Product Information

Anti-Flag® M2 Antibody, Mouse Monoclonal

Clone M2, Purified from Hybridoma Cell in bioreactor

B3111

Product Description

Monoclonal ANTI-FLAG® M2 is a purified immunoglobulin, IgG1, monoclonal antibody, purified from culture supernatant of hybridoma cells, that binds to FLAG® fusion proteins.[1] Unlike ANTI-FLAG® M1 antibody, the M2 antibody will recognize the FLAG® sequence at the N-terminus, Met-N-terminus, C-terminus, or at an internal site of FLAG® fusion proteins. Monoclonal ANTI-FLAG® M2 is useful for identification and capture of FLAG® fusion proteins by common immunological procedures such as Western blots and immunoprecipitation. It is also useful for affinity purification of FLAG® fusion proteins when bound to a solid support.

Monoclonal ANTI-FLAG® M2 binding is not calcium dependent.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 15 mM sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet on the product page online at SigmaAldrich.com for information regarding hazards and safe handling practices.

Storage/Stability

Store the undiluted antibody at – 20 °C in working aliquots. Repeated freezing and thawing is not recommended.

NOTE: Overtime, small amounts of purified antibodies can precipitate from solution due to intermolecular hydrophobic interactions. If a precipitate is observed in this product, briefly centrifuge the vial to pellet the precipitate. Withdraw the desired volume of antibody solution from the clear supernatant for use. This should not alter the performance of the purified antibody in Western blot or immunoprecipitation applications.

Procedure

Improved Western Blot Method for Detecting FLAG® Fusion Proteins using Monoclonal ANTI-FLAG® M2.

1. Separate FLAG® fusion proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5–10 µg of total lysate protein per lane.
2. Transfer proteins from the gel to an Immobilon®-P or other polyvinylidene difluoride (PVDF) membrane. Nitrocellulose membranes can be used, but typically result in less sensitivity.
3. Wash the blot in at least 0.5 mL/cm^2 of Milli-Q® water for 2–3 minutes with mild agitation.
4. Block the blot with at least 0.5 mL/cm^2 of Phosphate.

   Buffered Saline (PBS), with 5% nonfat milk, for 60 minutes at room temperature with agitation (about 50–60 rpm).
5. Remove the blocking agent and wash once with 0.5 mL/cm^2 of PBS.
6. Add Monoclonal ANTI-FLAG® M2 to a final concentration of 10 µg/mL to the blot in at least 0.5 mL/cm^2 of PBS-TWEEN® 20, with 0.5% nonfat milk and incubate at room temperature for 60 minutes.

   NOTE: Using less Monoclonal ANTI-FLAG M2 antibody may help to reduce background and cross-reactivity.
   See the "Troubleshooting Guide."
7. Remove the Monoclonal ANTI-FLAG® M2 solution and wash once with at least 0.5 mL/cm^2 of PBS.
8. Add Anti-Mouse IgG–Peroxidase (Cat. No. A2304) or equivalent, to at least 0.5 mL/cm^2 of PBS-TWEEN® 20, with 0.5% nonfat milk. Incubate the blots with shaking at room temperature for 60 minutes.

9. Wash the blot eight times for a total of 20 minutes in Phosphate Buffered Saline (PBS), plus 0.05% TWEEN® 20.

Immunofluorescence

Monoclonal ANTI-FLAG® M2 may be used in immunofluorescent procedures. A typical concentration for use is 20 µg/mL.[2]

Product Profile

Protein concentration (E_{280}): 3.8 – 4.2 mg/mL.

Antigenic binding site: N-Asp-Tyr-Lys-Asp-Asp-Asp-AspLys-C.

Specificity: Monoclonal ANTI-FLAG® M2 detects a single band of protein on a Western blot from HEK 293 cells over-expressing FLAG® proteins, crude cell lysate.

Sensitivity: Monoclonal ANTI-FLAG® M2 detects 2 ng of FLAG-BAP™ fusion protein on a dot blot using chemiluminescent detection.

Note: To obtain best results, it is recommended that each individual user determine working dilution by titration assay.

References

1. Brizzard, B.L., et al., Immunoaffinity purification of FLAG® epitope-tagged bacterial alkaline phosphatase using a novel monoclonal antibody and peptide elution. BioTechniques, 16, 730-735 (1994)
2. Ciaccia, A.V., and Price, E.M., IBI FLAG® Epitope, 1, 4-5 (1992)
3. Bjerrum, O.J., and Heegaard. N.H.H., CRC Handbook of Immunoblotting of Proteins, Volume I, Technical Descriptions, CRC Press, (1988) p. 229-236
4. Dunbar, B.S. (ed.) Protein Blotting: A Practical Approach, IRL Press, NY, p. 67-70 (1994)
5. Fortin, A., et al., A 56- to 54-kilodalton non grata signal in immunoblot analysis using the horseradish peroxidase chemiluminescence system. Biochem. Cell Biol., 72, 239-243 (1994)
6. Harlow, E., and Lane, D., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988, Product Code A2926
7. Layton, Curtis J., Peter L. McMahon, and William J. Greenleaf. "Large-scale, quantitative protein assays on a high-throughput DNA sequencing chip." Molecular cell 73.5 (2019): 1075-1082.
10. Develop the blots using suitable ECL™ substrate for 1 minute.

8. Bi, Xinyan, and Kun-Lin Yang. "Liquid crystals decorated with linear oligopeptide FLAG® for applications in immunobiosensors." Biosensors and Bioelectronics 26.1 (2010): 107-111.
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11. Roosild, Tarmo P., Samantha Castronovo, and Senyon Choe. "Structure of anti-FLAG® M2 Fab domain and its use in the stabilization of engineered membrane proteins." Acta Crystallographica Section F: Structural Biology and Crystallization Communications 62.9 (2006): 835-839.

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