (Q61L). Anti-HA agarose slurry (6 µl) was incubated with 50 µl HA-tagged positive control lysate from the kit (Lane 1) or 500 µl cell lysate from Rac1 (Q61L) and HA-Pak1-PBD co-transfected cells (Lane 2), HA-Pak1-PBD transfected cells (Lane 3) or non-transfected cells (Lane 4). IP and co-IP reactions were performed at 4°C overnight. The Western blot was first probed with anti-Rac1 antibody (panel A) and then reprobed with anti-HA antibody (panel B).

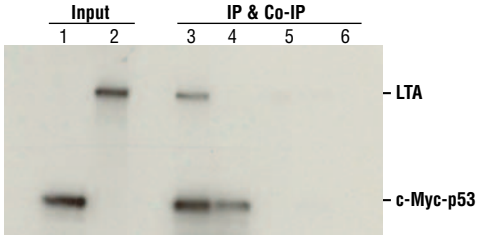


Figure 3. Co-IP of SV40 large T-antigen (LTA) with c-Myc-tagged p53. LTA and c-Myc-p53 were expressed and ^{35}S-labeled *in vitro* (Lane 1 and 2). Before IP and co-IP, the lysates (5 µl each) of LTA and c-Myc-p53 (Lanes 3 and 6), c-Myc-p53 alone (Lane 4) or LTA alone (Lane 5) were incubated at 30°C for 1 hour. Anti-c-Myc agarose (5 µg antibody in 10 µl slurry) (Lanes 3, 4 and 5) or plain agarose slurry (Lane 6) was added to the corresponding sample. IP and co-IP reactions were performed at 4°C overnight. IP and co-IP products were eluted and separated on 12% SDS-PAGE. The ^{35}S-labeled proteins were detected by fluorography.

PRODUCT #	DESCRIPTION	PKG. SIZE
23610	**ProFound™ HA-Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with an HA tag. The kit is supplied complete with an HA-tagged positive control lysate containing over-expressed GST-HA.* Includes: Product # 23612, ProFound™ HA-Tag IP/Co-IP Application Set Product # 23613, HA-Tagged Positive Control	Kit 500 µl
23615	**ProFound™ Mammalian HA-Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with an HA tag. The kit is supplied complete with a mammalian cell lysis buffer and an HA-tagged positive control lysate containing over-expressed GST-HA.* Includes: Product # 23617, ProFound™ Mammalian HA-Tag IP/Co-IP Application Set Product # 23613, HA-Tagged Positive Control	Kit 500 µl
23620	**ProFound™ c-Myc-Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with a c-Myc peptide tag. Kit is supplied complete with a c-Myc-tagged positive control lysate containing over-expressed GST-c-Myc.* Includes: Product # 23622, ProFound™ c-Myc Tag IP/Co-IP Application Set Product # 23633, c-Myc Tagged Positive Control	Kit 500 µl
23625	**ProFound™ Mammalian c-Myc Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with a c-Myc peptide tag. Kit is supplied complete with a mammalian cell lysis buffer and a c-Myc-tagged positive control lysate containing over-expressed GST-c-Myc.* Includes: Product # 23627, ProFound™ Mammalian c-Myc Tag IP/Co-IP Application Set Product # 23633, c-Myc Tagged Positive Control	Kit 500 µl

Tools for MBP-Tagged Targets

Reacti-Bind™ Dextrin Coated Plates

- Bind recombinantly expressed fusion proteins containing MBP, allowing ELISA analysis of the proteins
- Lower detection limit is 5 ng of purified MBP
- Easy quantitation of antibodies raised against MBP-fused proteins
- Simple format for protein:protein interaction studies
- Detergents used to lyse cells don't inhibit binding to pre-coated plates as they do with plain polystyrene
- Pre-blocked to reduce nonspecific binding

Factor Xa for Fusion Tag Removal

Many fusion proteins are often engineered to contain a cleavage site targeted by proteases. Factor Xa is extremely specific for the Ile-Glu-Gly-Arg sequence. By engineering this sequence between the tag and target molecules, the two can be cleaved using enzymatic digestion. Pierce Factor Xa has an activity of >130 U/mg.

