FLAG®

A Proven System for Detection and Purification of Proteins

The FLAG Expression System is an established way to express, purify, and detect recombinant fusion proteins. FLAG and 3×FLAG® have proven utility in numerous applications such as Western blotting, immunocytochemistry, immunoprecipitation, flow cytometry, protein purification, and in the study of protein-protein interactions, cell ultrastructure, and protein localization. These small hydrophilic tags facilitate superior detection and purification of recombinant fusion proteins when using our highly specific and sensitive ANTI-FLAG® antibodies.

- Proven: Cited in more than 2,000 publications
- Ultrasensitive: Antibodies detect as little as 100 femtomoles of protein
- Comprehensive: Complete systems in multiple formats

Cat. No.	Quantity	Product Description	Characteristics	Applications
FLAG Affinity Gels				
A4596	1 ml 5 ml 10 ml 25 ml	ANTI-FLAG M1 Agarose Affinity Gel	**Specificity:** N-terminal FLAG fusion proteins. Binding Ca^{2+}-dependent; the complex dissociates in the absence of calcium ions. Does not bind to Met-FLAG fusion proteins, will not recognize unprocessed, cytoplasmically expressed proteins **Binding Capacity:** ≥0.6 mg protein per ml gel, binding requires Ca^{2+} **Elution:** FLAG peptide; glycine, pH 3.5; EDTA **Form:** Suspension of beaded agarose in 50% glycerol containing 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, 0.02% (w/v) sodium azide	■ Immunoprecipitation ■ Purification of N-terminal FLAG fusion proteins
A2220	1 ml 5 ml 10 ml 25 ml	ANTI-FLAG M2 Agarose Affinity Gel	**Specificity:** N-terminal, Met-N-terminal, C-terminal FLAG fusion proteins, 3×FLAG fusion proteins **Binding Capacity:** ≥0.6 mg per ml gel **Elution:** FLAG peptide; glycine, pH 3.5; 3×FLAG Peptide **Form:** Suspension of beaded agarose in 50% glycerol containing 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, 0.02% (w/v) sodium azide, freezer safe	■ Immunoprecipitation ■ Purification of FLAG and 3×FLAG fusion proteins
F2426	1 ml 5 × 1 ml	EZview™ Red ANTI-FLAG® M2 Affinity Gel	**Specificity:** N-terminal, Met-N-terminal, C-terminal FLAG fusion proteins, 3×FLAG fusion proteins **Binding Capacity:** ≥0.6 mg per ml gel **Elution:** FLAG peptide glycine, pH 3.5; 3×FLAG peptide **Form:** Suspension of red colored beaded agarose in phosphate buffered saline containing 50% glycerol and 0.0015% Kathon® CG/IPCII as an antimicrobial preservative	■ Immunoprecipitation
FLAG Peptides				
F3290	4 mg 25 mg	FLAG Peptide	**Sequence:** Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys **MW:** 1013 **Form:** Lyophilized powder	■ Elution of FLAG fusion proteins from the ANTI-FLAG M1 and M2 affinity resins **Working Concentration:** ■ 100 µg/ml is commonly used to elute FLAG fusion proteins
F4799	4 mg 25 mg	3×FLAG Peptide	**Sequence:** Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys **Note:** The Asp-Tyr-Lys-Xaa-Xaa-Asp motif is repeated three times in the peptide; the eight amino acids at the C-terminus make up the classic FLAG sequence (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) **MW:** 2861.9 **Form:** Lyophilized powder	■ Elution of 3×FLAG fusion proteins from ANTI-FLAG M2 affinity gels **Working Concentration:** ■ 100 µg/ml is commonly used to elute 3×FLAG fusion proteins

Cat. No.	Quantity	Product Description	Characteristics	Applications
FLAG Antibodies				
F3040	200 µg 1 mg 5 mg	ANTI-FLAG M1 Monoclonal Antibody, Purified IgG	**Specificity:** N-terminal FLAG. Binding is Ca^{2+}- dependent; the complex dissociates in the absence of calcium ions. Does not bind to Met-FLAG fusion proteins; will not recognize unprocessed, cytoplasmically expressed proteins **Form:** Solution in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% sodium azide	■ Immunoprecipitation ■ Immunocytochemistry ■ Western blotting ■ EIA **Working Dilution:** ■ 10 µg/ml by indirect Western blotting (chemiluminescent)
F3165	200 µg 1 mg 5 mg	ANTI-FLAG M2 Monoclonal Antibody, Purified IgG	**Specificity:** N-terminal, Met-N-terminal, Carboxy-terminal, or internal. Binding is not Ca^{2+}-dependent **Form:** Solution in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% sodium azide	■ Immunoprecipitation ■ Immunocytochemistry ■ Western blotting ■ EIA **Working Dilution:** ■ 10 µg/ml by indirect Western blotting (chemiluminescent)
F1804	200 µg 1 mg 5 mg	Monoclonal ANTI-FLAG M2, antibody produced in mouse, affinity purified	**Specificity:** N-terminal, Met-N-terminal, Carboxyl-terminals, or internal. Binding is not Ca^{2+}-dependent	■ Immunoprecipitation ■ Immunocytochemistry ■ Western blotting ■ EIA **Working Dilution:** ■ 10 µg/ml by indirect Western blotting (chemiluminescent)
F4042	200 µg 1 mg 5 mg	ANTI-FLAG M5 Monoclonal Antibody	**Specificity:** Met-N-terminal FLAG. Useful for detecting cytoplasmically expressed Met-FLAG fusion proteins in mammalian crude cell extracts, but not recommended for fusion proteins expressed in *E. coli*. Binding is not Ca^{2+}-dependent **Form:** Solution in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% sodium azide	■ Western blotting **Working Dilution:** ■ 10 µg/ml by indirect Western blotting (chemiluminescent)
F2555	200 µl	Rabbit Monoclonal ANTI-FLAG, ascites fluid	**Specificity:** N-terminal and C-terminal FLAG fusion proteins	■ Immunoprecipitation ■ Immunocytochemistry ■ Western blotting ■ EIA **Working Dilution:** 1:1,000-1:2,000 by indirect Western blotting (chemiluminescent)
F7425	200 µg	Rabbit ANTI-FLAG Polyclonal Antibody	**Specificity:** Reacts with N-terminal, Met-N-terminal, and C-terminal FLAG fusion proteins. Binding is not Ca^{2+}-dependent **Form:** Solution of affinity isolated antibody in 10 mM phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide	■ Immunoprecipitation ■ Immunocytochemistry ■ Western blotting ■ Dot blotting **Working Dilution:** ■ 5 µg/ml by indirect immunofluorescence ■ 2.5 µg/ml by indirect Western blotting (chemiluminescent)

See FLAG Vectors, page 188.

FLAG

Cat. No.	Quantity	Product Description	Characteristics	Applications
FLAG® Antibody Conjugates				
F9291	200 µg 1 mg 5 × 1 mg	ANTI-FLAG® BioM2 Antibody, Biotin Conjugate	**Specificity:** N-terminal, Met-N-terminal or C-terminal of FLAG fusion proteins. Binding is not Ca^{2+}-dependent **Form:** Solution in 50% glycerol, 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% sodium azide. The conjugate protein concentration is ~1 mg/ml	■ Western blotting ■ Immunocytochemistry **Working Dilution:** ■ 10 µg/ml by indirect Western blotting (chemiluminescent)
F2922	200 µg 1 mg	ANTI-FLAG BioM5 Monoclonal Antibody, Biotin Conjugate	**Specificity:** Met-N-terminal FLAG fusion proteins. Binding is not Ca^{2+}-dependent. ANTI-FLAG BioM5 is **not** recommended for detection of FLAG fusion proteins in *E. coli* **Form:** Solution in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% sodium azide	■ Western blotting ■ Immunocytochemistry **Working Dilution:** ■ 2 µg/ml by indirect Western blotting (chemiluminescent)
A9469	200 µg 1 mg 5 × 1 mg	ANTI-FLAG M2-Alkaline Phosphatase	**Specificity:** N-terminal, Met-N-terminal, or C-terminal of FLAG fusion proteins. Especially useful in detection of FLAG fusion proteins expressed in murine host, where secondary anti-mouse antibodies may cause cross-reactivity. Binding is not Ca^{2+}-dependent **Form:** Purified immunoglobulin solution in Tris buffered saline containing 50% glycerol plus stabilizer and preservative. The conjugate protein concentration is ~1 mg/ml	■ Western blotting ■ ELISA **Working Dilution:** ■ 1:20,000 by indirect ELISA
A8592	200 µg 1 mg 5 × 1 mg	ANTI-FLAG M2-Peroxidase	**Specificity:** N-terminal, Met-N-terminal or C-terminal of FLAG fusion proteins. Especially useful in detection of FLAG fusion proteins expressed in murine host, where secondary anti-mouse antibodies may cause cross-reactivity. Binding is not Ca^{2+}-dependent **Form:** Solution in phosphate buffered saline containing 50% glycerol plus preservative and stabilizer. The conjugate protein concentration is ~1 mg/ml	■ Immunocytochemistry ■ Immunohistochemistry ■ ELISA ■ Western blotting **Working Dilution:** ■ 1:20,000 by indirect ELISA ■ 1:100-1:1,000 by immunocytochemistry
F4049	200 µg 1 mg 5 × 1 mg	ANTI-FLAG M2 Monoclonal Antibody-FITC	**Specificity:** N-terminal, Met-N-terminal or C-terminal of FLAG fusion proteins. Binding is not Ca^{2+}-dependent **Form:** Solution in 10 mM sodium phosphate, 150 mM NaCl, 1% bovine serum albumin, 0.1% sodium azide, pH 7.4	■ Fluorescent immunocytochemistry ■ Fluorescent immunohistochemistry ■ Flow cytometry **Working Dilution:** ■ 10 µg/ml by indirect immunofluorescence using mammalian cells fixed with methanol:acetone
A9594	200 µg 1 mg 5 × 1 mg	ANTI-FLAG M2-Cy™3 Conjugate	**Specificity:** N-terminal, Met-N-terminal or C-terminal of FLAG fusion proteins. Especially useful in detection of FLAG fusion proteins expressed in murine host, where secondary anti-mouse antibodies may cause cross-reactivity. Binding is not Ca^{2+}-dependent **Form:** Purified immunoglobulin in phosphate buffered saline plus 1% BSA and preservative. The conjugate protein concentration is ~1 mg/ml	■ Immunocytochemistry **Working Dilution:** ■ 10 µg/ml by direct immunofluorescence using mammalian cells fixed with methanol:acetone

Cat. No.	Quantity	Product Description	Characteristics	Applications
Secondary Antibodies				
A9044	2 ml	Rabbit Anti-Mouse IgG, (whole molecule) Peroxidase Conjugate	**Specificity:** Mouse IgG Binds all mouse Igs **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal as a preservative	**Working Dilution:** ■ 1:6,000-8,000 by dot blotting ■ 1:40,000 by direct ELISA ■ 1:200 by immunohisto-chemistry (formalin-fixed, paraffin-embedded sections) ■ 1:80,000 by indirect Western blotting (chemiluminescent)
A9917	1 ml	Goat Anti-Mouse IgG, Fab Fragment Peroxidase Conjugate, Adsorbed with Human IgG	**Specificity:** Mouse IgG Fab Immunospecific purification removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the Fab fragment of mouse IgG. The antibody preparation is solid phase adsorbed with human IgG to ensure minimal cross-reactivity in tissue or cell preparations **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal as a preservative	**Working Dilution:** ■ 1:8,000 by dot blotting ■ 1:60,000 by direct ELISA ■ 1:150 by immunohisto-chemistry (formalin-fixed, paraffin-embedded sections) ■ 1:80,000 by indirect Western blotting (chemiluminescent)
A3682	1 ml	Goat Anti-Mouse IgG, Fab Fragment Peroxidase Conjugate, Adsorbed with Human IgG and Rat Serum Proteins	**Specificity:** Mouse IgG Immunospecific purification removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the Fab fragment of mouse IgG. The antibody preparation is solid phase adsorbed with human IgG and rat serum proteins to ensure minimal cross-reactivity in tissue or cell preparations. Binds all mouse Igs **Form:** Solution in 0.01 M phosphate buffered saline pH 7.4, containing 0.01% thimerosal	**Working Dilution:** ■ 1:4,000 by dot blotting ■ 1:40,000 by direct ELISA ■ 1:150 by immunohisto-chemistry (formalin-fixed, paraffin-embedded sections) ■ 1:80,000 by indirect Western blotting (chemiluminescent)

View recent literature at **sigma.com/flaglit**

FLAG

Cat. No.	Quantity	Product Description	Characteristics	Applications
FLAG®-Control Proteins				
P5975	0.1 mg	Met-FLAG BAP Control Protein	N-terminal Met-FLAG-BAP control protein is a 468 amino acid N-terminal Met-FLAG fusion protein of *E. coli* bacterial alkaline phosphatase (BAP) **MW:** 49.4 kDa	■ Control protein for ANTI-FLAG M2 and M5 monoclonal antibodies in Western blotting, ELISA, immunoprecipitation, fluorescence microscopy, light microscopy, FACS, and in immunoaffinity chromatography with the ANTI-FLAG M2 affinity gel
P2104	0.1 mg	Amino-terminal Met-3×FLAG-BAP™ Control Protein	A 482 amino acid N-terminal FLAG fusion protein of *E. coli* bacterial alkaline phosphatase (BAP) **MW:** 49.9 kDa	■ Control protein for ANTI-FLAG M2 monoclonal antibody in Western blotting, ELISA, immunoprecipitation, and immunoaffinity chromatography
P7457	0.1 mg	Carboxy-terminal FLAG-BAP™ Control Protein	A 466 amino acid C-terminal FLAG fusion protein of *E. coli* bacterial alkaline phosphatase (BAP) **MW:** 49.1 kDa	■ Control protein for the ANTI-FLAG M2 monoclonal antibody in immunological procedures such as Western blotting, ELISA, immunoprecipitation, fluorescence microscopy, light microscopy, FACS, and immunoaffinity chromatography
P7582	0.1 mg	Amino-terminal FLAG-BAP Control Protein	A 467 amino acid N-terminal FLAG fusion protein of *E. coli* bacterial alkaline phosphatase (BAP) **MW:** 49.3 kDa	■ Control protein for ANTI-FLAG M1 and M2 monoclonal antibodies in Western blotting, ELISA, immunoprecipitation, fluorescence microscopy, light microscopy, FACS, and in immunoaffinity chromatography

FLAG Purification

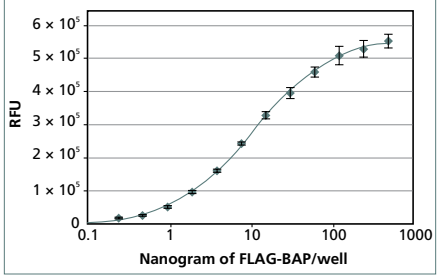
ANTI-FLAG HS M2 Coated Plates

ANTI-FLAG High Sensitivity 96-well plates provides a convenient, ready-to-use, platform for the capture and detection of FLAG fusion proteins. The ANTI-FLAG M2 coating can detect as little as 1 ng/well with a capacity of up to 300 ng/well of FLAG fusion protein. Suitable for screening for expression, study of protein:protein interactions, and ELISA assays. The ANTI-FLAG high sensitivity M2 coated multiwell plate can be used to detect N-terminal, Met-N-terminal, internal, and C-terminal FLAG and 3×FLAG fusion proteins.

