DETECTION AND PURIFICATION

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 3xFLAG 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. Read more about the FLAG system in the Cloning and Expression Section.

Product Number	Package Size	Description	Characteristics	Applications
FLAG Affinity Gels				
A 4596	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
A 2220	1 ml 5 ml 10 ml 25 ml	ANTI-FLAG® M2 Agarose Affinity Gel (Freezer Safe)	**Specificity:** N-terminal, Met-N-terminal, C-terminal FLAG fusion proteins, 3xFLAG fusion proteins **Binding Capacity:** ≥ 0.6 mg per ml gel **Elution:** FLAG peptide; glycine, pH 3.5; 3xFLAG 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.	• Immunoprecipitation • Purification of FLAG and 3xFLAG fusion proteins
F 2426	1 ml 5 x 1 ml	EZview™ Red ANTI-FLAG® M2 Affinity Gel	**Specificity:** N-terminal, Met-N-terminal, C-terminal FLAG fusion proteins, 3xFLAG fusion proteins **Binding Capacity:** ≥ 0.6 mg per ml gel **Elution:** FLAG peptide glycine, pH 3.5; 3xFLAG 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				
F 3290	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
F 4799	4 mg 25 mg	3xFLAG™ 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 3xFLAG fusion proteins from ANTI-FLAG M2 affinity gels **Working Concentration:** 100 µg/ml is commonly used to elute 3xFLAG fusion proteins

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Product Number	Package Size	Description	Characteristics	Applications
FLAG Antibodies				
F 3040	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)
F 3165	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)
F 4042	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)
F 7425	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)
FLAG Antibody Conjugates				
F 9291	200 µg 1 mg 5 x 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 approximately 1 mg/ml.	• Western blotting • Immunocytochemistry **Working Dilution:** • 10 µg/ml by indirect Western blotting (chemiluminescent)
F 2922	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)
A 9469	200 µg 1 mg 5 x 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 approximately 1 mg/ml.	• Western blotting • ELISA **Working Dilution:** • 1:20,000 by indirect ELISA
A 8592	200 µg 1 mg 5 x 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 approximately 1 mg/ml.	• Immunocytochemistry • Immunohistochemistry • ELISA • Western blotting **Working Dilution:** • 1:20,000 by indirect ELISA • 1:100-1:1,000 by immunocytochemistry

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Product Number	Package Size	Description	Characteristics	Applications
FLAG Antibody Conjugates cont'd				
F 4049	200 µg 1 mg 5 x 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
A 9594	200 µg 1 mg 5 x 1 mg	ANTI-FLAG® M2-Cy3™ 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 approximately 1 mg/ml.	• Immunocytochemistry **Working Dilution:** • 10 µg/ml by direct immunofluorescence using mammalian cells fixed with methanol:acetone
ANTI-FLAG Secondary Antibodies				
A 9044	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)
A 9917	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)
A 3682	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)

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Product Number	Package Size	Description	Characteristics	Applications
FLAG-Control Proteins				
P 5975	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
P 2104	0.1 mg	Amino-terminal Met-3xFLAG-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
P 7457	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
P 7582	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

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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 (Figure 1) 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 3xFLAG fusion proteins.

Product Code	Description	Size
P 2983	ANTI-FLAG High Sensitivity Clear M2 coated 96-well plate	1 each 5 each

FLAG-Fusion Protein Detection Using StarBright® Green Substrate

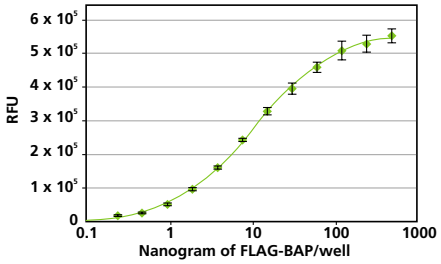


Figure 1: *Ten microliters of N-terminal FLAG-BAP Control protein (Prod. Code P 7582) diluted in Tris buffer with 1% BSA was incubated in an Anti-FLAG M2 coated clear 96-well plate (Prod. Code P 2983) for two hours at room temperature. The plate was washed four times with TBS with 0.05% TWEEN®-20 on 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-tagged 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-tagged proteins without the need for preliminary steps and calibrations. The affinity bound FLAG-tagged proteins can be efficiently eluted from the resin by acidic conditions or by competition with 3xFLAG® 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.

