

Product Information

3X FLAG® Peptide

F4799

Storage Temperature 2-8 °C

Product Description

The 3X FLAG® Peptide is a synthetic peptide of 23 amino acid residues, with a calculated molecular weight of 2,864 Da, where the Asp-Tyr-Lys-Xaa-Xaa-Asp motif¹ 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). The sequence of the 3X FLAG® Peptide is as follows:

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

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.^{2,3} Five column volumes of this working solution are sufficient to elute most 3X FLAG® fusion proteins. FLAG peptide (Cat. No. F3290) will not elute 3X FLAG® fusion proteins.

Other publications have used other concentrations of this 3X FLAG® Peptide at varying concentrations, where we have not necessarily tested those different conditions, such as:

- 200 ng/mL⁴
- 150 µg/mL⁵
- 0.2 mg/mL⁶
- 0.3 mg/mL (300 µg/mL)⁷
- 150 µM⁸
- 340 µM⁹
- 0.5 mg/mL¹⁰

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 mg/mL. Aliquot and store at -20 °C. Repeated freezing and thawing is not recommended.

Storage/Stability

Store the product at 2-8 °C.

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

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.

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. Allow the buffer to drain from the column. Leave residual TBS in the column to aid in packing the ANTI-FLAG® M2 affinity gel.

Packing the Column

1. Thoroughly suspend a vial of ANTI-FLAG® M2 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 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.

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.

Binding the 3X FLAG® Fusion Protein to the Column

1. 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 deoxyribonuclease I prior to loading on the column.
2. 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.
3. After binding, wash the column three times with 12 mL aliquots of TBS.

Elution of 3X FLAG® Fusion Proteins by Competition with 3X FLAG® Peptide:

1. Allow the column to drain completely.
2. 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 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.

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.

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.

References

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