Data Sheet

ANTI-FLAG® M1 Agarose Affinity Gel

A4596

Product Description

The FLAG® peptide sequence, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (DYKDDDDK), is one of the most widely used protein tags in recombinant protein expression and purification.[1-3] The ANTI-FLAG® M1 Agarose Affinity Gel is a covalent conjugate of a purified mouse IgG2B monoclonal antibody to agarose by a hydrazide linkage. The ANTI-FLAG® M1 antibody has a binding specificity for FLAG®-tagged proteins with the FLAG®-tag at their N-terminus. This product is useful for calcium-mediated purification of FLAG® fusion proteins.

The binding specificity is at the free N-terminus of the FLAG® sequence (N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C). Several theses[4-5] and dissertations[7-26] cite use of this product in their research protocols.

Reagent

The ANTI-FLAG® M1 Affinity Gel is supplied as a suspension in 50% glycerol containing 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4, and 0.02% (w/v) sodium azide.

Storage/Stability

Store the resin as supplied at –20 °C. Store columns of ANTI-FLAG® Affinity Gel as indicated in the following procedure. **Do not store the gel at freezing temperatures in the absence of glycerol**.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

Purification of FLAG® Fusion Proteins with ANTI-FLAG® M1 Affinity Gel

- Pre-equilibrate the column and all buffers. Perform all steps at room temperature.
- If there is a problem with proteases, perform column chromatography at 2-8 °C.
- Cellular debris and particulate matter can clog the column and must be removed prior to purification.
- Highly viscous samples containing chromosomal DNA or RNA can also clog the column. These samples should be sonicated or treated with nuclease to reduce the viscosity.
- Amino-terminal-FLAG-BAP™ positive control proteins can be used to verify the functionality of the gel.
- The ANTI-FLAG® M1 Affinity Gel is resistant to the following detergents: 5.0% TWEEN® 20, 5.0% TRITON® X-100, 0.1% NP-40®, 0.1% CHAPS, and 0.2% digitonin. This gel can also be used with 1.0 M NaCl or 1.0 M urea.
- **Do not use the gel in the presence of SDS, deoxycholate, or guanidine HCl**. This is not a comprehensive list of interfering substances. For general guidance, a Reagent Compatibility Table is provided on page 5.

A. Isolation of FLAG® Fusion Proteins from Yeast BJ3505 Broth

(Proceed to Section B, Column Set-Up, if you are not working in yeast.)

1. Grow 50 mL of culture in YP expression medium (1% dextrose, 3% glycerol, 1% yeast extract, and 2% peptone) using optimized conditions for secreted expression of FLAG® fusion protein.
2. Divide the culture into two sterile plastic centrifuge tubes and centrifuge at 10,000 × g for 5 minutes.
3. Pipette 20 mL of supernatant from each centrifuged tube into fresh sterile plastic centrifuge tubes. Carefully pipette the supernatant without transferring the cell pellet.
4. Centrifuge the supernatant at 15,000 × g for 15 minutes.
5. Pipette 15 mL from each centrifuged tube into a sterile plastic 50 mL storage tube. Keep on ice.

   Note: If you do not wish to store your protein, you may proceed directly to Step 7.

6. Storage of the FLAG® fusion protein (Optional):
   - Sterile-filter the centrifuged supernatant by passing it through a 0.45 µm filter.
   - 15 mL per filter may be processed before back pressure is too high from particulates clogging the filter.
   - Centrifuged culture broth from YP4 media cannot be sterilized using 0.45 µm filters, since they will become clogged.
   - The filtered supernatant may be stored on ice for up to a week before degradation of the FLAG® fusion protein begins to occur.
7. Buffer exchange into TBS/Ca buffer (50 mM Tris, pH 7.4, with 0.15 M NaCl and 10 mM $CaCl_2$) to insure high reproducible binding of the FLAG® fusion protein. Two methods are available:
   - Add 9 mL of centrifuged culture broth to 1 mL of 10× TBS/Ca (0.5 M Tris, pH 7.4, with 1.5 M NaCl and 100 mM $CaCl_2$).
   - Take 10 mL of centrifuged culture broth and buffer exchange into TBS/Ca on a Sephadex® G-25 desalting column.