Ordering Information

Cat. No.	Product Description	Quantity
P2983	ANTI-FLAG High Sensitivity Clear M2 coated 96-well plate	1 ea. 5 ea.

FLAG Fusion Protein Detection Using StarBright® Green Substrate



Ten microliters of N-terminal FLAG-BAP™ Control protein (Cat. No. P7582) diluted in Tris buffer with 1% BSA was incubated in an ANTI-FLAG M2 coated clear 96-well plate (Cat. No. P2983) for two hours at room temperature. The plate was washed four times with TBS with 0.05% TWEEN® 20 on a plate washer. One hundred microliters of StarBright Green substrate was added and the reaction was incubated at 37 °C for 10 minutes. Fluorescence was read on a Wallace Victor2™ plate reader. Results demonstrate that the ANTI-FLAG HS M2 coated plates can purify as little as 1 ng of fusion protein.

FLAG M Purification Kit

The FLAG M Purification Kit utilizes CelLytic™ M for rapid and efficient lysis, and protein extraction from mammalian cells, and the ANTI-FLAG M2 affinity gel for affinity purification of active FLAG fusion proteins. It can also be used for immunoprecipitation. The affinity purification is performed with ANTI-FLAG M2 affinity gel, which is a highly specific monoclonal antibody covalently attached to agarose resin. The use of an affinity resin allows for efficient binding of FLAG fusion proteins without the need for preliminary steps and calibrations. The affinity bound FLAG fusion proteins can be efficiently eluted from the resin by acidic conditions or by competition with 3×FLAG peptide. The eluted proteins can be analyzed for their activity, size, post-translational modifications, interactions, etc.

Sufficient for 3-5 uses of 1 ml affinity purification column.

Ordering Information

Cat. No.	Product Description	Quantity
CELLMM2	FLAG M Purification Kit	1 kit

Components
CelLytic M
10× Wash Buffer
Elution Buffer
3×FLAG Peptide
ANTI-FLAG M2-Agarose Affinity Gel
Amino-terminal FLAG-BAP Fusion Protein
Polypropylene chromatography column

Components
N-FLAG-BAP Control protein
ANTI-FLAG M2-Peroxidase (HRP) Conjugate
3,3′,5,5′-Tetramethylbenzidine (TMB) Substrate

ProteoQwest™ FLAG® Colorimetric Western Blotting Kit, TMB Substrate

Designed for colorimetric detection of as little as 1 ng of FLAG epitope-tagged fusion protein on Western blots. The colorimetric reaction occurs directly on the membrane and can be visually monitored for desired signal intensity; no darkroom, film, or imager is needed. A purified FLAG-tagged fusion protein, N-FLAG-BAP™, has been included to confirm proper performance of the kit. Immunostaining with the provided ANTI-FLAG® M2 monoclonal antibody-peroxidase conjugate eliminates non-specific background and simplifies the procedure compared to the use of unconjugated ANTI-FLAG antibodies with anti-mouse IgG secondary antibody peroxidase conjugates. In addition, superior results can be obtained for immunostaining blots from immunoprecipitation experiments, because there is no reaction between the ANTI-FLAG-HRP conjugate and the heavy and light antibody chains in the immunoprecipitation samples on the blot. This kit has sufficient reagents for 25 mini-gel sized (10 × 10 cm) blots.

Ordering Information

Cat. No.	Product Description	Quantity
PQ0300	ProteoQwest FLAG Colorimetric Western Blotting Kit, TMB Substrate	1 kit

Components
N-FLAG-BAP Control protein
ANTI-FLAG M2-Peroxidase (HRP) Conjugate
Chemiluminescent Peroxidase Substrate (CPS) Reagent
Chemiluminescent Peroxidase Substrate (CPS) Reaction Buffer
Tris Buffered Saline, pH 8.0 with 3% nonfat milk
Tris Buffered Saline with TWEEN® 20

ProteoQwest FLAG Chemiluminescent Western Blotting Kit, CPS Substrate

Designed for chemiluminescent detection of FLAG epitope-tagged fusion protein on Western blots. The chemiluminescent peroxidase substrate included in the kit provides high sensitivity chemiluminescent detection of as little as 0.1 ng of FLAG fusion protein. All of the components of the kit have been tested and the procedure has been optimized. Immunostaining with the provided ANTI-FLAG M2 monoclonal antibody-peroxidase conjugate eliminates non-specific background and simplifies the procedure compared to the use of unconjugated ANTI-FLAG antibodies with anti-mouse IgG secondary antibody peroxidase conjugates. The kit has sufficient reagents for immunostaining 12 mini-gel sized (10 × 10 cm) blots.

Ordering Information

Cat. No.	Product Description	Quantity
PQ0400	ProteoQwest FLAG Chemiluminescent Western Blotting Kit, CPS Substrate	1 kit

FLAG Immunoprecipitation Kit

Sigma's FLAG Immunoprecipitation Kit provides a rapid and efficient immunoprecipitation and elution of an active FLAG fusion protein.

Typically 70-90% of a bound FLAG fusion protein is released and retains biological activity. Immunoprecipitation is a powerful technique for the isolation of proteins or protein complexes. Immunoprecipitation consists of the following steps: cell lysis, binding of specific antigen to an antibody, antibody-antigen complex precipitation, precipitant wash, and antigen dissociation from the immune complex. Epitope-tagged proteins can be affinity purified and immunoprecipitated, using highly specific antibodies raised against their epitope. The use of such antibodies facilitates subsequent biochemical and immunological analysis.

Features and Benefits

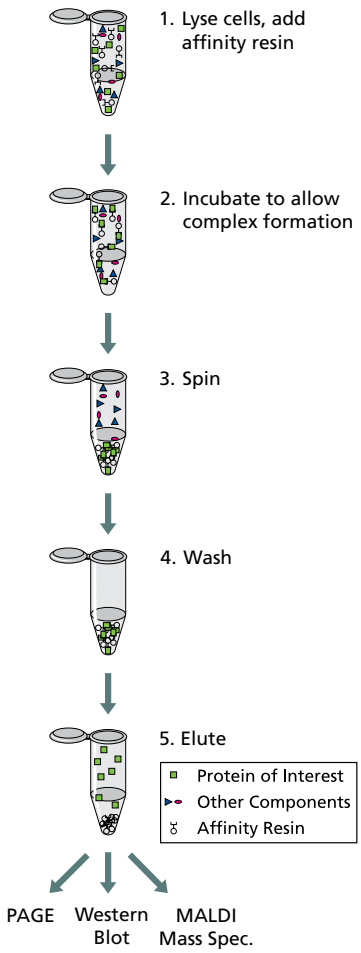
- Utilizes highly specific ANTI-FLAG M2 affinity gel
- No preliminary steps or calibrations
- 3×FLAG® peptide provides easy and gentle elution through direct competition

Ordering Information

Cat. No.	Product Description	Quantity
FLAGIPT1	FLAG Immunoprecipitation Kit	1 kit

Components
ANTI-FLAG M2 affinity gel
Elution Buffer
FLAG-BAP Control Protein
3×FLAG Peptide
Lysis Buffer
2× Sample Buffer
10× Wash Buffer

Affinity-based Molecular Pull-down Technique



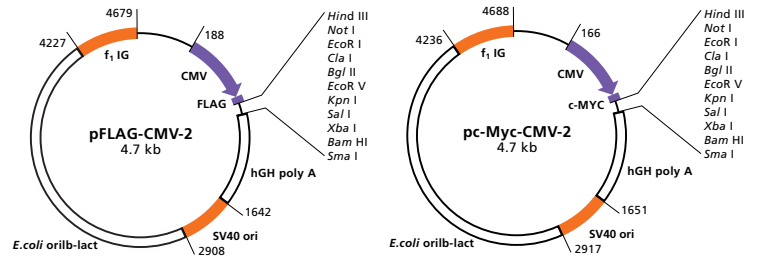
FLAG

Components
pFLAG-CMV-2 Expression Vector
pc-Myc-CMV-2 Expression Vector
pFLAG-CMV-2-p53 control plasmid
pc-Myc-CMV-2-Large T Antigen control plasmid
pc-Myc-CMV-2 BAP control plasmid
Verification Primer–MF
Verification Primer–MR

Immunoprecipitation Vector Kit

The Immunoprecipitation Vector Kit is for use with the FLAG® 96-Well Immunoprecipitation System. The kit provides expression vectors pFLAG-CMV™-2 and pc-Myc-CMV™-2 designed for the cloning and expression of protein interaction candidates as N-terminal FLAG and N-terminal c-Myc fusions. Control plasmids pFLAG-CMV-2-p53, pC-Myc-CMV-2-Large T antigen, and pC-Myc-CMV-2-BAP are supplied with the expression vectors. The control plasmids are intended for expression of positive and negative binding partners in the immunoprecipitation analysis. The kit also includes the MF-2 and MR-2 verification primers.

Expression Vector Design



Ordering Information

Cat. No.	Product Description	Quantity
COIPP	Immunoprecipitation Vector Kit	1 kit

HIS-Select®

A Highly Selective Chemistry for His-tagged Protein Purification

Sigma's HIS-Select products have the ability to purify His-tagged proteins quickly and with high selectivity. This is made possible by the **non-charged** linkage chemistry that attaches the chelate to the agarose bead matrix. Most other immobilized metal affinity chromatography (IMAC) systems for His-tagged protein purification utilize a charged chelate linkage. Extraneous charges on the resin will attract any oppositely charged amino acid in a protein, thereby, increasing non-specific binding. In addition, because the HIS-Select linker is **hydrophilic,** like the surface of most proteins, there is no interaction between the resin and non-specific proteins due to polarity of the resin.

The **highly pure tetradentate** chelate feature of the resin also aids in reducing non-specific binding and increasing binding capacity. Tetradentate chelates hold the metal ion at four coordination points opposed to three points like IDA type chelates. This makes the tetradentate chelate preferred over IDA type chelates because the metal ion is held tightly and this results in less metal leaching from the affinity gel.

These technologies combined, the non-charged, hydrophilic linker, and the highly pure tetradentate chelate, allow HIS-Select to provide **superior selectivity and binding capacity** for your His-tagged protein. Thus, HIS-Select allows you to eliminate time-consuming secondary purification and allows for true **one-step purification.**

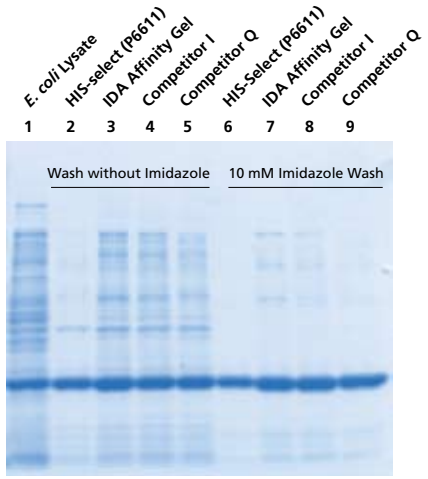
Features and Benefits

- Highly selective for higher purity
- Non-charged, hydrophilic linkage reduces non-specific binding
- Highly pure tetradentate chelate for higher binding capacity
- One-step purification

HIS-Select Requires Less Imidazole

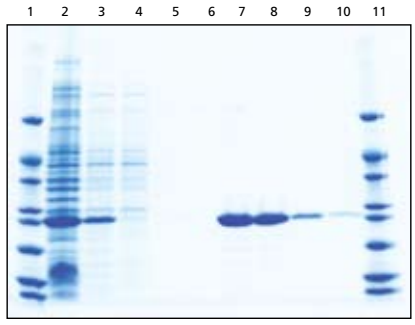
Imidazole has traditionally been added to the wash buffer to modulate non-specific binding when purifying His-tagged proteins with affinity gels. However, the HIS-Select technology is so selective that it requires much less **imidazole (0-10 mM)** in the wash buffer than has traditionally been used. Even with no imidazole in the wash buffer, the data clearly indicates that little non-specific binding occurs when using the HIS-Select Affinity Gel in lane 2 in comparison to the lanes 3-5. Furthermore, washing with the low concentration of 10 mM imidazole, non-specific binding has been virtually eliminated (lane 6) with the HIS-Select Affinity Gel. Using concentrations of imidazole higher than 10 mM will not further decrease non-specific binding.

Less Imidazole Required than Competition



Lysates from *E. coli* containing 8 mg of HIS-tagged protein per ml resin were loaded onto the resins in the presence of either no or 10 mM imidazole. These were incubated for 30 minutes at room temperature while rotating. Resins were washed three times with wash buffer (50 mM sodium phosphate, 300 mM NaCl, pH 8) and the His-tagged protein was eluted with 250 mM imidazole. Eluted protein was run on a gel and stained with EZBlue™ Gel Staining Reagent (G1041).