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

Product Code	Description	Size
CelL-M-M2	FLAG M Purification Kit	1 kit

FLAG® Y Purification Kit

The FLAG Y Purification Kit utilizes CelLytic™ Y for rapid and efficient lysis, and protein extraction from yeast cells, and the ANTI-FLAG® M2 affinity gel for affinity purification of active FLAG-tagged proteins. The affinity purification and immunoprecipitation 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-tagged proteins without the need for preliminary steps and calibrations. The affinity bound FLAG-tagged proteins can be efficiently eluted from the resin by acidic conditions or by competition with 3xFLAG® peptide. The eluted proteins can be analyzed for their activity, size, post-translational modifications, interactions, etc.

Sufficient for 50 immunoprecipitation reactions or one 1 ml affinity purification column.

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

Product Code	Description	Size
CelL-Y-M2	FLAG Y Purification Kit	1 kit

DETECTION AND PURIFICATION

Components
N-FLAG-BAP Control protein
ANTI-FLAG-M2-Peroxidase (HRP) Conjugate
3,3′5,5′-Tetramethylbezidine (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 x 10 cm) blots.

Product Code	Description	Size
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-tagged 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 x 10 cm) blots.

Product Code	Description	Size
PQ0400	ProteoQwest FLAG Chemiluminescent Western Blotting Kit, CPS Substrate	1 kit

DETECTION AND PURIFICATION

FLAG® Immunoprecipitation

FLAG® Immunoprecipitation Kit

Sigma's FLAG Immunoprecipitation Kit provides a rapid and efficient immunoprecipitation and elution of an active FLAG-tagged protein (Fig. 1).

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 & Benefits

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

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

Product Code	Description	Size
FLAG-IPT-1	FLAG Immunoprecipitation Kit	1 kit

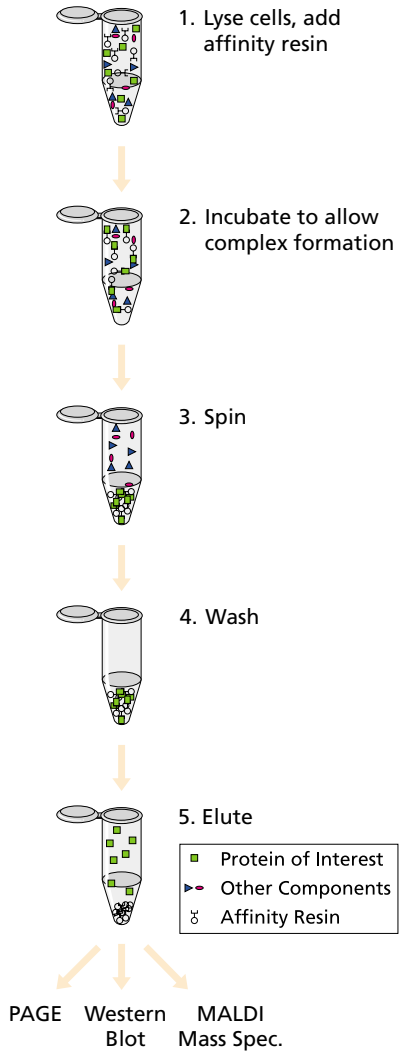


Figure 1. *Affinity-based molecular pull-down technique.*

DETECTION AND PURIFICATION

FLAG® 96-Well Immunoprecipitation System

Accurately Quantitate Protein-Protein Interactions in a 96-Well Format

The FLAG 96-Well Immunoprecipitation (IP) System is designed to facilitate the handling of multiple IP samples and eliminate the multiple centrifugation steps typically required when performing IP assays. Potential binding partners are first tagged with the FLAG and c-Myc epitopes. The bait protein is then captured on the ANTI-FLAG® M2 High Sensitivity 96-Well Plate. Finally, the interacting protein is detected by a colorimetric assay using an anti-c-Myc antibody.