B. Column Set-Up

1. Place the empty chromatography column on a firm support.
2. Attach a drainage tube to the column to control the flow rate. Limit the length of tubing to 25 cm.
3. Remove the top and bottom tabs and rinse the column twice with TBS (50 mM Tris with 150 mM NaCl, pH 7.4). Allow the buffer to drain from the column and leave residual TBS in the column to aid in packing the ANTI-FLAG® M1 Affinity Gel.

C. Packing the Column

1. Thoroughly suspend the vial of ANTI-FLAG® M1 Affinity Gel to make a uniform suspension of the gel beads.
2. Immediately transfer the suspension to the column.
3. Allow the gel bed to drain and rinse the vial with TBS.
4. Add the rinse to the column and allow the column to drain again. The gel bed will not crack when excess solution is drained under normal circumstances, but do not let the gel bed dry.

D. Washing the Column

Wash the gel by loading three sequential 5 mL aliquots of 0.1 M glycine HCl, pH 3.5, followed by three sequential 5 mL aliquots of TBS. Avoid disturbing the gel bed while loading. Let each aliquot drain completely before adding the next. Do not leave the column in glycine HCl buffer for longer than 20 minutes.

E. Binding FLAG® Fusion Proteins to the Column

1. Proper binding of FLAG® fusion proteins to the ANTI-FLAG® M1 affinity column requires 0.15 M sodium chloride at pH 7.0 as well as the presence of calcium. Before loading the lysate or culture supernatant onto the ANTI-FLAG® M1 affinity column, be sure that it contains at least 1 mM $CaCl_2$.

   Note: If the sample contains particulate material, centrifuge or filter prior to applying to the column. Viscous samples should be treated with DNase or sonicated prior to loading on the column.
2. Load the supernatant onto the column under gravity flow. Fill the column completely several times or attach a 12 mL column reservoir prior to loading for larger volumes. Depending upon the protein and flow rate, all of the antigen may not bind. Multiple passes over the column will improve the binding efficiency.
3. Wash the column three times with 12 mL aliquots of TBS/Ca (TBS containing 1 mM $CaCl_2$).

F. Elution of FLAG® Fusion Proteins

Three protocols are provided here as suggested protocols for elution of FLAG®-tagged proteins from this ANTI-FLAG® M1 Affinity Gel.

1. Elution of FLAG® Fusion Proteins by Acid Elution with Glycine:
   - Elute the bound FLAG® fusion protein from the column with six 1 mL aliquots of 0.1 M glycine HCl, at pH 3.5, into vials containing 15-25 µL of 1 M Tris, pH 8.0.
   - Do not leave the column in glycine-HCl buffer for longer than 20 minutes.
2. Elution of FLAG® Fusion Proteins by EDTA Chelating Agent:
   - Incubate the column with 1 mL of TBS/EDTA (TBS containing 2 mM EDTA) for 30 minutes to chelate the calcium ions.
   - Follow with 1 mL aliquots of TBS/EDTA at 10-minute intervals. Six elution aliquots are usually sufficient to elute the FLAG® fusion protein.
3. Elution of FLAG® Fusion Proteins by Competition with FLAG® Peptide:
   - Allow the column to drain completely.
   - Elute the bound FLAG®-tagged protein by competitive elution with five one-column volume aliquots of a solution with 100 µg/mL FLAG® peptide (Cat. No. F3290) in TBS.

G. Storing the Column

1. Wash the column three times with 5 mL of TBS/A (TBS containing 0.02 % sodium azide).
2. Then add another 5 mL of TBS/A.
3. Store at 2-8 °C without draining.