One Step Purification



Lane 1 Low Range SigmaMarker™ (M3913)
Lane 2 Whole Cell Extract in Sample Buffer
Lane 3 Whole Cell Extract with CelLytic™ B
Lane 4 Flow Through
Lane 5 Wash 1
Lane 6 Wash 2
Lane 7 Elute 1
Lane 8 Elute 2
Lane 9 Elute 3
Lane 10 Elute 4
Lane 11 Low Molecular Weight SigmaMarker (M3913)

HIS-Select® Nickel Affinity Gel

This is the tool of choice for small to medium scale purification of HIS-tagged proteins. The figure on the right shows a typical purification using HIS-Select Nickel Affinity Gel. Note that in elution lanes 7-10 there is virtually no non-specific binding.

Ordering Information

Cat. No.	Product Description	Quantity
P6611	HIS-Select Nickel Affinity Gel	5 ml 25 ml 100 ml 500 ml

Ask about bulk resin orders!

Selectivity: HIS-Select vs. Leading Competitor

E. coli lysates containing a HIS-tagged protein were purified using standard procedures. Proteins were bound at pH 8 in 50 mM sodium phosphate, 300 mM sodium chloride buffer containing 10 mM imidazole. Columns were washed with buffer containing 10 mM imidazole and eluted with buffer containing 250 mM imidazole.

HIS-Select HF Nickel Affinity Gel

HIS-Select HF (High Flow-Rate) Nickel Affinity Gel brings the superior selectivity of HIS-Select technology to a highly cross-linked agarose designed for higher flow rates and mechanical stability under pressure. This product is designed for production scale purification and FPLC™ applications.

Ordering Information

Cat. No.	Product Description	Quantity
H0537	HIS-Select HF Nickel Affinity Gel	10 ml 25 ml 100 ml 500 ml

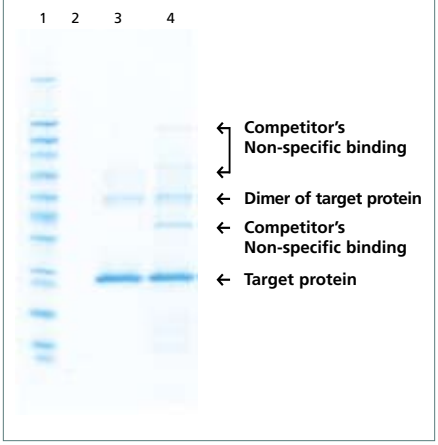
HIS-Select Cobalt Affinity Gel

For those who prefer to use cobalt as the affinity metal ion for HIS-tagged protein purification, our new HIS-Select Cobalt Affinity Gel allows for high purity, low non-specific binding and high binding capacity for your protein. HIS-Select Cobalt Affinity Gel also works well for purification of HIS-tagged proteins in native, denaturing, or mild reducing conditions.

Ordering Information

Cat. No.	Product Description	Quantity
H8162	HIS-Select Cobalt Affinity Gel	5 ml 25 ml 100 ml

Comparison of Cobalt Affinity Resins



Lane 1: SigmaMarker™, Wide Range (M4038)
Lane 2: Empty
Lane 3: HIS-Select Cobalt Affinity Gel (H8162) Elution
Lane 4: Competitor C Cobalt Metal Affinity Resin Elution
E. coli cell extract containing a HIS-tagged protein was purified using HIS-Select Cobalt Affinity Gel (H8162) and a leading competitor's Cobalt Metal Affinity Resin using standard protocols for each. Samples were run on a 4-20% Tris-Glycine gel and stained with EZ Blue™ Gel Staining Reagent (G1041).

EZview™ Red HIS-Select Nickel Affinity Gel

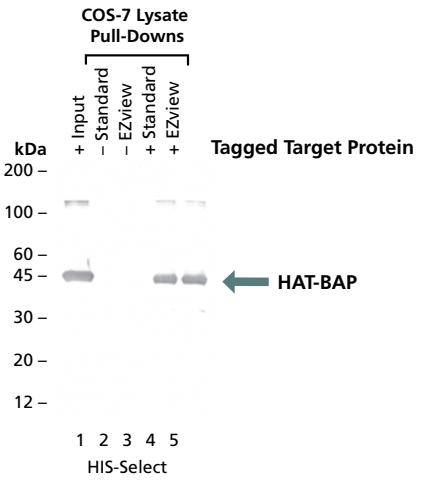
When performing small-scale affinity capture, such as molecular pull-down, the affinity matrix is difficult to see in the microcentrifuge tube with standard resins. Accidental aspiration of the resin leads to quantitative variability in results. The EZview Red Affinity Gel greatly reduces the risk of pellet loss. EZview resins perform as well as conventional non-colored affinity gels, but allow the user to easily differentiate pellet from supernatant. This correlates to more accurate data because less target protein is lost.

Ordering Information

Cat. No.	Product Description	Quantity
E3528	EZview Red HIS-Select Nickel Affinity Gel	1 ml 5 × 1 ml

See more EZview Red Affinity gels on page 111.

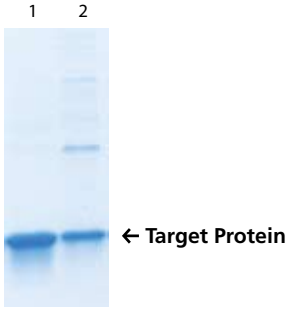
Direct Affinity Capture of Tagged Proteins



Target proteins were spiked (+), or not spiked (–), into COS-7 cell lysates and captured using either standard resin or EZview HIS-Select HC Nickel affinity beads. Western blots of 12% SDS-PAGE gels are shown. Blots were probed with Anti-HAT™ tag antibody plus alkaline phosphatase conjugated secondary antibody and developed with BCIP/NBT.

Results:
Lane 1: Control target protein
Lanes 2 & 3: EZview and standard affinity beads show no detectable background
Lanes 4 & 5: EZview and standard affinity beads show equal binding capacity

HIS-Select Spin Columns Yield a Purer Target Protein



Lysates from *E. coli* containing 300 µg of HIS-tagged protein were purified using HIS-Select Spin Columns and a competitor's spin column. HIS-Select Spin Columns have little non-specific binding as compared to competitor.

Lane 1: HIS-Select Spin Column

Lane 2: Competitor Q Spin Column

HIS-Select Nickel Spin Columns

Pre-packed and ready-to-use HIS-Select Spin Columns allow for fast and consistent small-scale purification of HIS-tagged protein from crude cell extracts. The Spin Columns contain a matrix of silica particles and use the HIS-Select technology to attain high protein purity and low non-specific binding. Spin Columns allow for fast purification in approximately 15 minutes!

Ordering Information

Cat. No.	Product Description	Quantity
H7787	HIS-Select Nickel Spin Columns	10 ea. 50 ea.

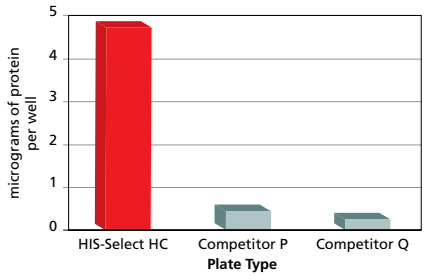
HIS-Select Nickel Filter Plate

Filter plates incorporate the speed, consistency, and convenience of spin columns into a multi-sample format. This pre-packed 96-sample plate is useful for small-scale histidine-tagged protein purification from crude cell extracts in less than 15 minutes. Filter plates are also compatible with centrifugation, vacuum-manifold, and robotics systems. 96 Deepwell 2 ml collection plates (P7616 or P1492) are also available for use with HIS-Select Filter plates.

Ordering Information

Cat. No.	Product Description	Quantity
H0413	HIS-Select Filter Plate	1 ea. 5 × 1 ea.

HIS-Select HC Plates Bind More Protein Than the Competition



20 µg of pure His-tagged protein was added to the wells of the HIS-Select HC 96-well Plate, and two competitor plates for capture of target protein. All plates were incubated for 4 hours at room temperature. The protein was eluted with 250 mM imidazole and quantified with the Bicinchoninic Acid Kit for Protein Determination Kit (BCA1).

HIS-Select HC Nickel-Coated 96-Well Plates

HIS-Select High Capacity (HC) Nickel Coated Plates are coated with a proprietary, high-density nickel chelate matrix. This patented coating allows for greater per-well binding capacity than any other commercial histidine-binding plates and low non-specific binding. The 96-well plates can capture ≥4 µg protein per well and the 384-well plates capture ≥2 µg protein per well.

Ordering Information

Cat. No.	Product Description	Quantity
S5563	HIS-Select HC Nickel-Coated 96-Well Plates	1 ea. 5 ea.

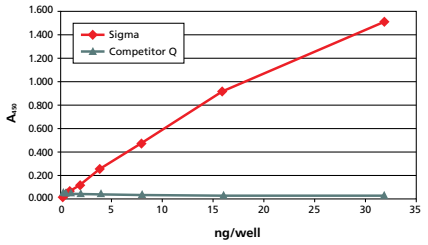
HIS-Select® HS Nickel-Coated 96-Well Plates

HIS-Select High Sensitivity (HS) Nickel Coated Plates are designed for highly accurate low-level detection of HIS-tagged proteins. The captured proteins can be detected using standard enzyme-linked assay (ELISA) techniques. These plates are pretreated to reduce non-specific binding and are sensitive enough to capture as little as 1 ng/well of HIS-tagged protein.

Ordering Information

Cat. No.	Product Description	Quantity
S5688	HIS-Select HS Nickel-Coated 96-Well Plates	1 ea. 5 ea.

Comparison with Leading Competitor's Plate



HIS-Select HS nickel coated 96-well plate comparison with a competitor's plate for capture of HAT-BAP target protein from cell lysates.

HIS-Select Wash and Elution Buffer

HIS-Select Wash Buffer is a pre-made solution of 10 mM imidazole that is optimized to reduce non-specific binding of proteins during wash steps. HIS-Select Elution Buffer is pre-made solution of 250 mM imidazole that is optimized to elute histidine-containing target proteins. Both products are compatible with HIS-Select purification products and other Immobilized Affinity Chromatography Systems in native conditions.

Ordering Information

Cat. No.	Product Description	Quantity
H5288	HIS-Select Wash Buffer	500 ml 1 L
H5413	HIS-Select Elution Buffer	250 ml 500 ml

Imidazole

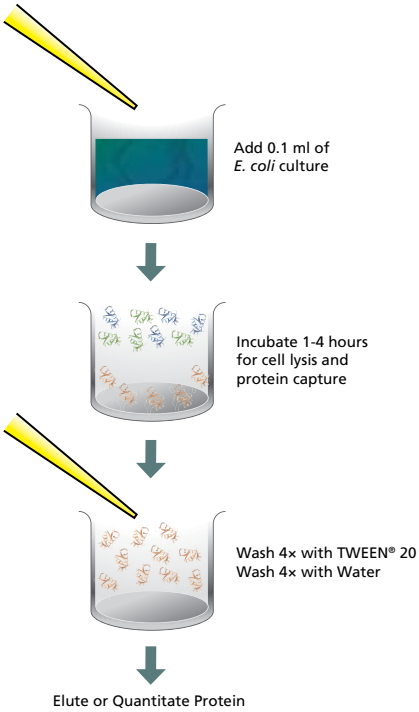
Wash and elution buffer for HIS-tagged protein purification with HIS-Select affinity gels, magnetic beads, cartridges, spin columns, and plates.

DNase, RNase, protease ..... none detected

Ordering Information

Cat. No.	Product Description	Quantity
I5513	Imidazole, for molecular biology, minimum 99%	5 g 25 g 100 g

iLAP Procedure



One-step Cell Lysis and Purification

100 µl of *E. coli* culture expressing HIS-tagged protein was added to each well of the iLAP plate. Plate was incubated at room temperature for 2 hours to lyse the cells and capture the protein. Plate was washed and protein from one random well was eluted with 250 mM imidazole for analysis. Gel was stained with ProteoSilver™ Silver Stain Kit (PROTSIL1).
Lane 1: *E. coli* culture lysate
Lane 2: Eluted protein

HIS-Select® iLAP® Plates

Integrated Lysis and Affinity Purification (iLAP)

Sigma's HIS-Select iLAP 96-well Plates are coated with both a cell lysis reagent and the HIS-Select nickel chelate matrix. This patented technology allows for cells to be lysed and the HIS-tagged protein to be captured all in one step. Cell lysis and protein extraction is highly efficient and eliminates the need to harvest cells from the culture. The HIS-Select coating is highly selective for HIS-tagged proteins, reduces non-specific binding, and has a high binding capacity of ≥4 µg protein/well. This makes these plates ideal for rapid colony screening and protein:protein interaction assays.

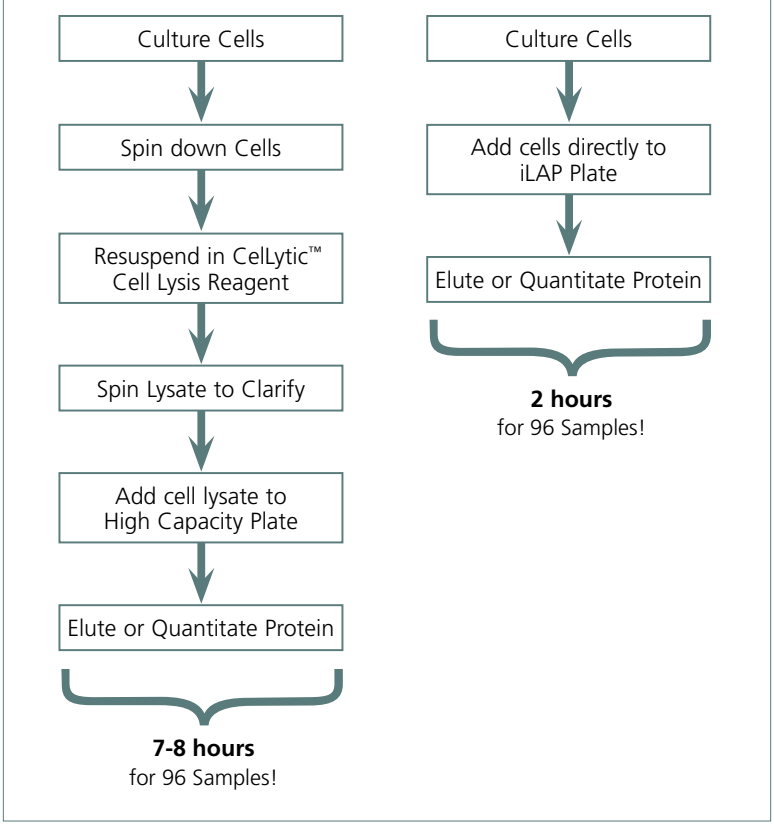
Features and Benefits

- One-step cell lysis and HIS-tagged protein purification
- Efficient cell lysis without harvesting cells from the culture
- HIS-Select – highly selective for HIS-tagged proteins, less non-specific binding, and high binding capacity
- Ideal for rapid colony screening

Ordering Information

Cat. No.	Product Description	Quantity
H9412	HIS-Select iLAP Plates	1 ea. 5 × 1 ea.