The core kit (HT-COIP-1), IP Vector (COIP-P) and IP Detection (COIP-D) kits are designed specifically for Co-IP and validation of protein-protein interactions. Kits within the system are optimized for use together, yet can be used individually as appropriate for greatest flexibility. The core kit (HT-COIP-1) alone can be the basis for a multitude of user defined applications. The IP Vector kit (COIP-P) includes two expression vectors; one for a N-terminal FLAG fusion, the other for a N-terminal c-Myc fusion. Control plasmids and verification primers are included to verify each step of expression. The IP Detection kits include the ingredients for colorimetric detection of protein-protein interactions in a high throughput format.

Features & Benefits

- 3 Kits make up an integrated and complete system yet allow flexibility
- 96-well format greatly increases throughput
- Timely ELISA format for protein-protein interactions
- ANTI-FLAG M2 antibody is covalently coupled to the surface of the well, preventing any interference on Western blots

ELISA Analysis of the intereaction of FLAG-p53 and c-Myc-large T Antigen captured on ANTI-FLAG M2 Plates showing quantitative detection of c-Myc large T antigen.

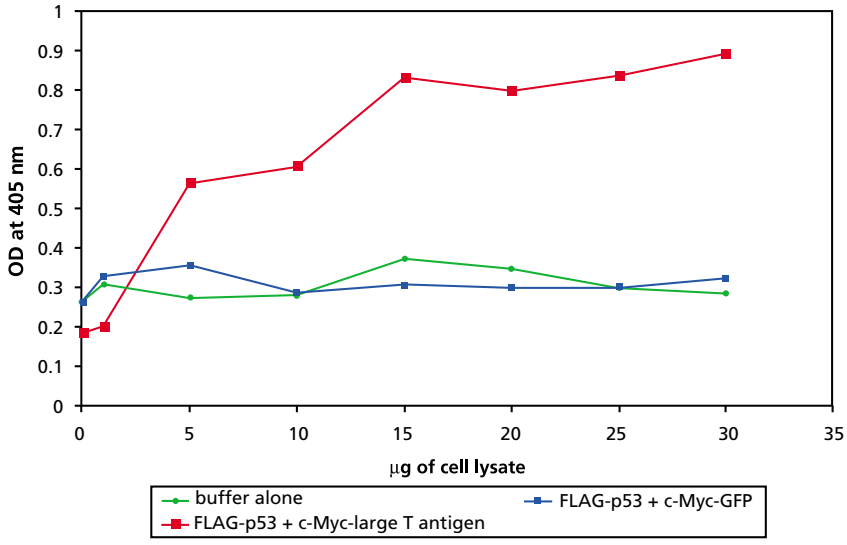
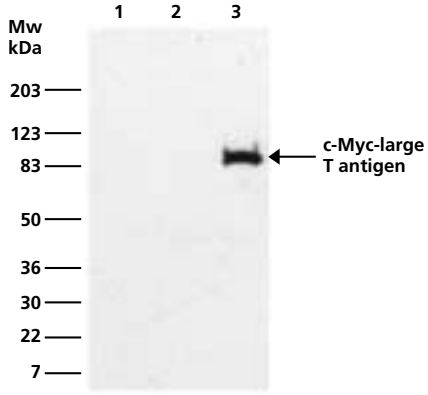


Figure 2. *COS-7 cells were co-transfected with FLAG-p53 and c-Myc-large T antigen and FLAG-p53 and c-Myc-GFP. Various amounts of cell lysate were incubated with the wells of the FLAG M2 plate. Detection of the exogenous large T antigen was performed in an ELISA procedure using alkaline phosphatase-conjugated anti-c-Myc antibody (1:100).*

Co-Immunoprecipitation of FLAG-p53 and c-Myc-large T antigen on FLAG M2 plate showing specific detection of the interaction.



