Product Information

Monoclonal ANTI-FLAG® M2, Clone M2

Produced in Mouse, Purified Immunoglobulin

F3165

Product Description

Monoclonal ANTI-FLAG® M2 is a purified immunoglobulin, IgG1, monoclonal antibody, isolated from murine ascites fluid, 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 immuno-precipitation. It is also useful for affinity purification of FLAG® fusion proteins when bound to a solid support.

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

Reagent

This product is supplied in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% sodium azide.

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.

Preparation Instructions

Dilute the antibody solution from 0.5–10 µg/mL in Tris Buffered Saline, pH 8.0, with 3% nonfat milk (Cat. No. T8793). Adjust the antibody concentration to maximize detection sensitivity and to minimize background.

Storage/Stability

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

Note: Over time, 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 Tris Buffered Saline, pH 8.0, with 3% nonfat milk, or 50 mM Tris, 0.138 M NaCl, 2.7 mM KCl, pH 8.0, containing 30 mg/mL nonfat dry milk, for 30 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 TBS (Cat. No. T6664).
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 TBS with 3% nonfat dry milk and incubate at room temperature for 30 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 TBS.
8. Add Anti-Mouse IgG–Peroxidase (Cat. No. A9044) or equivalent, to at least 0.5 mL/cm^2 of TBS with 3% nonfat dry milk. Use the concentrations listed in Table 1. These concentrations are recommended starting concentrations for the antibodies used in Western blotting. Incubate the blots with shaking at room temperature for 30 minutes.

Table 1.

Antibody Concentrations

ANTI-FLAG® M2 primary antibody	Substrate	Secondary antibody concentration
0.5-10 µg/mL	ECL+™	1:80,000
0.5-10 µg/mL	ECL™	1:10,000

9. Wash the blot eight times for a total of 20 minutes in Tris Buffered Saline with TWEEN® 20, pH 8.0 (50 mM Tris, 0.138 M NaCl, 2.7 mM KCl, pH 8.0, plus 0.05% TWEEN® 20, Cat. No. T9039).
10. Develop the blots with the appropriate substrate for 5 minutes.
11. Expose BioMax™ light film to the blot. Exposure times range from 30 seconds to 10 minutes. It is best to do a quick exposure of 10–30 seconds to determine what exposure time is needed. If the signal is too intense even at the short exposure times, let the signal decay from 1–8 hours or longer if necessary, and then re-expose the film.

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}): 2.0 – 5.0 mg/mL

Antigenic binding site:

N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C

Specificity: Monoclonal ANTI-FLAG® M2 detects a single band of protein on a Western blot from an *E. coli* crude cell lysate.

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

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

References

1. Brizzard, B.L. *et al.*, *BioTechniques*, **16(4)**: 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, pp. 229-236 (1988).
4. Dunbar, B.S. (ed.), *Protein Blotting: A Practical Approach*. IRL Press at Oxford University Press (Oxford, UK / New York, NY), pp. 67-70 (1994).
5. Fortin, A. *et al*., *Biochem. Cell Biol*., **72(5-6)**: 239-243 (1994).
6. Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*, pp. 726 (1989).
7. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY) (1988).
8. Pampori, N.A. *et al*., *BioTechniques*, **18(4)**: 589-590 (1995).

Problem	Possible Cause	Solution
Fusion protein not detected	Protein not expressed	Verify nucleic acid sequence of FLAG® in vector construct. If sequence is present, attempt to optimize expression.
	Target protein poorly represented in sample	Positive controls should always be included. If the positive control works, the sample may not contain the FLAG® fusion protein of interest or it may be present at concentrations too low to detect. Immunoprecipitation with Monoclonal ANTI-FLAG® M2 Affinity Gel (Cat. No. A2220) may be required for low FLAG® fusion protein concentrations. Positive controls available from Sigma: • Amino-terminal FLAG-BAP™ Fusion Protein (Cat. No. P7582) • Carboxy-terminal FLAG-BAP™ Fusion Protein (Cat. No. P7457) • Amino-terminal Met-FLAG-BAP™ Fusion Protein (Cat. No. P5975)
	Defective detection reagents	Run appropriate controls to ensure performance. Use 10 ng/lane of a control FLAG-BAP™-fusion protein as a positive control. If no signal is obtained with the control, repeat the procedure using a newer lot of antibody-HRP conjugate and freshly prepared reagents.
	Inadequate exposure time using chemiluminescent system	If no signal is seen, expose for longer times. We recommends trying 30-second to 10-minute exposure times.
	Inappropriate film	Switch to film designated for chemiluminescent detection such as Kodak BioMax™ Light.
	No target protein present on membrane	Verify transfer by visualizing proteins on the membrane using a Ponceau S solution (Cat. No. P7170). If possible, a positive control should always be run to ensure that the components are functioning. Prestained protein markers (such as Cat. No. C1992 and C4861) may also be used to verify complete transfer.
	Antigen is covered by blocking reagent due to overblocking	Masking of a signal can occur if the blocking reagent (such as casein or gelatin blocking buffers, Cat. No. C7594 or G7663, respectively) is used at too high a concentration. A dilution of 1:1 to 1:3 may be done to decrease the concentration. If the problem persists, use TBS with 3% non-fat dry milk (Cat. No. T8793).
	Antibody concentration not optimal	Determine optimal working dilution for ANTI-FLAG® antibody by titration. Consider using more antibody if no signal or weak signal is detected. Also, antibody used at too high a concentration can also cause inhibition of signal especially in chemiluminescent detection systems.

Problem	Possible Cause	Solution
Cross-reactivity	Cellular extract concentration is too high	2.5 to 10 µg per lane of total lysate protein is usually enough to obtain a good signal. Load less cellular extract or serially dilute the cell extract to obtain the optimal signal to noise ratio.
	ANTI-FLAG® M2 antibody concentration is too high	Dilute ANTI-FLAG® M2 antibody from 0.1 to 0.5 µg/mL. Use TBS with 3% non-fat dry milk as diluent.
	Secondary antibody concentration is too high	We recommend initial dilutions of 1:10,000 for ECL™ and 1:80,000 for ECL+™. Further dilutions may be necessary
	ANTI-FLAG® M2 antibody cross-reacts with naturally occurring FLAG-like epitopes	Increasing the temperature to 37 °C during the blocking, binding and wash steps may reduce cross-reactivity. Lysates from mock-transfected controls (transfected with plasmid without insert DNA) will help distinguish the FLAG-fusion proteins from other cross-reacting proteins.

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