Procedure Comparison



HIS-Select® iLAP Column

The HIS-Select iLAP (Integrated Lysis and Affinity Purification) Columns combine cell lysis and protein purification steps into one. These single-use, disposable columns are designed for the one-step purification of histidine-tagged proteins directly from 5 ml of bacterial culture. The single-step method uses Sigma's patented iLAP technology, which allows quick and simple histidine-tagged protein purification from recombinant clones. Each column contains nickel chelate resin for the purification, as well as five lysis reagent tablets, which include all the necessary detergents and enzymes needed for efficient cell lysis and protein extraction while eliminating the need to harvest the cells from culture. Each column is capable of purifying at least 1 mg of histidine-tagged protein directly from 5 ml of bacterial culture in less than one hour. The procedure provided can be used to extract soluble proteins directly from growing cells and results in a nearly pure fusion protein preparation following elution from the column. This makes these columns ideal for confirming expression of a histidine-tagged target protein, for performing protein-protein interaction assays, or for rapidly screening colonies or multiple constructs (cultures).

Ordering Information

Cat. No.	Product Description	Quantity
H9913	HIS-Select iLAP Column	1 ea. 25 ea.

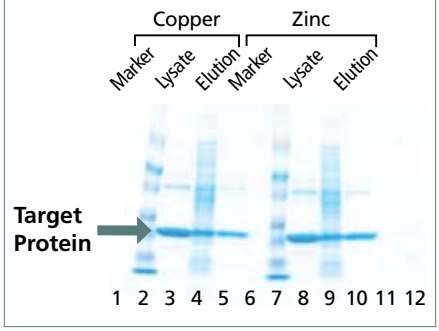
IMAC-Select Affinity Gel

IMAC-Select Affinity Gel is an immobilized metal affinity chromatography (IMAC) product. While most commonly used for purifying proteins it can also be used for purifying peptides. The gel matrix consists of 6% beaded agarose derivatized with a proprietary, patent pending, quadridentate chelate. IMAC-Select Affinity Gel is durable and can capture proteins that have an affinity for various metal ions. Although some naturally occurring proteins have affinities for some metals, many are given a recombinant histidine tag so that the protein of interest will have an affinity for weak to borderline Lewis acids, such as nickel or cobalt. Exposed cysteine and tryptophan residues may also bind, but are not as commonly used as histidine. Other metal ions, such as iron or gallium, may be used to purify phosphoproteins. IMAC-Select Affinity Gel is supplied as a metal-free gel and allows researchers to add a chelating metal of choice. The gel can also be used to remove metal contamination from a protein or peptide solution. The resin will have a metal binding capacity of 10 to 30 µmole/ml of resin.

Ordering Information

Cat. No.	Product Description	Quantity
I1408	IMAC-Select Affinity Gel	5 ml 25 ml 100 ml

Purification of Histidine-tagged Recombinant Protein



A 28 kDa histidine-tagged recombinant protein was purified from *E. coli* lysates using IMAC-Select Affinity Gel charged with either copper or zinc. Lanes 3 and 8 are the recombinant protein. Lanes 4 and 9 are the *E. coli* lysates containing the target protein. Lanes 5 and 10 are the purified protein after IMAC. Molecular Weight Marker used is ColorBurst™ Marker (Cat. No. C1992).

Components
CelLytic M
5× Phosphate Buffer (250 mM Sodium Phosphate, pH 8, 1.5 M NaCl)
2.5 M Imidazole
EZview Red HIS-Select HC Nickel Affinity Gel
5% TWEEN® 20
2.5% Hexadecyltrimethylammonium Bromide (CTAB)
Protease Inhibitor Cocktail

HIS-Select M Affinity Capture Kit

This kit provides all the components needed for the extraction and rapid small-scale affinity capture of HIS-tagged proteins from mammalian cells. The EZview™ Red HIS-Select Nickel Affinity Gel provided in the kit exhibits high selectivity of HIS-tagged proteins and the very low non-specific binding of other proteins. Plus the red color allows for less pellet loss during aspiration when performing immunoprecipitation or molecular-pull down experiments. The CelLytic™ M component is a quick and effective lysis buffer for both adherent and suspension cells that express the HIS-tagged protein. The kit is sufficient for 50 affinity capture purifications and a highly detailed protocol for affinity capture is provided.

Ordering Information

Cat. No.	Product Description	Quantity
EHM1	HIS-Select M Affinity Capture Kit	1 kit

Components
CelLytic M
5× Phosphate Buffer (250 mM Sodium Phosphate, pH 8, 1.5 M Nacl)
2.5 M Imidazole
HIS-Select Nickel Affinity Gel
5% TWEEN 20
2.5% Hexadecyltrimethylammonium Bromide (CTAB)
Polypropylene chromatography columns

HIS-Select M Purification Kit

This kit provides all the components needed for the extraction and rapid affinity purification of HIS-tagged proteins from mammalian cells. The HIS-Select Nickel Affinity Gel provided in the kit exhibits high selectivity of HIS-tagged proteins and the very low non-specific binding of other proteins. The Affinity Gel allows for one-step purification of at least 15 mg of an ~30 kDa HIS-tagged protein per ml of affinity gel. The CelLytic M component is a quick and effective lysis buffer for both adherent and suspension cells that express the histidine-tagged protein. The kit is sufficient for 5 × 1 ml affinity purification columns and a highly detailed protocol for affinity purification is provided.

Ordering Information

Cat. No.	Product Description	Quantity
HMP1	HIS-Select M Purification Kit	1 kit

Components
CelLytic Y
5× Phosphate Buffer (250 mM Sodium Phosphate, pH 8, 1.5 M NaCl)
2.5 M Imidazole
HIS-Select Nickel Affinity Gel
5% TWEEN 20
2.5% Hexadecyltrimethylammonium Bromide (CTAB)
Polypropylene chromatography columns

HIS-Select Y Purification Kit

This kit provides all the components needed for the extraction and rapid affinity purification of HIS-tagged proteins from yeast cells. The HIS-Select Nickel Affinity Gel provided in the kit exhibits high selectivity of HIS-tagged proteins and the very low non-specific binding of other proteins. The Affinity Gel allows for one-step purification of at least 15 mg of an ~30 kDa HIS-tagged protein per ml of affinity gel. The CelLytic Y component is a quick and effective lysis buffer for yeast cells that express the HIS-tagged protein. The kit is sufficient for 5 × 1 ml affinity purification columns and a highly detailed protocol for affinity purification is provided.

Ordering Information

Cat. No.	Product Description	Quantity
HYP1	HIS-Select Y Purification Kit	1 kit

Purify!

SIGMA Life Science

True one-step protein purification with Sigma's HIS-Select® Technology

Sigma's HIS-Select Resins utilize a proprietary nickel chelate linkage for highly selective histidine-tagged protein purification.

- High binding capacity
- Target protein enrichment from a single pass
- Reduced non-specific binding for higher purity

To learn more, visit ***sigma-aldrich.com/his_select***

- Affinity Resins
- 96-Well Plates
- Spin Columns
- Complete Kits
- Bulk Resins

Our Innovation, Your Research — Shaping the Future of Life Science

HIS-Select® Quick Reference Guide




	Nickel Affinity Gel	High Flow (HF) Nickel Affinity Gel	Cobalt Affinity Gel	HIS-Select Spin Columns
Catalog Number	**P6611**	**H0537**	**H8162**	**H7787**
Package Sizes	5 ml, 25 ml, 100 ml, 500 ml	10 ml, 25 ml, 100 ml, 500 ml	5 ml, 25 ml, 100 ml	10 ea., 50 ea.
Application	■ Gravity Flow Column ■ Small-Med. Scale	■ FPLC™ ■ Production Scale	■ Gravity Flow Column ■ Small-Med. Scale	■ Mini Prep ■ Process by Centrifugation or Vacuum
Scale per Sample	100 µg–10 g	100 µg–100 g	100 µg–10 g	■ 150 µg ■ 600 µl/load
Binding Capacity	15 mg/ml	15 mg/ml	15 mg/ml	150 µg/column
Matrix	6% Beaded Agarose	6% Beaded Agarose, Highly Cross-linked	6% Beaded Agarose	Silica Particles (Porous, Spherical)
Bead Size	45–165 µm	45–165 µm	45–165 µm	~20 µm
Exclusion Limit (MW) or Pore Size	4×10^6 Da	4×10^6 Da	4×10^6 Da	0.1 µm ($\sim 10 \times 10^6$ Da)
Max. Linear Flow Rate (Max. pressure)	150 cm/hr (5 psi)	3000 cm/hr (200 psi)	150 cm/hr (5 psi)	N/A
Recommended Flow Rate for 1 × 2 cm Column	1 ml/min	5 ml/min	1 ml/min	N/A
Recommended Binding Time/Speed	N/A	N/A	N/A	Equivalent to ~80 × *g* Centrifugation
Optimal pH Stability	3-10	3-10	3-10	3-10
Physical Form	50% suspension in 30% ethanol	50% suspension in 30% ethanol	50% suspension in 30% ethanol	Pre-packed dry matrix in spin column
Antimicrobial Agent	30% ethanol	30% ethanol	30% ethanol	N/A
Storage	2-8 °C	2-8 °C	2-8 °C	2-8 °C
Recommended Imidazole Conc. for Load/Wash	0-10 mM	0-10 mM	0-10 mM	0-5 mM
Recommended Imidazole Conc. for Elution	250 mM	250 mM	250 mM	250 mM




EZview™ Red Affinity Gel	High Sensitivity (HS) Nickel Coated Strip Plates	High Capacity (HC) Nickel Coated Plates 96-well	iLAP® High Capacity (HC) Nickel Coated Plates 96-well
E3528	**S5688**	**S5563**	**H9412**
1 ml, 5 × 1 ml	1 ea., 5 ea.	1 ea., 5 × 1 ea.	1 ea., 5 × 1 ea.
■ Mini Prep Pull-Downs	■ High Throughput Screen (ELISA) ■ Compatible with Robotics	■ High Throughput, High Capacity Screening ■ Multi-Sample Mini Prep ■ In-Well Protein Assays ■ Compatible with Robotics	■ Direct Cell Lysis and Purification of Target Protein in Well ■ High Throughput, High Capacity Screening ■ Multi-Sample Mini Prep ■ In-Well Protein Assays ■ Compatible with Robotics
10–500 µg per pull-down	■ 1 ng–1 µg/well ■ 96-well: 200 µl/well	■ 1–4 µg/well ■ 96-well: 200 µl/well	■ 1–4 µg/well ■ 96-well: 200 µl/well
15 mg/ml	Sensitivity: ≤1 ng/well	96-well: ≥4 µg/well	≥4 µg/well
6% Beaded Agarose	Clear Polystyrene Plate; Proprietary Coating	Clear Polystyrene Plate; Proprietary Coating	Clear Polystyrene Plate; Proprietary Coatings for Lysis and Purification
45–165 µm	N/A	N/A	N/A
4 × 10^6 Da	N/A	≥300 kDa	≥300 kDa
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
1 hour at 4 °C	1 hour at 25 °C	96-well; 4 hours at 25 °C	1-4 hours at 25 °C
3-10	3-10	3-10	3-10
50% suspension in 30% ethanol	Clear 96-well flat-bottom, polystyrene strip plate	Clear 96-well flat-bottom, polystyrene plates	Clear 96-well flat-bottom, polystyrene plates
30% ethanol	N/A	chlorhexidine	N/A
2-8 °C	2-8 °C	2-8 °C	2-8 °C
0-10 mM	0-5 mM	0-1 mM	0-1 mM
250 mM	N/A	200 mM	200 mM

HIS-Select® Quick Reference Guide




	HIS-Select iLAP Column	Nickel Filter Plates 96-well	HIS-Select Wash Buffer	HIS-Select Elution Buffer
Catalog Number	**H9913**	**H0413**	**H5288**	**H5413**
Package Sizes	1 ea., 25 ea.	1 ea., 5 ea.	500 ml, 1 L	250 ml, 500 ml
Application	■ Direct Cell Lysis and Purification of Histidine-tagged Protein in a Prepacked Column ■ Small Scale ■ Gravity	■ Small Scale ■ High Throughput Screening ■ Process by Centrifugation or Vacuum ■ Compatible with Robotics	Optimized to reduce non-specific binding of proteins during wash steps when purifying histidine containing proteins with HIS-Select purification products or other immobilized metal affinity chromatography (IMAC) products	Optimized to elute histidine-containing target proteins from HIS-Select purification products or other immobilized metal affinity chromatography (IMAC) products
Scale per Sample	100 µg–2 mg	■ 1 mg/well ■ 1 ml/load	N/A	N/A
Binding Capacity	2 mg/column	1 mg/well	N/A	N/A
Matrix	6% Beaded Agarose Tableted Lysis Reagents	Silica Particles (Porous, Spherical)	N/A	N/A
Bead Size	45–165 µm	~20 µm	N/A	N/A
Exclusion Limit (MW) or Pore Size	4×10^6 Da	0.1 µm ($\sim 10 \times 10^6$ Da)	N/A	N/A
Max. Linear Flow Rate (Max. pressure)	N/A	N/A	N/A	N/A
Recommended Flow Rate for 1 × 2 cm Column	Gravity Flow	N/A	N/A	N/A
Recommended Binding Time/Speed	N/A	Equivalent to ~270 × *g* Centrifugation	N/A	N/A
Optimal pH Stability	6.0-7.5	3-10	N/A	N/A
Physical Form	Tablets in 5.5 ml polypropylene column	Pre-packed dry matrix in 96-well filter plate	Liquid	Liquid
Antimicrobial Agent	N/A	N/A	N/A	N/A
Storage	2-8 °C	2-8 °C	Room Temperature	Room Temperature
Recommended Imidazole Conc. for Load/Wash	0-10 mM	0-5 mM	N/A	N/A
Recommended Imidazole Conc. for Elution	250 mM	250 mM	N/A	N/A

EZview™ Red – High Visibility Affinity Gel

A major disadvantage of affinity-based molecular pull-down and immunoprecipitation procedures is that the affinity matrix is difficult to see in the microcentrifuge tube following centrifugation steps.

