

## Product Information

### Purified 3xFLAG® peptide

≥ 95% (HPLC), lyophilized powder

Product Number **SAE0194**

### Product Description

This product is a synthetic peptide of 23 amino acid residues of a calculated molecular mass of 2,864 Da, with the following sequence,

N-Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-C

The Asp-Tyr-Lys-Xaa-Xaa-Asp motif<sup>1</sup> 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-Lys).

This product is for use in competitive elution of 3X FLAG fusion proteins from the ANTI-FLAG M2 monoclonal antibody in solution or bound to agarose on the ANTI-FLAG M2 agarose affinity gel.

A working concentration of 100 µg/mL is commonly used to elute 3X FLAG fusion proteins from the ANTI-FLAG M2 affinity gel. Five column volumes of this working solution are sufficient to elute most 3X FLAG fusion proteins. 3X FLAG peptide will elute ~50% of the control 3X FLAG-BAP (bacterial alkaline phosphatase) fusion protein (Amino-terminal Met-3XFLAG™-BAP Control Protein, Catalog Number P2104) bound to ANTI-FLAG M2 affinity gel.

**Note:** FLAG peptide (Catalog Number F3290) will **not** elute 3X FLAG fusion proteins.

### Preparation Instructions

To prepare a stock solution, dissolve in TBS (50 mM Tris-HCl, pH 7.4, with 150 mM NaCl) at a concentration of 5 µg/mL. Aliquot and store at -20 °C. Repeated freezing and thawing is not recommended.

### 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.

### Storage/Stability

Store the lyophilized product at -20 °C. For extended storage after reconstitution, keep at -20 °C in working aliquots. Avoid repeated freeze-thaw cycles.

### Procedure

#### Peptide Elution of 3X FLAG Fusion Protein from ANTI-FLAG M2 Affinity Gel

**Note:** Affinity chromatography may be performed at room temperature. If, however, the 3X FLAG fusion protein is unstable or sensitive to protease, chromatography should be performed at 2-8 °C.

1. Column Set-Up
  - Place the empty chromatography column on a firm support.
  - Attach a drainage tube to the column to control the flow rate. Limit the length of tubing to 25 cm.
  - Remove the top and bottom tabs and rinse the column twice with TBS. Allow the buffer to drain from the column and leave residual TBS in the column to aid in packing the ANTI-FLAG M2 affinity gel.
2. Packing the Column
  - Thoroughly suspend a vial of ANTI-FLAG M2 affinity gel to make a uniform suspension of the gel beads.
  - Immediately transfer the suspension to the column.
  - Allow the gel bed to drain and rinse the vial with TBS.
  - Add the rinse to the column and allow it to drain again. The gel bed will not crack when excess solution is drained under normal circumstances, but do not let the gel bed run dry.
3. 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 for longer than 20 minutes.
4. Binding the 3X FLAG Fusion Protein to the Column
  - Proper binding of FLAG fusion proteins to

the ANTI-FLAG M2 affinity gel requires physiological ionic strength and neutral pH. (Note: If the sample contains particulate material, centrifuge or filter prior to applying to the column. Viscous samples should be sonicated or treated with DNase I prior to loading on the column.)

- Load the sample onto the column under gravity flow. Fill the column completely several times for large 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.
  - After binding, wash the column three times with 12 mL aliquots of TBS.
5. Elution of 3X FLAG Fusion Proteins by Competition with 3X FLAG Peptide:
- Allow the column to drain completely.
  - Elute the bound 3X FLAG-BAP or the 3X FLAG fusion protein of interest by competitive elution with five one-column volume aliquots of a solution containing 100 µg/mL of 3X FLAG peptide in TBS.
  - Note: Column packing quality, flow rate, and specific properties of the 3X FLAG fusion protein may influence the efficiency of protein elution.

6. Recycling the Column:

- 3X FLAG peptide may not elute all of the 3X FLAG fusion protein bound to ANTI-FLAG M2 affinity gel.
- It is recommended the column be regenerated immediately after use by washing with three 5 mL aliquots of 0.1 M glycine HCl, pH 3.5.
- The column should be immediately re-equilibrated in TBS until the effluent is at neutral pH.
- Note: Do not leave the column in glycine HCl for longer than 20 minutes.

7. Storing the Column:

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

**References**

1. Miceli, R. *et al.*, *J. Immunol. Methods*, **167(1-2)**, 279-287 (1994).

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