PRODUCT #	DESCRIPTION	PKG. SIZE
15141	**Reacti-Bind™ Dextrin Coated Plates** 96-Well Plate in an 8-Well Strip Plate Format	5 plates
32521	**Factor Xa (Bovine)**	50 µg

Complementary Products

Coated/Activated Microplates

Product #	Description	Pkg. Size
15131	**Reacti-Bind™ Protein G Coated Plates, 96-Well Format**	5 plates
15130	**Reacti-Bind™ Protein A Coated Plates, 96-Well Format**	5 plates
15134	**Reacti-Bind™ Goat Anti-Mouse Coated Plates, 96-Well Format**	5 plates
15135	**Reacti-Bind™ Goat Anti-Rabbit Coated Plates, 96-Well Format**	5 plates

Binding/Elution Buffer Components

Product #	Description	Pkg. Size
28372	**BupH™ Phosphate Buffered Saline Packs**	40 packs
28374	**BupH™ Modified Dulbecco's Phosphate Buffered Saline Packs**	40 packs
28376	**BupH™ Tris Buffered Saline Packs**	40 packs
28320	**Surfact-Amps™ 20** Active Ingredient: Tween®-20 (10%)	6 x 10 ml
21004	**ImmunoPure® IgG Elution Buffer**	1 liter
29129	**ImmunoPure® D-Biotin**	1 g

Substrates

Product #	Description	Pkg. Size
34021	**ImmunoPure® TMB Substrate Kit**	400 ml
34022	**1-Step™ Turbo TMB-ELISA**	250 ml
37615	**1-Step™ ABTS**	250 ml
37620	**Phosphatase Substrate Kit (Contains PNPP)**	Kit
34012	**1-Step™ Chloronaphthol (4CN)**	250 ml
34018	**1-Step™ TMB-Blotting**	250 ml
34065	**ImmunoPure® Metal Enhanced DAB Substrate Kit**	Kit
34034	**ImmunoPure® Fast Red TR/AS-MX Substrate Kit**	Kit
34042	**1-Step™ NBT/BCIP**	250 ml
34080	**SuperSignal® West Pico Chemiluminescent Substrate**	500 ml
34075	**SuperSignal® West Dura Extended Duration Substrate**	100 ml
34095	**SuperSignal® West Femto Maximum Sensitivity Substrate**	100 ml

Biotinylation Reagents

Product #	Description	Pkg. Size
21217	**EZ-Link® Sulfo-NHS-Biotin**	50 mg
21333	**EZ-Link® Iodoacetyl-LC-Biotin**	50 mg
21335	**EZ-Link® Sulfo-NHS-LC-Biotin**	100 mg
20217	**EZ-Link® NHS-Biotin**	100 mg
21336	**EZ-Link® NHS-LC-Biotin**	50 mg
21340	**EZ-Link® Biotin-LC-Hydrazide**	50 mg
21900	**EZ-Link® Biotin-BMCC**	50 mg
29987	**EZ-Link® Photoactivatable Biotin**	0.5 mg

Accessories

Product #	Description	Pkg. Size
89849	**Protein Desalting Spin Columns** • Fast – samples can be processed in less than 5 minutes • Convenient – no cumbersome preparation steps are required • High protein recovery – provide greater than 95% salt retention	25/pkg.
89862	**Protein Desalting Spin Columns**	25/pkg.
69700	**Handee™ Spin Cup Columns**	50 units
69705	**Handee™ Mini-Spin Columns Plus Accessories** *(Does not include microcentrifuge tubes.)*	25 units
69710	**Handee™ Resin Separators**	25 units
69715	**Handee™ Microcentrifuge Tubes, 1.5 ml**	72 tubes

U.S. patent pending on SwellGel® Technology.
SuperSignal® Technology is protected by U.S. Patent # 5,503,741.
B-PER® Technology is protected by U.S. Patent # 6,174,704.
U.S. patent pending on Optiprep™ Technology.
Tween® is a trademark of ICI Americas.

Protein Chemistry

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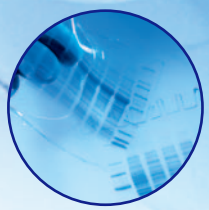
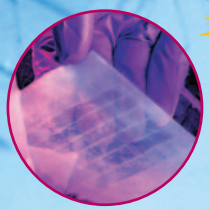
Get great results in record time.

Get to know Pierce GelCode™ Gel Staining Products – the world standard for ease-of-use, speed, performance and economy in life science laboratories.



GelCode™ Blue Stain

GelCode™ SilverSNAP® Stain II



GelCode™ E-Zinc® Stain

www.piercenet.com

GelCode™ Blue Stain Reagent

- No destaining required with this Coomassie dye-based SDS-PAGE gel stain
- Exceptional results in just 2 hours

Product #	Description	Pkg. Size
24590	GelCode™ Blue Stain Reagent	500 ml
24592	GelCode™ Blue Stain Reagent	3.5 liters

NEW!

GelCode™ SilverSNAP® Stain Kit II

- Easy-to-use, sensitive and reliable stain
- More user flexibility in fixing and staining steps
- Low uniform backgrounds aid in band detection

Product #	Description	Pkg. Size
24612	GelCode™ SilverSNAP® Stain Kit II	Kit

GelCode™ E-Zinc® Reversible Stain Kit

- Rapid staining, easily reversed and sensitivity comparable to silver
- Great results in 15 minutes

Product #	Description	Pkg. Size
24582	GelCode™ E-Zinc® Reversible Stain Kit	Kit

PIERCE

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