To facilitate more speed and accuracy, Sigma has developed a dye-conjugated agarose for our EZview Red Affinity Gels. The vivid red color of the affinity beads provides high visibility that allows easy differentiation of the pellet from the supernatant; therefore, reducing the risk of accidental aspiration of the pellet and allowing for less tedious manipulations.

Features and Benefits

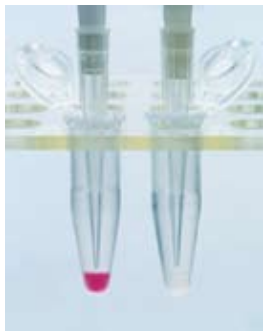
- Increase gel visibility
- Maximize yields by avoiding sample loss
- Perform small-scale affinity capture with confidence and efficiency

Ordering Information

Cat. No.	Product Description	Quantity
E6654	EZview Red Anti-c-Myc Affinity Gel	1 ml 5 × 1 ml
F2426	EZview Red ANTI-FLAG® M2 Affinity Gel	1 ml 5 × 1 ml
E6779	EZview Red Anti-HA Affinity Gel	1 ml 5 × 1 ml
P6486	EZview Red Protein A Affinity Gel	1 ml 5 × 1 ml
E3403	EZview Red Protein G Affinity Gel	1 ml 5 × 1 ml
E5529	EZview Red Streptavidin Affinity Gel	1 ml 5 × 1 ml
E3528	EZview Red HIS-Select® HC	1 ml 5 × 1 ml
E6406	EZview Red Glutathione Affinity Gel	1 ml 5 × 1 ml

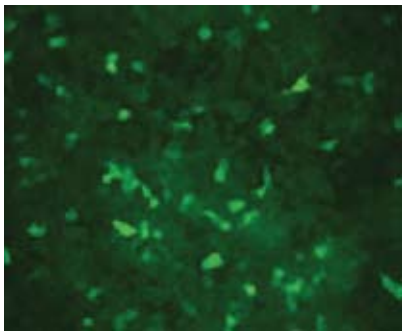
EZview Red Protein A Affinity Gel

EZview Standard



EZview Red Protein A Affinity Gel has enhanced visibility compared to standard, non-colored Protein A Agarose.

Anti-MAT Monoclonal Antibody Used for Immunostaining to Detect MAT-tagged Proteins Expressed in Mammalian Cells



Adherant 293T cells were transfected with a FLAG-MAT-Tag-MAPK expression vector. After two days the cells were fixed and permeablilized with 3% paraformaldehyde and 0.5% TRITON® X-100. The cells were stained with 5 µg/ml Anti-MAT monoclonal antibody and developed with Anti-Mouse IgG (Fab Specific)-FITC conjugate at a 1:40 dilution. Staining of the fusion protein can be seen in the cytoplasm and nuclei of the transfected cells.

Metal Affinity Tag System

The MAT-Tag™ System (**m**etal **a**ffinity **t**ag) has recently been developed by Sigma for the expression, purification, and detection of recombinant fusion proteins. This system utilizes a small, novel, seven amino acid (HNHRHKH) sequence created for purification of recombinant MAT fusion proteins using HIS-Select® Nickel and Cobalt Affinity Gels. Many of our vectors make use of the MAT tag in combination with the well-known FLAG® tag. This allows for convenient purification with the IMAC technology and the superior sensitivity of the antibody-based FLAG system for detection. MAT tag containing vectors are offered in formats for N-terminal or C-terminal tagging.

In addition, a very sensitive detection system utilizing a highly specific monoclonal antibody has also been developed that can detect both N- and C- terminal MAT-tagged proteins.

The novel MAT-tag system allows investigators additional flexibility for studying protein expression, structure, modification, function, and protein-protein interactions.

Features and Benefits

- Complete protein expression system
- Higher selectivity for higher purity
- Compatible with HIS-Select Products
- Superior antibody detection

MAT Bacterial Expression Vectors *tac* Promoter System

Vectors utilizing the strong *tac* promoter (a hybrid of the *E. coli trp* and *lac* promoters) offer protein expression levels in excess of 10 mg/L of culture when using IPTG as a de-repressor. These vectors can be used to express protein in any established *E. coli* host.

pTAC-MAT-Tag-1 (5.4 kb)

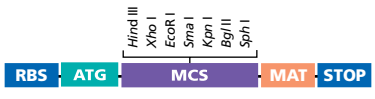
pTAC-MAT-Tag™-1

Cytoplasmic expression of N-terminal MAT fusion proteins under the *tac* promoter. Supplied with a pTAC-MAT-Tag-2+BAP Control Vector.

Ordering Information

Cat. No.	Product Description	Quantity
E5530	pTAC-MAT-Tag-1 Expression Vector	10 µg

pTAC-MAT-Tag-2 (5.4 kb)



pTAC-MAT-Tag-2

Cytoplasmic expression of C-terminal MAT fusion proteins under the *tac* promoter. Supplied with a pTAC-MAT-Tag-2+BAP Control Vector.

Ordering Information

Cat. No.	Product Description	Quantity
E5405	pTAC-MAT-Tag-2 Expression Vector	10 µg

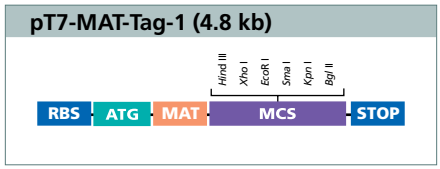
See MAT Antibody, page 127.

MAT-Tag Bacterial Expression Vectors
T7 Promoter System

The pT7-MAT vectors offer the very strong T7/*lac* promoter. These expression vectors produce even higher yields of recombinant proteins than the *tac* promoter system. However, the T7 promoter is known for background ("leaky") expression, which can be a drawback when recombinant proteins are toxic to the host cell. Therefore, Sigma's vectors contain the *lac* operator (*lacO*) sequences immediately downstream from the promoter to reduce leaky expression. Unlike the *tac* promoter system, pT7 vectors must be expressed in hosts containing a source of the T7 polymerase such as (DE3) lysogenic strains.

pT7-MAT-Tag-1

Cytoplasmic expression of N-terminal MAT fusion proteins under the T7/*lac* promoter. Supplied with a pT7-MAT-Tag-2+BAP Control Vector.

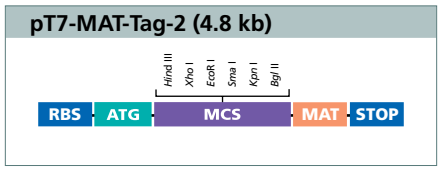


Ordering Information

Cat. No.	Product Description	Quantity
E5780	pT7-MAT-Tag-1 Expression Vector	10 µg

pT7-MAT-Tag-2

Cytoplasmic expression of C-terminal MAT fusion proteins under the T7/*lac* promoter. Supplied with a pT7-MAT-Tag-2+BAP Control Vector.

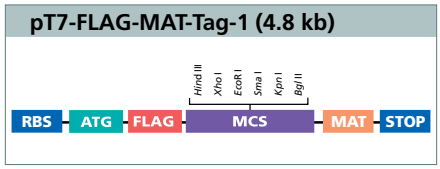


Ordering Information

Cat. No.	Product Description	Quantity
E5655	pT7-MAT-Tag-2 Expression Vector	10 µg

pT7-FLAG-MAT-Tag-1

Cytoplasmic expression of N-terminal Met-FLAG, C-terminal MAT dual tagged fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-MAT-Tag-1+BAP Control Vector.

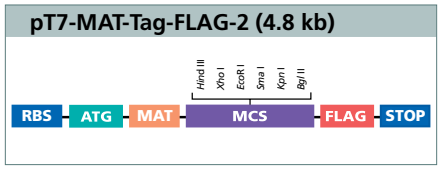


Ordering Information

Cat. No.	Product Description	Quantity
E5280	pT7-FLAG-MAT-Tag-1 Expression Vector	10 µg

pT7-MAT-Tag-FLAG-2

Cytoplasmic expression of N-terminal MAT, C-terminal FLAG dual tagged fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-MAT-Tag-1+BAP Control Vector.

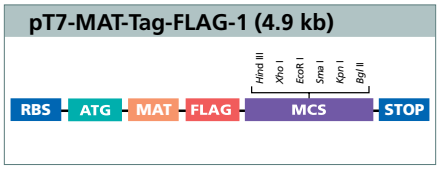


Ordering Information

Cat. No.	Product Description	Quantity
E4905	pT7-MAT-Tag-FLAG-2 Expression Vector	10 µg

MAT Bacterial Expression Vectors
T7 Promoter System

The recognition sequence for enterokinase, Asp-Asp-Asp-Asp-Lys, is found at the C-terminal end of the FLAG® epitope tag. Removal of the FLAG is possible in all fusion proteins containing an N-terminal FLAG sequence. Dual tag fusion proteins may also be cleaved with enterokinase for removal of one or more tags, depending on the position of FLAG in the protein sequence.

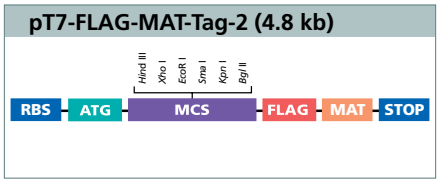


pT7-MAT-Tag™-FLAG-1

Cytoplasmic expression of N-terminal MAT-FLAG dual tagged fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-MAT-Tag-1+BAP Control Vector.

Ordering Information

Cat. No.	Product Description	Quantity
E5155	pT7-MAT-Tag-FLAG-1 Expression Vector	10 µg



pT7-FLAG-MAT-Tag-2

Cytoplasmic expression of C-terminal FLAG-MAT dual tagged fusion proteins under the T7/*lac* promoter. Supplied with a pT7-FLAG-MAT-Tag-1+BAP Control Vector.

Ordering Information

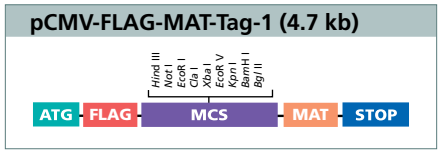
Cat. No.	Product Description	Quantity
E5030	pT7-FLAG-MAT-Tag-2 Expression Vector	10 µg

MAT Mammalian Expression Vectors
Transient Expression

CMV vectors contain the pMB1 (derivative of pBR322) origin for replication in bacterial cells, the β-lactamase gene for ampicillin resistance selection in bacteria, hGH, polyA, and the f1 origin.

pCMV-FLAG-MAT-Tag-1

Transient cytoplasmic expression of N-terminal Met-FLAG, C-terminal MAT dual tagged fusion proteins under the CMV promoter. Supplied with pCMV-FLAG-MAT-Tag-1+MAPK1 Control Vector.



Ordering Information

Cat. No.	Product Description	Quantity
C5864	pCMV-FLAG-MAT-Tag-1 Expression Vector	20 µg

pCMV-MAT-Tag-FLAG-1

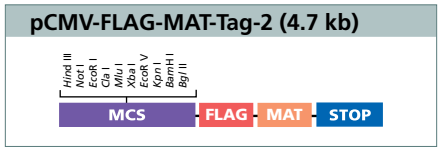
Transient cytoplasmic expression of N-terminal MAT-FLAG dual tagged fusion proteins under the CMV promoter. Supplied with pCMV-FLAG-MAT-Tag-1+ MAPK1 Control Vector.

Ordering Information

Cat. No.	Product Description	Quantity
C5989	pCMV-MAT-Tag-FLAG-1 Expression Vector	20 µg

pCMV-FLAG-MAT-Tag-2

Transient cytoplasmic expression of C-terminal FLAG-MAT dual tagged fusion proteins under the CMV promoter. Supplied with pCMV-FLAG-MAT-Tag-1+ MAPK1 Control Vector.