Western blot using Anti-c-Myc

Lane 1: 30 µg cell lysate with no plasmids

Lane 2: 30 µg co-IP of cell lysate co-transfected with FLAG-p53 and c-Myc-GFP

Lane 3: 30 µg co-IP of cell lysate co-transfected with FLAG-p53 and c-Myc-large T antigen

Cell lysates, prepared from COS-7 cells that were co-transfected with FLAG-p53 and c-Myc-large T antigen, were incubated in the wells of the FLAG M2 plate. Eluted proteins were subjected to Western blotting with anti-c-Myc antibody. The interacting p53 -large T antigen complex was captured via the FLAG tag on p53 and detected via the c-Myc tag on the large T antigen.

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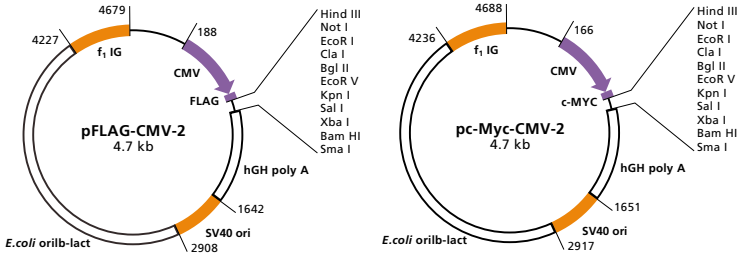
Components
pFLAG-CMV-2 Expression Vector
pc-Myc-CMV-2 Expression Vector
pFLAG-CMV-2-p53 control plasmid
pc-Myc-CMV-2-Large T Antigen control plasmid
pc-Myc-CMV-2 BAP control plasmid
Verification Primer–MF
Verification Primer–MR

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.



Product Code	Description	Size
COIP-P	Immunoprecipitation Vector Kit	1 kit

Components
ANTI-FLAG M2 Capture Plate
Amino-terminal FLAG-BAP fusion protein
10x Wash Buffer
Lysis Buffer
2x Sample Buffer
SealPlate Film

FLAG® 96-Well Immunoprecipitation Kit

The FLAG 96-Well Immunoprecipitation Kit is the core kit of a system to validate protein-protein interactions in a convenient 96-well format. The kit contains the ANTI-FLAG® 96-well plate and all reagents necessary for capture and elution of FLAG fusion proteins. This flexible kit can be the basis for user defined assays as well as use as part of the FLAG 96-well IP System.

Product Code	Description	Size
HT-COIP-1	FLAG 96-Well Immunoprecipitation Kit	1 kit

Components
Monoclonal Anti-c-Myc, Clone 9E10, Alkaline Phosphatase Conjugate
Tris-Buffered saline, pH 8.0, with 3% Nonfat Milk
SIGMA *FAST*™ p-nitrophenyl Phosphate Tablets

Immunoprecipitation Detection Kit

The Immunoprecipitation Detection Kit contains the reagents needed for colorimetric detection of protein-protein interactions on the 96-well ANTI-FLAG® M2 plate. The supplied Anti-c-Myc alkaline phosphatase conjugated antibody recognizes and binds to the c-Myc epitope tag on the co-expressed binding partner of the FLAG-tagged fusion protein immobilized on the plate. The conjugated antibody hydrolyzes the supplied p-Nitrophenyl Phosphate Disodium (pNPP) substrate to produce a yellow-colored end product that can be read spectrophotometrically at 405 nm.

Product Code	Description	Size
COIP-D	Immunoprecipitation Detection Kit	1 kit