3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

ANTI-FLAG® M1 Agarose Affinity Gel

Catalog Number **A4596**
Storage Temperature –20 °C

Product Description
ANTI-FLAG® M1 Agarose Affinity Gel is a purified mouse IgG2B monoclonal antibody covalently attached to agarose by hydrazide linkage. It is useful for calcium-mediated purification of FLAG® fusion proteins.

Binding specificity:
Free N-Terminus of FLAG sequence
N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C

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 (PBS/A).

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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.

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

Pre-equilibrate the column, all buffers, and 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. It 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.

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 supernatent from each centrifuged tube into fresh sterile plastic centrifuge tubes. Carefully pipette the supernatent without transferring the cell pellet.

4. Centrifuge the supernatent at 15,000 × *g* for 15 minutes.

5. Pipette 15 ml from each centrifuged tube into a sterile plastic 50 ml storage tube and keep on ice.
Note: If you do not wish to store your protein, proceed directly to Step 7.

6. Storage of the FLAG fusion protein (Optional) – Filter sterilize the centrifuged supernatent 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 supernatent 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:

   a. 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 $CaCl_2$).

   b. 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. Select one of the three following procedures for elution.

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 fusion protein by competitive elution with five one-column volume aliquots of a solution containing 100 µg/ml FLAG peptide (Catalog Number F3290) in TBS.

G. Storing the Column – Wash the column three times with 5 ml of TBS/A (TBS containing 0.02 % sodium azide) then add another 5 ml of TBS/A and store at 2–8 °C without draining.

H. Recycling the Column – It is recommended the column be regenerated immediately after use by washing with three 5 ml aliquots of glycine HCl, pH 3.5. The column should be immediately re-equilibrated in TBS until the effluent is at neutral pH.

Notes: When *E. coli* periplasmic extracts are applied to the column it may be possible to reuse the column as many as 20 times.

When *E. coli* crude cell extracts are applied to the column it may be reused 3 times before loss of binding capacity is observed.

The number of cycles observed will be dependent on variables such as sample condition.

Do not leave the column in glycine HCl buffer for longer than 20 minutes.

AH,PHC 09/10-1