Ordering Information

Cat. No.	Product Description	Quantity
C6114	pCMV-FLAG-MAT-Tag-2 Expression Vector	20 µg

MAT Baculovirus Transfer Vectors

pPolh-MAT-Tag™ are baculovirus transfer vectors used for producing Metal Affinity Tag (MAT-Tag) fusion proteins in insect cells. The pPolh-MAT-Tag vectors contain the strong viral polyhedrin (polh) promoter for high-level expression of target genes during the very late phase of infection. The vectors also contain a high copy of bacterial origin of replication and an ampicillin resistance gene (amp^r) for easy propagation in *E. coli* host cells.

pPolh-MAT-Tag-1 Features

Feature	Map Position
AcNPV sequence (ORF 603)	1-1146
Recommended 5′ primer sequence binding site	1079-1100
polh Promoter	1076-1145
MAT	1167-1187
MCS	1188-1246
Recommended 3′ primer sequence binding site	1300-1320
M13 origin	2576-3229
polyA	1599-1604
AcNPV Sequence (ORF1629)	1286-2629
β-lactamase (amp^r)	3616-4473
pUC ori	4624-5267

pPolh-MAT-Tag-2 Features

Feature	Map Position
AcNPV sequence (ORF 603)	1-1146
Recommended 5′ primer sequence binding site	1079-1100
polh Promoter	1076-1145
MCS	1148-1211
MAT	1212-1232
Recommended 3′ primer sequence binding site	1295-1315
M13 origin	2571-3224
polyA	1594-1599
AcNPV Sequence (ORF1629)	1281-2624
β-lactamase (amp^r)	3611-4468
pUC ori	4619-5262

Ordering Information

Cat. No.	Product Description	Quantity
T6699	pPolh-MAT-Tag-1 Transfer Vector Expression of N-terminal MAT fusion proteins in insect cells	20 µg
T6574	pPolh-MAT-Tag-2 Transfer Vector Expression of C-terminal MAT fusion proteins in insect cells	20 µg

Detection and Purification Selection Guide

Cat. No.	Quantity	Product Description	Characteristics	Applications
Polyhistidine/Histidine				
H1029	0.2 ml 0.5 ml	Monoclonal Anti-PolyHistidine, Clone HIS-1	**Specificity:** N-terminal Polyhistidine fusion proteins **Note:** Weakly recognizes C-terminal Polyhistidine fusion proteins **Form:** Ascites fluid with 15 mM sodium azide as a preservative	■ Western blotting ■ Immunoprecipitation ■ Dot blotting ■ ELISA **Working Dilution:** ■ 1:3,000 by indirect Western blotting using lysate of induced bacteria expressing a polyhistidine tagged protein
A5588	0.5 ml	Monoclonal Anti-PolyHistidine, Clone HIS-1, Alkaline Phosphatase Conjugate	**Specificity:** N-terminal polyhistidine fusion proteins **Note:** Weakly recognizes C-terminal polyhistidine fusion proteins **Form:** Solution in 0.05 M Tris buffer, pH 8.0, containing 1% BSA, 1.0 mM $MgCl_2$, 50% glycerol, and 15 mM sodium azide	■ Western blotting ■ Dot blotting ■ ELISA **Working Dilution:** ■ 1:2,000 by Western blotting (colorimetric) using lysates of *E. coli* induced to express polyhistidine tagged protein
A7058	1 vl	Monoclonal Anti-PolyHistidine, Clone HIS-1, Peroxidase (HRP) Conjugate	**Specificity:** N-terminal polyhistidine fusion proteins **Note:** Weakly recognizes C-terminal polyhistidine fusion proteins **Form:** Lyophilized powder. Reconstitution with 0.5 ml of water results in a solution of 0.01 M sodium phosphate buffered saline containing 1% BSA and 0.01% thimerosal	■ Western blotting ■ Dot blotting ■ ELISA **Working Dilution:** ■ 1:2,000 by Western blotting using lysate of induced bacteria expressing poly-histidine tagged protein
HA				
A2095	1 ml	Anti-HA Agarose Affinity Gel	**Specificity:** N-terminal or C-terminal HA-tagged (YPYDVPDYA) fusion proteins **Binding Capacity:** 30-50 nmoles of HA tagged fusion protein per 1 ml of settled resin **Elution:** At least 3.5 nmoles of HA-tagged fusion protein per ml of settled resin, as determined using HA-tagged fusion protein of 120 kDa and low pH elution buffer **Form:** Suspension 50% (v:v) in 0.01 M phosphate buffered saline, containing 15 mM sodium azide. Specific antibody concentration is 2.0-2.4 mg/ml settled resin	■ Immunoprecipitation ■ Immunoaffinity purification
E6779	1 ml	EZview™ Anti-HA Agarose Affinity Gel	**Specificity:** N-terminal or C-terminal HA-tagged (YPYDVPDYA) fusion proteins **Binding Capacity:** 0.4 mg HA-tagged fusion protein per ml of affinity gel **Form:** Suspension of red colored beaded agarose in phosphate buffered saline containing 50% glycerol and 0.0015% Kathon® CG/IPCII as an antimicrobial preservative	■ Immunoprecipitation
I2149	0.5 mg 1 mg	HA Peptide	**Sequence:** Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala **MW:** 1102.2 **Form:** Lyophilized powder	Sequence used in recombinant HA epitope tagged proteins. Epitope recognized by anti-HA monoclonal antibodies
H9658	0.2 ml	Monoclonal Anti-HA Tag, Clone HA-7	**Specificity:** N-terminal or C-terminal HA-tagged (YPYDVPDYA) fusion proteins **Form:** Mouse ascites fluid with 15 mM sodium azide as a preservative	■ ELISA ■ Immunocytochemistry ■ Immunoprecipitation ■ Western blotting **Working Dilution:** ■ 1:10,000 by Western blotting (colorimetric)

Detection and Purification Selection Guide

Cat. No.	Quantity	Product Description	Characteristics	Applications
HA *(con't)*				
H6908	0.2 ml 0.5 ml	Anti-HA Tag, Affinity Isolated Rabbit Antibody	**Specificity:** N-terminal and C-terminal HA-tagged (YPYDVPDYA) fusion proteins **Form:** Affinity isolated rabbit antibody in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide	■ Western blotting ■ Immunoprecipitation ■ Immunocytochemistry **Working Dilution:** ■ 1:50 by indirect immunofluorescence using HA-tagged fusion protein transfected cells ■ 1:200 by immunoprecipitation using HA-tagged fusion protein from cell lysates ■ 1:1,000 by Western blotting (colorimetric) using HA-tagged fusion protein transfected cell extracts
B9183	100 µg	Monoclonal Anti-HA, Biotin Conjugate antibody produced in mouse	**Specificity:** N- and C-terminal HA-tagged fusion proteins **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative	■ Indirect immunoblotting (chemiluminescent) **Working Dilution:** ■ 0.25-0.50 µg/ml using HA-tagged fusion proteins in transiently transfected mammalian cell extracts
A5477	500 µg	Monoclonal Anti-HA-Alkaline Phosphatase Conjugate	**Specificity:** N-terminal and C-terminal HA-tagged (YPYDVPDYA) fusion proteins **Form:** Purified immunoglobulin solution in 0.05 M Tris buffer, pH 8.0, containing 1% bovine serum albumin, 1 mM $MgCl_2$, 50% glycerol, and 15 mM sodium azide as a preservative	■ Western blotting **Working Dilution:** ■ 1:4,000 on mammalian cell extracts expressing HA-tagged fusion proteins
H6533	1 vl	Monoclonal Anti-HA Tag, Clone HA-7, Peroxidase Conjugate	**Specificity:** N-terminal or C-terminal HA-tagged (YPYDVPDYA) fusion proteins **Form:** Lyophilized powder and should be reconstituted with 0.5 ml of water	■ Western blotting **Working Dilution:** ■ 1:4,000-8,000 by Western blotting (colorimetric)
H7411	100 µg	Monoclonal Anti-HA, FITC Conjugate antibody produced in mouse	**Specificity:** N- and C-terminal HA fusion proteins **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative	■ Immunocytochemistry **Working Dilution:** ■ 1-5 µg/ml using HA-tagged fusion protein transfected into mammalian cells fixed with paraformaldehyde
H9037	200 µg	Anti-HA, Rhodamine Conjugate	**Specificity:** N-terminal and C-terminal HA-tagged (YPYDVPDYA) fusion proteins **Form:** Purified immunoglobulin solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative	■ Immunofluorescence **Working Dilution:** ■ 10-15 µg/ml on mammalian cells expressing HA-tagged fusion proteins
c-Myc				
A7470	1 ml	Anti-c-Myc Agarose Affinity Gel	**Specificity:** N-terminal or C-terminal c-Myc tagged (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) fusion proteins. **Binding Capacity:** 2 nmoles c-Myc fusion protein per 1 ml a settled resin **Elution:** 1.5 nmoles c-Myc fusion protein per 1 ml a settled resin **Form:** 50% (v/v) Suspension in 0.01 M phosphate buffered saline, containing 15 mM sodium azide	■ Immunoprecipitation ■ Immunoaffinity purification
E6654	1 ml 5 × 1 ml	EZview™ Red Anti-c-Myc Affinity Gel	**Specificity:** c-Myc tagged fusion proteins **Form:** Suspension of red colored beaded agarose in phosphate buffered saline containing 50% glycerol and 0.0015% Kathon® CG/IPCII as an antimicrobial preservative	■ Immunoprecipitation

Cat. No.	Quantity	Product Description	Characteristics	Applications
c-Myc *(con't)*				
M2435	4 mg 25 mg	c-Myc Peptide	c-Myc Peptide is a synthetic peptide corresponding to amino acids 410-419 of the C-terminal of human c-Myc **Sequence:** N-Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-C **MW:** 1203.3 **Form:** Lyophilized powder	■ Inhibition of antibody staining by c-Myc antibodies **Titer:** ■ 5-10 µg/ml for inhibition of antibody staining in Western blotting
M4439	0.1 ml	Monoclonal Anti-c-Myc, Clone 9E10, purified	**Specificity:** N-terminal or C-terminal c-Myc tagged (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) fusion proteins **Form:** Purified IgG in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide	■ Screening for expression ■ Immunoprecipitation ■ Immunocytochemistry ■ Immunohistochemistry ■ ELISA ■ Western blotting **Working Dilution:** ■ Minimum dilution of 1:5,000 by immuno-blotting of *E. coli* extract expressing recombinant c-Myc-tagged fusion protein
M5546	0.2 ml 0.5 ml	Monoclonal Anti-c-Myc, Clone 9E10	**Specificity:** N-terminal or C-terminal c-Myc tagged (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) fusion proteins **Form:** Mouse ascites fluid with 15 mM sodium azide as a preservative	■ Western blotting ■ Immunoprecipitation of c-Myc-tagged fusion proteins but not native or denatured c-Myc protein from cell lysate ■ Immunohistochemistry ■ Electron microscopy ■ ELISA **Working Dilution:** ■ 1:100 by Western blotting (colorimetric) using a c-Myc-tagged fusion protein
C3956	0.2 mg	Anti-c-Myc, Polyclonal, Affinity Isolated Rabbit Antibody	**Specificity:** N-terminal or C-terminal c-Myc tagged (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) fusion proteins. Developed using a peptide corresponding to amino acids 408-425 of the human c-Myc proto-oncogene, conjugated to maleimide-activated KLH through a C-terminal added cysteine residue **Form:** Affinity isolated antibody at ~0.5 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide	■ Western blotting ■ Immunoprecipitation ■ Immunocytochemistry **Working Dilution:** ■ By Western blotting, at least 1.0 µg/ml of the antibody can detect c-Myc fusion proteins in cell extracts from transfected cultures as well as bacterial lysates ■ 5-10 µg/ml for indirect immunofluorescence staining in methanol: acetone fixed transiently transfected cells
B7554	100 µg	Monoclonal Anti-c-Myc Biotin Conjugate antibody produced in mouse	**Specificity:** N-terminal or C-terminal c-Myc tagged (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) fusion proteins **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative	■ Indirect immunoblotting (chemiluminescent) **Working Dilution:** ■ 0.05-0.1 µg/ml using Extract of 293T cells expressing c-Myc tagged fusion protein

Detection and Purification Selection Guide

Cat. No.	Quantity	Product Description	Characteristics	Applications
c-Myc *(con't)*				
A5963	0.5 ml	Monoclonal Anti-c-Myc, Clone 9E10, Alkaline Phosphatase Conjugate	**Specificity:** N-terminal or C-terminal c-Myc tagged (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) fusion proteins **Form:** Purified immunoglobulin solution in 0.05 M Tris buffer, containing 1% bovine serum albumin, 1 mM $MgCl_2$, 50% glycerol, and 15 mM sodium azide	■ Western blotting **Working Dilution:** ■ 1:100 by Western blotting (colorimetric) using a c-Myc tagged fusion protein
A5598	0.5 mg	Anti-c-Myc, Peroxidase conjugate, Affinity Isolated Antibody	**Specificity:** N-terminal or C-terminal c-Myc tagged (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) fusion proteins **Form:** Affinity isolated rabbit antibody in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal	■ Screening for expression ■ Western blotting **Working Dilution:** ■ Minimum dilution of 1:5,000 by immuno-blotting of *E. coli* extract expressing recombinant c-Myc-tagged fusion protein
F2047	100 µg	Monoclonal Anti-c-Myc FITC Conjugate antibody produced in mouse, Clone 9E10	**Specificity:** N-terminal or C-terminal c-Myc tagged (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) fusion proteins **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative	■ Immunocytochemistry **Working Dilution:** ■ 5.0 µg/ml using mammalian cells transfected with c-Myc tagged fusion, protein fixed with paraformaldehyde
C6594	0.5 ml	Monoclonal Anti-c-Myc, Clone 9E10, Cy™3 Conjugate	**Specificity:** N-terminal or C-terminal c-Myc tagged (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) fusion proteins **Form:** Purified mouse immunoglobulin supplied in 0.01 M sodium phosphate buffered saline, containing 1% bovine serum albumin and 15 mM sodium azide	■ Immunofluorescent Immunocytochemistry ■ Immunofluorescent Immunohistochemistry **Working Dilution:** ■ 1:50 by direct immuno-fluorescence using formalin-fixed, paraffin-embedded human colon carcinoma tissue
T7				
T8823	200 µg	Monoclonal anti-T7 tag, Clone T7tag, purified	**Specificity:** T7-tagged (MASMTGGQQMG) fusion proteins **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative	■ Western blotting **Working Dilution:** ■ 1-2 µg/ml on bacterial extracts expressing recombinant T7-tagged fusion protein
T3699	1 vl	Monoclonal anti-T7 tag, Peroxidase Conjugate	**Specificity:** T7-tagged (MASMTGGQQMG) fusion proteins **Form:** Lyophilized powder. Reconstitution with 0.5 ml of water results in a solution of 0.01 M sodium phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 0.01% thimerosal	■ Western blotting **Working Dilution:** ■ 1:1,000 on 250-500 ng of purified T7-tagged fusion protein
HSV				
H4640	4 mg 25 mg	HSV Peptide	**Sequence:** N-Lys-Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp-C **MW:** 1367.4 HSV Tag Peptide is a synthetic peptide corresponding to amino acids 290-300 of glycoprotein D of herpes simplex virus types I and II, with added N-terminal lysine **Form:** Lyophilized powder	■ Inhibition of antibody staining by HSV antibodies **Working Dilution:** ■ 5-10 µg/ml for inhibition of antibody staining in Western blotting
H6030	200 µg	Anti-HSV, Affinity Isolated Rabbit Antibody	**Specificity:** HSV-tagged fusion proteins **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide	■ Western blotting ■ Immunoprecipitation **Working Dilution:** ■ 2.5 µg/ml by Western blotting