H. Recycling the Column

1. It is recommended the column be regenerated immediately after use by washing with three 5 mL aliquots of glycine HCl, pH 3.5.
2. The column should be **immediately** re-equilibrated in TBS until the effluent is at neutral pH.

General Notes

1. When *E. coli* periplasmic extracts are applied to the column, it may be possible to reuse the column as many as 20 times.
2. When *E. coli* crude cell extracts are applied to the column, the column may be reused 3 times before loss of binding capacity is observed.
3. The number of cycles observed will be dependent on variables such as sample condition.
4. **Do not leave the column in glycine-HCl buffer for longer than 20 minutes.**

References

1. Terpe, K., *Appl. Microbiol. Biotechnol.*, **60(5)**, 523-533 (2003).
2. Einhauer, A., and Jungbauer, A., *J. Biochem. Biophys. Methods*, **49(1-3)**, 455-465 (2001).
3. Chubet, R.G., and Brizzard, B.L., *BioTechniques*, **20(1),** 136-141 (1996).
4. Munjal, Neera, "Bioprocessing of microalgae *C. reinhardtii* for production and purification of single chain antibody fragment". Texas A&M University, M.S. thesis, p. 24 (December 2014).
5. Quinones, Kathryn Marie, "Bioprocessing of recombinant proteins produced in the chloroplast of *Chlamydomonas reinhardtii*". Texas A&M University, M.S. thesis, p. 32 (August 2015).
6. DeRango-Adem, Eva Francesca, "Macromolecular Interactome of *Tetrahymena* CHD Family Chromatin Remodelers". University of Toronto, M.Sc. thesis, p. 50 (October 2017).
7. Heymann, Gregory Seth, "C-Terminus of HSP70 Interacting Protein as a Regulator of Parkin Translocation". University of Toronto, M.Sc. thesis, p. 22 (2019).
8. Jabbar, Javard, "POLR2A/RPB1 subunit of RNA polymerase II interacts with NTD-MED14 containing core mediator complex to facilitate basal and activator driven transcription". Bilkent University, M.Sc. thesis, p. 21 (June 2020).
9. Direkze, Shamindra Gerald, "Characterisation of Transcriptional Mediator Subunit, MED17 and its Regulation by Cyclins". University of London, Ph.D. dissertation, p. 120 (2006).
10. Cabrera, Ilva Esther, "The Role of G Protein Signaling Components in Growth and Development of the Filamentous Fungus, *Neurospora crassa*". University of California Riverside, Ph.D. dissertation, p. 149 (December 2015).
11. Ning, Wenjing, "Functionalized membranes for protein purification and proteolysis prior to mass spectrometry analysis". Michigan State University, Ph.D. dissertation, p. 28 (2016).
12. Ragazzini, Roberta, "Identification of a tissue-specific cofactor of polycomb repressive complex 2". Université Pierre et Marie Curie, Ph.D. dissertation, p. 106 (2017).
13. DiChiara, Andrew Stephen, "Type I Collagen Proteostasis". Massachusetts Institute of Technology, Ph.D. dissertation, p. 149 (February 2018).
14. Kulkarni, Sayali Vishwas, "Bioprocessing of microalgae for extraction of high-value products". Texas A&M University, Ph.D. dissertation, p. 101 (August 2018).
15. Ravi, Ayswarya, "Evaluation of mixed-mode chromatography resins for isolation of recombinant therapeutic proteins". Texas A&M University, Ph.D. dissertation, p. 43 (August 2019).
16. Laurien, Lucie Henriette, "The role of RIPK1 auto-phosphorylation at S166 in cell death and inflammatory signaling". Universität zu Köln, Ph.D. dissertation, p. 29 (2021).