Cat. No.	Quantity	Product Description	Characteristics	Applications
V5				
A7345	1 ml	Anti-V5 Agarose Affinity Gel	**Specificity:** V5-tagged (GKPIPNPLLGLDST) fusion proteins. Developed using a synthetic peptide corresponding to amino acids 95-108 of the P/V proteins of paramyxovirus SV5, conjugated to KLH **Binding Capacity:** 2.5 nmoles of V5-fusion protein per 1 ml **Form:** 50% (v/v) Suspension in 0.01 M phosphate buffered saline, containing 15 mM sodium azide	■ Immunoaffinity purification ■ Immunoprecipitation
V7754	4 mg 25 mg	V5 Peptide	V5 Peptide is a synthetic peptide corresponding to amino acids 95-108 of non-structural protein V and to RNA polymerase α subunit (P protein), of paramyxovirus SV5 with an N-terminal cysteine **Sequence:** N-Cys-Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr-C **MW:** 1524.8 **Form:** Lyophilized powder	■ Inhibition of antibody staining by Anti-V5-Tag antibodies **Working Dilution:** ■ 5-10 µg/ml for inhibition of antibody staining in Western blotting
V8012	50 µg	Monoclonal Anti-V5 Clone V5-10	**Specificity:** V5 tagged (GKPIPNPLLGLDST) fusion proteins expressed in transfected mammalian cells or produced by *in vitro* translation. Developed using a synthetic peptide corresponding to amino acid residues (95-108) of the P/V proteins of the paramyxovirus SV5, conjugated to KLH **Form:** Supplied at ~1 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide	■ Western blotting ■ Immunocytochemistry ■ ELISA **Working Dilution:** ■ 0.5-1 µg/ml by Western blotting ■ 1-2 µg/ml by immunocytochemistry on transfected mammalian cells fixed with methanol: acetone
V8137	0.2 mg	Anti-V5, IgG Fraction of Rabbit Antiserum	**Specificity:** V5-tagged (GKPIPNPLLGLDST) fusion proteins. Developed in rabbit using a synthetic peptide corresponding to amino acids 95-108 of the P/V proteins of paramyxovirus SV5, conjugated to KLH **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide	■ Western blotting ■ Immunoprecipitation ■ Immunocytochemistry **Working Dilution:** ■ 2.5 µg/ml by Western blotting using a V5 tagged fusion protein
V2260	1 vl	Monoclonal Anti-V5 Clone V5-10, Peroxidase Conjugate	**Specificity:** V5-tagged (GKPIPNPLLGLDST) fusion proteins **Form:** Lyophilized powder. Reconstitution with 0.5 ml of water results in a solution of 0.01 M sodium phosphate buffered saline containing 1% BSA and 0.01% thimerosal	■ Western blotting **Working Dilution:** ■ 1:4,000-8,000 by Western blotting

Detection and Purification Selection Guide

Cat. No.	Quantity	Product Description	Characteristics	Applications
GST				
A5838	0.5 ml	Anti-Glutathione-S-Transferase, IgG Fraction of Rabbit Antiserum, Alkaline Phosphatase Conjugate	**Specificity:** Recognizes native and denatured-reduced forms of purified GST or GST fusion proteins. Specific for GST from *Schistosoma japonicum,* and does not recognize GST from rat, rabbit, porcine, or bovine liver, or from human placenta when tested by ELISA **Form:** Rabbit IgG fraction of antiserum supplied in 0.05 M Tris buffer, pH 8.0, containing 1 mM $MgCl_2$, 1% BSA, and 15 mM sodium azide	■ Western blotting ■ Dot blotting ■ ELISA **Working Dilution:** ■ 1:2,000 by Western blotting (colorimetric) using lysates of *E. coli* induced to express recombinant GST
A7340	0.5 ml	Anti-Glutathione-S-Transferase, IgG Fraction of Rabbit Antiserum, Peroxidase Conjugate	**Specificity:** Recognizes native and denatured-reduced forms of purified GST or GST fusion proteins. Specific for GST from *Schistosoma japonicum,* and does not recognize GST from rat, rabbit, porcine, or bovine liver, or from human placenta when tested by ELISA **Form:** The product is supplied as IgG fraction of rabbit antiserum in 0.01 M phosphate buffered saline, pH 7.4, containing 0.01% thimerosal	■ Western blotting ■ Dot blotting ■ ELISA **Working Dilution:** ■ 1:1,000 by Western blotting (colorimetric) using lysates of *E. coli* induced to express recombinant GST
Maltose Binding Protein (MBP)				
M6295	0.2 ml 0.5 ml	Monoclonal Anti-Maltose Binding Protein, Clone MBP-17	**Specificity:** Non-reduced and denatured-reduced forms of purified MBP or MBP fusion proteins **Form:** Mouse ascites fluid with 15 mM sodium azide as a preservative	■ Western blotting ■ Dot blotting ■ ELISA **Working Dilution:** ■ 1:4,000 by Western blotting (colorimetric) using purified, recombinant MBP
A3963	0.5 ml	Monoclonal Anti-Maltose Binding Protein, Clone MBP-17, Alkaline Phosphatase Conjugate	**Specificity:** Non-reduced and denatured-reduced forms of purified MBP or MBP fusion proteins **Form:** Purified mouse antibody in 0.05 M Tris buffer, containing 1% bovine serum albumin, 50% glycerol, and 15 mM sodium azide	■ Western blotting ■ Dot blotting ■ ELISA **Working Dilution:** ■ 1:400 by Western blotting (colorimetric) using purified, recombinant MBP
A4213	1 vl	Monoclonal Anti-Maltose Binding Protein, Clone MBP-17, Peroxidase Conjugate	**Specificity:** Non-reduced and denatured-reduced forms of purified MBP or MBP fusion proteins **Form:** The antibody conjugate is provided as a lyophilized powder and should be reconstituted with 0.5 ml of water	■ Western blotting ■ Dot blotting ■ ELISA **Working Dilution:** ■ 1:1,000 by Western blotting (colorimetric) using purified, recombinant MBP
Cellulose Binding Domain (CBD)				
C5473	0.2 ml	Monoclonal Anti-Cellulose Binding Domain (CBD_{clos}), Clone CBD-8	**Specificity:** CBD_{clos} (CBD family IIIa, from *Clostridium cellulovorans,* 17 kDa) **Form:** Ascites fluid with 15 mM sodium azide as a preservative	■ Western blotting **Working Dilution:** ■ 1:20,000 by Western blotting using a recombinant 17 kDa fragment of the cellulose complex from *Clostridium cellulovorans*

Cat. No.	Quantity	Product Description	Characteristics	Applications
VSV-G				
A1970	1 ml	Anti-VSV-G Agarose Affinity Gel	**Specificity:** VSV-G tagged fusion proteins **Binding Capacity:** At least 15 nmoles of VSV-G tagged fusion protein per ml of settled resin **Elution:** At least 5 nmoles of a VSV-G tagged fusion protein per ml of settled resin, as determined using VSV-G tagged fusion protein of 120 kDa and low pH elution buffer **Form:** 50% (v/v) Suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative	■ Immunoprecipitation ■ Immunoaffinity purification
V7887	4 mg 25 mg	VSV-G Peptide	VSV-G Peptide is a synthetic peptide corresponding to the C-terminal of vesicular stomatitis virus-glycoprotein **Sequence:** N-Tyr-Thr-Asp-Ile-Glu-Met-Asn-Arg-Leu-Gly-Lys-C **MW:** 1339.5 **Form:** Lyophilized powder	■ Inhibition of antibody staining by VSV-G antibodies **Titer:** ■ 10-15 µg/ml for inhibition of antibody staining in Western blotting
V5507	0.2 ml 0.5 ml	Monoclonal Anti-VSV Glycoprotein, Clone P5D4	**Specificity:** The antibody recognizes an epitope containing the five carboxy-terminal amino acids of vesicular stomatitis virus glycoprotein (VSV-G). In infected cells, the antibody localizes the immature forms of VSV-G in the rough endoplasmic reticulum (RER) and in the cisternae of Golgi complex, as well as mature VSV-G at the cell surface and in the budding virus. The antibody does not stain the secreted form of VSV-G that lacks the membrane and the cytoplasmic domain. It recognizes native as well as denatured forms of VSV-G tagged proteins **Form:** Provided as mouse ascites fluid with 15 mM sodium azide as a preservative	■ Western blotting ■ Immunoprecipitation ■ Immunocytochemistry ■ Immunoelectron microscopy **Working Dilution:** ■ 1:1,000 by Western blotting (colorimetric) using whole cell extracts expressing VSV-G tagged fusion protein
V4888	0.2 mg	Anti VSV-G, Affinity Isolated Rabbit Antibody	**Specificity:** N-terminal or C-terminal VSV-G tag **Form:** Affinity isolated antibody at ~1.0 mg/ml in solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide	■ Western blotting ■ Immunoprecipitation ■ Immunofluorescence **Working concentration:** ■ Minimum 0.5 µg/ml by immunoblotting or immunoprecipitation of recombinant VSV-G-tagged fusion proteins ■ Minimum 1.0 µg/ml by immunofluorescence
A5977	1 vl	Monoclonal anti VSV-G, Peroxidase Conjugate, Clone P5D4	**Specificity:** The antibody recognizes an epitope containing the five carboxy-terminal amino acids of vesicular stomatitis virus glycoprotein (VSV-G). Recognizes native as denatured forms of VSV-G tagged proteins **Form:** The product is supplied as a lyophilized powder. Reconstitution with 0.5 ml of water results in a solution of 0.01 M sodium phosphate buffered saline, pH 7.4, containing 1% BSA and 0.01% thimerosal	■ Western blotting **Working Dilution:** ■ 1:1,000 on 20-50 ng of a purified VSV-G tagged fusion protein (chemiluminescence)
C7706	0.2 ml 0.5 ml	Monoclonal Anti-VSV Glycoprotein, Clone P5D4, Cy3 Conjugate	**Specificity:** See details for this clone in the description for V5507 **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide	■ Immunocytochemistry **Working Dilution:** ■ 1:10,000 by direct immunofluorescence using COS-7 cells transfected with a VSV-G tagged vinculin construct

Detection and Purification Selection Guide

Cat. No.	Quantity	Product Description	Characteristics	Applications
Thioredoxin				
A2582	1 ml	Anti-Thioredoxin Agarose Conjugate, IgG Fraction of Rabbit Antiserum	**Specificity:** The antibody is specific for natural *Escherichia coli* and recombinant thioredoxin **Binding Capacity:** Binds a minimum of 0.4 mg of thioredoxin per ml of settled resin **Form:** 50% (v/v) Suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide	■ Immunoaffinity purification ■ Immunoprecipitation
T0803	0.2 ml 0.5 ml	Anti-Thioredoxin, IgG Fraction of Rabbit Antiserum	**Specificity:** The antibody is specific for natural *Escherichia coli* and recombinant thioredoxin. It may be used to identify the expression of thioredoxin fusion proteins **Form:** Solution supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide	■ Western blotting ■ Dot blotting **Working Dilution:** ■ 1:5,000 by dot blotting using purified recombinant thioredoxin ■ 1:5,000 by Western blotting (colorimetric) using *E. coli* extract
β-Galactosidase				
G4644	1 vl	Anti-β-Galactosidase, Developed in Mouse, Fractionated Antiserum	**Specificity:** Developed against purified β-galactosidase from *E. coli*. The antibody may be used to detect β-galactosidase expression by the *lacZ* reporter gene in P-element enhancer lines in *Drosophila* **Form:** Lyophilized from 0.01 M phosphate buffered saline, pH 7.2. Reconstitute with 2 ml of water	■ Western blotting **Working Dilution:** ■ 1:1,000 by Western blotting (colorimetric) using non-reduced β-galactosidase
G6282	0.2 ml 0.5 ml	Monoclonal Anti-β-Galactosidase, Clone GAL-40, Mouse IgM	**Specificity:** Developed against purified β-galactosidase from *E. coli*. The antibody may be used for detection of β-galactosidase expressed by the *E. coli lacZ* gene encoded in many cloned gene sequences; serves as an indicator for fusion proteins encoded by an inserted DNA sequence **Form:** Mouse ascites fluid with 15 mM sodium azide as a preservative	■ Western blotting ■ Dot blotting ■ Immunocytochemistry **Working Dilution:** ■ 1:1,000 by Western blotting (colorimetric) using denatured-reduced *E. coli* β-galactosidase
B0271	0.2 ml 0.5 ml	Monoclonal Anti-β-Galactosidase, Clone GAL-13, Mouse IgG1, Biotin Conjugate	**Specificity:** Developed against purified β-galactosidase from *E. coli*. This antibody reacts with soluble β-galactosidase without causing loss of enzymatic activity. It is not recommended for Western blotting; it does not recognize denatured or reduced β-galactosidase **Form:** The conjugate is supplied as a liquid in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15 mM sodium azide as a preservative	■ ELISA. The antibody may be used for amplification in immunoenzymatic staining by preparing a β-galactosidase anti-β-galactosidase (BGABG) soluble complex ■ Dot blotting on native purified or crude galactosidase **Working Dilution:** ■ 1:2,000 by dot blotting
G8021	0.2 ml 0.5 ml	Monoclonal Anti-β-Galactosidase, Clone GAL-13, Mouse IgG1	**Specificity:** Developed against purified β-galactosidase from *E. coli*. This antibody reacts with soluble β-galactosidase without causing loss of enzymatic activity. It is not recommended for Western blotting; it does not recognize denatured or reduced β-galactosidase **Form:** Mouse ascites fluid with 15 mM sodium azide as a preservative	■ Immunocytochemistry ■ ELISA ■ Dot blotting **Working Dilution:** ■ 1:2,000 by indirect ELISA using mouse primary antibody, bridging antibody and *E. coli* β-galactosidase