Troubleshooting Guide

Problem	Possible Cause	Solution
No signal is observed.	FLAG® fusion protein is not present in the sample.	• Make sure the protein of interest contains the FLAG®-tag by immunoblot or dot blot analyses. • Prepare fresh lysates. Avoid using frozen lysates. • Use appropriate protease inhibitors in the lysate or increase their concentrations to prevent degradation of the FLAG® fusion protein.
	Washes are too stringent.	• Reduce the number of washes. • Avoid adding high concentrations of NaCl to the mixture. • Use solutions that contain less or no detergent.
	Incubation times are inadequate.	Increase the incubation times with the affinity resin (from several hours to overnight).
	Interfering substance is present in sample.	• Lysates with high concentrations of dithiothreitol (DTT), 2-mercaptoethanol, or other reducing agents may destroy antibody function, and must be avoided. • Excessive detergent concentrations may interfere with the antibody-antigen interaction. Detergent levels in buffers may be reduced by dilution.
	Detection system is inadequate.	If Western blotting detection is used: • Check primary and secondary antibodies using proper controls to confirm binding and reactivity. • Verify that the transfer was adequate by staining the membrane with Ponceau S. • Use fresh detection substrate or try a different detection system.
Background is too high.	Proteins bind nonspecifically to the ANTI-FLAG® monoclonal antibody, the resin beads, or the microcentrifuge tubes.	• Pre-clear lysate with Mouse IgG-Agarose (Cat. No. A0919) to remove nonspecific binding proteins. • After suspending beads for the final wash, transfer entire sample to a clean microcentrifuge tube before centrifugation.

Reagent Compatibility Table

Reagent	Effect	Comments
Chaotropic agents (for example, urea, guanidine HCl)	Denatures the immobilized M1 antibody	• Do not use any reagent that contains chaotropic agents, since chaotropic agents will denature the M1 antibody on the resin and destroy its ability to bind the FLAG® fusion proteins. • If necessary, low concentrations of urea (1 M or less) can be used.
Reducing agents (such as DTT, DTE, 2-mercapto-ethanol)	Reduces the disulfide bridges holding the M1 antibody chains together	Do not use any reagent that contains reducing agents, since reducing agents will reduce the disulfide linkages in the M1 antibody on the resin and destroy its ability to bind FLAG® fusion proteins.
TWEEN® 20, 5% or less	Reduces nonspecific protein binding to the resin	May be used up to recommended concentration of 5%, but do not exceed.
TRITON™ X-100, 5% or less	Reduces nonspecific protein binding to the resin	May be used up to recommended concentration of 5%, but do not exceed.
IGEPAL® CA-630, 0.1% or less	Reduces nonspecific protein binding to the resin	May be used up to recommended concentration of 0.1%, but do not exceed.
CHAPS, 0.1% or less	Reduces nonspecific protein binding to the resin	May be used up to recommended concentration of 0.1%, but do not exceed.
Digitonin, 0.2% or less	Reduces nonspecific protein binding to the resin	May be used up to recommended concentration of 0.2%, but do not exceed.
Sodium chloride, 1.0 M or less	Reduces nonspecific protein binding to the resin by reducing ionic interactions	May be used up to recommended concentration of 1.0 M, but do not exceed.
Sodium dodecyl sulfate	Denatures the immobilized M1 antibody	• Do not use any reagent that contains sodium dodecyl sulfate in the loading and washing buffers, since sodium dodecyl sulfate will denature the M1 antibody on the resin and destroy its ability to bind FLAG® fusion proteins. • Sodium dodecyl sulfate is included in the sample buffer for removal of protein for immunoprecipitation. However, after contact with sodium dodecyl sulfate, the resin cannot be reused.
0.1 M glycine HCl, pH 3.5	Elutes FLAG® protein from the resin	Do not leave the column in glycine HCl for longer than 20 minutes. Longer incubation times will begin to denature the M1 antibody.
Deoxycholate	Interferes with M1 binding to FLAG® proteins	Do not use any reagent that contains deoxycholate, since deoxycholate will inhibit the M1 antibody from binding to FLAG® fusion proteins.

Notice

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