Cat. No.	Quantity	Product Description	Characteristics	Applications
Alkaline Phosphatase				
A2951	0.2 ml 0.5 ml	Monoclonal Anti-Human Placental Alkaline Phosphatase, Clone 8B6	**Specificity:** In SDS gels, the product reacts with both Regan and Nagao isozymes of human placental alkaline phosphatase (hPLAP, 130 kDa, 67/130 kDa). By RIA, the antibody binds to hPLAP with an affinity constant of 5×10^9 M^{-1}. It does not react with PLAP-like enzymes **Form:** Mouse ascites fluid with 15 mM sodium azide as a preservative	■ Immunohistochemistry (frozen sections) ■ Immunocytochemistry ■ RIA ■ ELISA ■ Western blotting **Working Dilution:** ■ 1:4,000 by immunohisto-chemistry (formalin-fixed, paraffin-embedded sections) using human placenta
A2080	1 ml	Monoclonal Anti-Alkaline Phosphatase, Human Placental, Agarose, Clone 8B6	**Specificity:** In SDS gels, the antibody reacts with both Regan and Nagao isozymes of human placental alkaline phosphatase (hPLAP, 130 kDa, 67/130 kDa). By RIA, the antibody binds to hPLAP with an affinity constant of 5×10^9 M^{-1}. It does not react with PLAP-like enzymes **Form:** 50% (v/v) Suspension in 0.1 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide. The purified immunoglobulin is immobilized on agarose, at 2 mg antibody per ml bed volume	■ Immunoprecipitation ■ Immunoaffinity purification
Green Fluorescent Protein (GFP)				
G6539	0.2 ml 0.5 ml	Monoclonal Anti-Green Fluorescent, Clone GFP-20	**Specificity:** The antibody was developed using a GFP-tagged fusion protein. The antibody reacts with fusion proteins expressed by prokaryotic expression vectors **Form:** Mouse ascites fluid with 15 mM sodium azide as a preservative	■ Western blotting ■ Dot blotting ■ ELISA **Working Dilution:** ■ 1:2,000 by Western blotting (colorimetric)
G1544	100 µg	Anti GFP (N-ter), developed in rabbit, affinity isolated antibody	**Specificity:** GFP fusion proteins. The epitope resides within amino acids 3-17 of the Green Fluorescent Protein **Form:** Solution of affinity isolated antibody at ~1.0 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative	■ Western blotting ■ Immunoprecipitation **Working Dilution:** ■ 0.25-0.5 µg/ml by Western blotting of GFP fusion proteins expressed in mammalian cell extracts (chemiluminescence) ■ 1.0-2.5 µg by Immuno-precipitation using a GFP fusion protein from transfected mammalian cell lysates
Protein A				
P6486	1 ml 5 × 1 ml	EZview™ Red Protein A Affinity Gel	**Specificity:** Fc portion of the IgG antibodies **Form:** Suspension of red colored beaded agarose in phosphate buffered saline containing 50% glycerol and 0.0015% Kathon® CG/IPCII as an antimicrobial preservative	■ Immunoprecipitation

Detection and Purification Selection Guide

Cat. No.	Quantity	Product Description	Characteristics	Applications
Streptavidin *(Please see the Sigma Life Science catalog for a complete listing)*				
E5529	1 ml 5 × 1 ml	EZview™ Red Streptavidin Affinity Gel	**Specificity:** Biotinylated compounds **Form:** Suspension of red colored beaded agarose in phosphate buffered saline containing 50% glycerol and 0.0015% Kathon® CG/IPCII as an antimicrobial preservative	■ Small scale affinity capture
S6940	1 ea. 5 ea.	SigmaScreen™ Streptavidin HC Coated Plates	**Specificity:** Biotinylated compounds **Capacity:** ≥300 pmol biotin/well **Note:** Proprietary high density coating	■ Protein-Protein interactions ■ High throughput immuno-affinity purification
M5432	5 ea.	SigmaScreen Streptavidin 96-Well Clear Plates	**Specificity:** Biotinylated compounds **Sensitivity:** ≤1 ng/well biotinylated compound **Blocking Agent:** Proprietary blocking agent, at 200 µl/well, both reduces background and improves stability	■ Protein-Protein interactions ■ ELISA ■ High throughput immunoaffinity
BK200	1 kit	Biotinylation Kit, Cleavable	Complete kit for biotinylation of proteins containing buffers, biotinylation reagent, gel filtration resin, reducing agent, and affinity resin. Allows for removal of biotin moiety due to cleavable linker, facilitating recovery of protein after affinity capture on avidin/streptavidin support	■ Components sufficient for minimum 200 mg of protein
S2890	0.25 mg 1 mg	Streptavidin-Alkaline Phosphatase Conjugate	**Specificity:** Biotinylated compounds **Note:** Optimal working dilution should be determined emperically by trying a range of dilutions **Form:** Lyophilized powder	■ Western blotting ■ ELISA ■ Immunocytochemistry ■ Immunohistochemistry
S2438	0.25 mg	Streptavidin-Peroxidase Polymer, Ultrasensitive	**Specificity:** Biotinylated compounds **Note:** Multiple active biomolecules on each polymer chain increase the biotin binding capacity and amplify the peroxidase enzyme signal. Recommended use of 5 µg/ml	■ Western blotting ■ ELISA ■ Immunocytochemistry ■ Immunohistochemistry
S3762	0.1 mg 0.5 mg 1 mg	Streptavidin-FITC Conjugate	**Specificity:** Biotinylated compounds **Form:** The product is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide	■ Fluorescent detection of biotinylated compounds
S6402	1 ml	Streptavidin-Cy™3 Conjugate	**Specificity:** Biotinylated compounds **Form:** Optimal working dilution should be determined emperically by trying a range of dilutions from a 1 mg/ml stock in buffer	■ Fluorescent Immunohistochemistry ■ Fluorescent Immunocytochemistry ■ Flow cytometry

Cat. No.	Quantity	Product Description	Characteristics	Applications
Two Hybrid System				
G3042	0.2 mg	Anti-GAL4 DNA-BD (Binding Domain), Affinity Isolated Antibody produced in rabbit	**Specificity:** Recombinant GAL4 DNA-BD **Form:** Solution of affinity isolated antibody at ~0.8 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide	■ Western blotting **Working Dilution:** ■ Minimum 2.5 µg/ml by immunoblotting of GAL4 DNA-BD fusion protein in *S. cerevisiae* extract
G9293	0.2 ml	Anti-GAL4 (Activation domain), Affinity Isolated Antibody produced in rabbit	**Specificity:** GAL4 DNA activation domain fusion proteins **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumine and 15 mM sodium azide as a preservative	■ Western blotting (chemiluminescence) **Working Dilution:** ■ 0.5 µg/ml on *S. cerevisiae* extracts expressing GAL4-AD fusion proteins
V4388	0.2 ml	Anti-VP16, IgG Fraction of Antiserum	**Specificity:** Amino acids 463-476 of VP16 **Form:** IgG fraction of rabbit antiserum provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide	■ Immunoprecipitation ■ Immunoblotting **Working Dilution:** ■ Minimum dilution of 1:1,000 by immunoblotting of transfected mammalian extracts expressing recombinant VP16-tagged fusion protein ■ Minimum dilution of 1:1,000 by immunoprecipitation of transfected mammalian extracts expressing recombinant VP16-tagged fusion protein
B9808	0.2 mg	Anti-B42 Antibody produced in rabbit	**Specificity:** B42 tagged fusion proteins **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative	■ Indirect immunoblotting (chemiluminescent) **Working Dilution:** ■ 0.5-1.0 µg/ml using 25 ng of purified recombinant B42 fusion protein
L0415	100 µg	Anti-Lex A Antibody produced in rabbit	**Specificity:** Lex A tagged fusion proteins **Form:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative	■ Indirect immunoblotting (chemiluminescent) **Working Dilution:** ■ 1 µg/ml using extracts of Galactose-induced *S. cerevisiae* expressing Lex A from GAL1 promoter
MAT-Tag™ Antibody				
M6693	200 µg	Monoclonal Anti-MAT-Tag Antibody produced in mouse	**Specificity:** N-terminal and C-terminal MAT-Tag fusion proteins	■ Immunoprecipitation ■ Immunocytochemistry ■ Western blotting ■ EIA **Working Dilution:** ■ 1 µg/ml by indirect Western blotting (chemiluminescent)

Detection and Purification Selection Guide

Cat. No.	Quantity	Product Description	Characteristics	Applications
Miscellaneous				
B7786	0.2 ml 0.5 ml	Anti-fd Bacteriophage, IgG Fraction of Rabbit Antiserum	**Specificity:** The antibody binds specifically to phage coat proteins of fd phage or M13 phage and thus may act as a capture antibody when coated directly on multiwell plates or as a primary detection antibody for specifically captured fd or M13 phage **Form:** IgG fraction of rabbit antiserum supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide	■ ELISA ■ May be useful in sorting large phage display libraries (antibody, peptide, etc.) with the expressed proteins fused to either the gene III or to the gene VIII protein of the filamentous phage **Working Dilution:** ■ 1:1,000-1:8,000 by Indirect ELISA using 5×10^7 phage/ml or 5×10^{10} phage/ml, respectively
B2661	0.5 ml	Anti-fd Bacteriophage-Biotin Conjugate, Rabbit, IgG Fraction of Antiserum	**Specificity:** The antibody binds specifically to phage coat proteins of fd phage or M13 phage and thus may act as a capture antibody when coated directly on multiwell plates or as a primary detection antibody for specifically captured fd or M13 phage **Form:** IgG fraction of rabbit antiserum at ~3.5 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as preservative	■ ELISA ■ May be useful in rapidly sorting large phage display libraries (antibody, peptide, etc.) with the expressed proteins fused to either the gene III or to the gene VIII protein of the filamentous phage. It may be used as a reagent in ''phage ELISA'' offering sensitive and specific activity for the detection of recombinant phage **Working Dilution:** ■ 1:500-1:1,000 by indirect ELISA using 10^{10}-10^{11} phage/ml coated wells
C9336	0.5 ml	Anti-Chloramphenicol Acetyl Transferase (CAT), Rabbit, IgG Fraction of Antiserum	**Specificity:** Developed against a bacterial chloramphenicol acetyltransferase (CAT). The antibody identifies recombinant CAT as a predominant band of 26 kDa in eukaryotic cells transfected with a plasmid bearing the CAT gene **Antigen MW:** 26 kDa **Form:** IgG fraction of rabbit antiserum supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide	■ Western blotting ■ Immunocytochemistry **Working Dilution:** ■ 10 µg/ml by Western blotting (colorimetric) ■ 10 µg/ml by indirect immunofluorescence
L2164	0.2 ml	Monoclonal Anti-Luciferase	**Specificity:** Monoclonal Anti-Luciferase recognizes recombinant luciferase in transfected eukaryotic cells **Form:** Purified immunoglobulin (IgG1) at ~2.0 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide	■ Western blotting ■ Immunocytochemistry **Working Dilution:** ■ 2-4 µg/ml by immunoblotting using whole extracts of transfected 293T cells expressing luciferase ■ 20-40 µg/ml by immunocytochemistry using methanol: acetone fixation of transfected 293T cells expressing luciferase

Cat. No.	Quantity	Product Description	Characteristics	Applications
Miscellaneous *(con't)*				
L0159	0.5 ml	Anti-Luciferase, Firefly, Rabbit, IgG Fraction of Antiserum	**Specificity:** Anti-Luciferase is developed in rabbits using firefly *(Photinus pyralis)* luciferase as immunogen **Form:** IgG fraction of antiserum supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide	■ Immunocytochemistry **Working Dilution:** ■ 10 µg/ml by indirect immunofluorescence using eukaryotic cells transfected with a plasmid bearing the luciferase gene
C7988	200 µg	Monoclonal Anti-Cre, Clone 7-23, Purified immunoglobulin	**Specificity:** Cre-recombinase protein (~38 kDa) The antibody epitope resides within the most carboxy terminal 29 amino acids of the protein **Form:** Purified immunoglobulin solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative	■ Western blotting ■ Immunoprecipitation ■ Immunohistochemistry ■ Immunocytochemistry ■ Flow cytometry **Working Dilution:** ■ 0.5-1.0 µg/ml by Western blotting on recombinant Cre recombinase
D0942	100 µg	Anti-DHFR (C-ter), developed in rabbit, affinity isolated antibody	**Specificity:** DHFR and DHFR fusion proteins. The epitope resides within amino acids 171-185 of mouse DHFR **Form:** Solution of affinity isolated antibody at ~1.0 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative	■ Western blotting ■ Immunoprecipitation **Working Dilution:** ■ 0.5-1.0 µg/ml by Western blotting on 50-100 ng of purified recombinant DHFR (chemiluminescence) ■ 0.5-1.0 µg by Immunoprecipitation using 100-200 ng of purified DHFR
D1067	100 µg	Anti-DHFR (N-ter), developed in rabbit, affinity isolated antibody	**Specificity:** DHFR and DHFR fusion proteins. The epitope resides within amino acids 27-40 of mouse DHFR **Form:** Solution of affinity isolated antibody at ~1.0 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative	■ Western blotting ■ Immunoprecipitation **Working Dilution:** ■ 0.5-1.0 µg/ml by Western blotting on 100 ng of purified recombinant DHFR (chemiluminescence) ■ 0.5-1.0 µg by Immunoprecipitation using 100-200 ng of purified DHFR

Epitope Tag Removal

Enterokinase

Cat. No.	Product Description	Recognition Peptide
E5144	Highly specific serine protease used for the removal of the FLAG® peptide from fusion proteins. It is supplied as a NaCl form, lyophilized from deionized water	LYS-X of FLAG N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-X*-C **Peptides are resistant to cleavage if proline occupies posistion X.*

Enterokinase Removal Kit

Cat. No.	Product Description	Components
PRKE	Designed as research tool for the removal of bovine enterokinase from mixtures containing a fusion protein cleaved by the enzyme. Using the kit, removal of essentially all enterokinase is accomplished by binding with immobilized rabbit antibodies to calf intestine enterokinase followed by spin filtration	Anti-Enterokinase-Agarose Conjugate, 1.5 ml 20× Wash Buffer, 4 ml Spin Filters, 10 each

Thrombin CleanCleave™ Kit

Cat. No.	Product Description	Recognition Peptide
RECOMT	Bovine thrombin immobilized on 4% beaded agarose designed for cleavage of recombinant fusion proteins. This format circumvents the need for removal of thrombin by chromatographic techniques. The ligand density allows for fast and efficient cleavage; the resin can be reused multiple times with only minimal loss in cleavage efficiency	P4-P3-Pro-Arg/Lys-X-P1′-P2′ **P4 and P3 are hydrophobic residues, P1′ and P2′ are non-acidic residues, and Arg/Lys-X-P1′ is the scissile bond.* P2-Arg/Lys-X-P1′ **Where P2 or P1′ is glycine and Arg/Lys-X-P1′ is the scissle bond.*