Product Information

Monoclonal ANTI-FLAG® M2, Clone M2

Produced in Mouse, Affinity Isolated Antibody

F1804

Storage Temperature −20 °C

Product Description

Monoclonal ANTI-FLAG® M2 is a mouse-derived, affinity-purified IgG1 monoclonal antibody that binds to fusion proteins containing a FLAG® peptide sequence.[1] The M2 antibody will recognize a FLAG® peptide sequence at the N-terminus, Met-N-terminus, C-terminus, or internal sites of a fusion protein. Binding of the M2 antibody is not dependent on calcium.

Monoclonal ANTI-FLAG® M2 is useful for detection, identification, and capture of fusion proteins that contain a FLAG® peptide sequence by common immunological procedures, such as Western blotting, immunofluorescence, and immunoprecipitation.

Reagent

Supplied at a concentration of ~ 1 mg of protein per mL of solution, and formulated in 50% glycerol, 10 mM sodium phosphate, and 150 mM NaCl, pH 7.4. The formulation contains no antimicrobial preservatives.

Antigenic binding site

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

Specificity

The monoclonal antibody detects only the target protein band(s) on a Western blot from an *E. coli*, plant or mammalian crude cell lysate.

Sensitivity

The monoclonal antibody detects as little as 2 ng of target protein by dot blot. The Western blot is tested down to 10 ng, but may detect lower using the procedure detailed below.

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

Immediately prior to use, dilute the monoclonal antibody in Tris-buffered saline (TBS), pH 8.0, with 3% nonfat milk (Cat. No. T8793). Dilutions in the described procedures are provided as guidelines. Adjust the antibody concentration to maximize detection sensitivity and minimize background.

Storage

Store the undiluted antibody at −20 °C in working aliquots. The product, as formulated, will not freeze when stored at the recommended temperature.

Note: Over time, small amounts of purified antibodies can precipitate due to intermolecular hydrophobic interactions. If 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 most applications.

Procedures

Western Blot Immunostaining[2-5]

Note: this procedure is based on chemiluminescent detection using Chemiluminescent Peroxidase Substrate-1 (Cat. No. CPS160). Dilutions of both primary and secondary antibodies may require optimization when using other substrates or conditions.

1. Separate fusion proteins containing a FLAG® peptide sequence from sample lysates using a standard SDS-PAGE protocol. Load 2.5–10 µg of total protein lysate per lane.
2. Transfer proteins from the gel to a nitrocellulose membrane, Immobilon®-P, or other polyvinylidene difluoride (PVDF) membrane. The PVDF membrane may provide greater downstream sensitivity.
3. Wash the blot in at least 0.5 mL/cm^2 of purified water for 2–3 minutes employing gentle agitation (50–60 rpm).
4. Block the blot with at least 0.5 mL/cm^2 of Tris Buffered Saline (TBS), pH 8.0, with 3% nonfat milk for 30 minutes at room temperature, employing gentle agitation.
5. Remove the blocking agent and wash once with 0.5 mL/cm^2 of Tris buffered saline, pH 8.0 (Cat. No. T6664).
6. Add the desired concentration of monoclonal antibody to the blot. A final antibody concentration of 1 µg/mL (1:1,000 dilution of the antibody as supplied) in at least 0.5 mL/cm^2 of TBS with 3% nonfat milk is suggested. Incubate at room temperature for 30 minutes employing gentle agitation.

   Note: Dilutions must be optimized for different substrates and systems.
7. Decant off the Monoclonal ANTI-FLAG® M2 solution and wash once with at least 0.5 mL/cm^2 of TBS, pH 8.0.
8. Add the secondary antibody in the form of Anti-Mouse IgG-Peroxidase (Cat. No. A9044) or equivalent. The concentration of secondary antibody must be optimized based on the substrate being used. For detection using Chemiluminescent Peroxidase Substrate-1, a final secondary antibody dilution of 1:30,000 should be employed. Specifically, it is suggested the antibody as supplied be diluted in at least 0.5 mL/cm^2 of TBS with 3% nonfat milk. Incubate the blot employing gentle agitation at room temperature for 30 minutes.
9. Wash the blot at least three times for a total of 15 minutes (5 minutes per wash) in TBS with 0.05% TWEEN® 20, pH 8.0 (Cat. No. T9039). Agitate gently, employing at least 0.5 mL/cm^2 of wash solution.
10. Develop the blot using Chemiluminescent Peroxidase Substrate-1 or an equivalent reagent for 5 minutes**. Do not agitate** the blot during this incubation step. Drain briefly and wrap in plastic film.
11. Expose BioMax® Light film to the blot for a range of times from several seconds up to 10 minutes. It is recommended that a quick exposure of 10–30 seconds be performed to determine the optimal exposure time needed. If the signal is too intense even at the short exposure times, allow the signal to decay over a 1–8 hour period (or longer if necessary), and then re-expose the film.

Indirect Immunofluorescent Cytochemical Staining

Monoclonal ANTI-FLAG® M2 may be utilized in immunocytochemical staining procedures when used in conjunction with a labeled secondary antibody (indirect).[6] A generic procedure for adherent cell staining is described, using immunofluorescence, employing an Anti-Mouse IgG-FITC conjugate as the label.

1. Grow and transfect cells on coverslips.
2. Fix the cells by incubation with phosphate buffered saline, pH 7.4 (Cat. No. P3813), containing 4% paraformaldehyde (Cat. No. P6148), and 4% sucrose (Cat. No. S1888) for 15 minutes at room temperature.
3. Wash the fixed cells with PBS for 5 minutes. Repeat once.
4. Permeabilize the cells by incubation with 0.25% TRITON™ X-100 (Cat. No. T9284) in PBS for 5 minutes.
5. Wash the cells with PBS for 5 minutes. Repeat once.
6. Block by incubation with 10% bovine serum albumin (Cat. No. A9647) in PBS (10% BSA/PBS) for 30 minutes at 37 °C.
7. Incubate with Monoclonal ANTI-FLAG® M2 diluted in the range of 1:500 to 1:2,000 in 3% BSA/PBS for 2 hours at 37 °C.
8. Wash with PBS for 5 minutes. Repeat twice.
9. Incubate with the secondary antibody, Anti-Mouse IgG- FITC (Cat. No. F9137) at a 1:1,000 dilution in 3% BSA/PBS for 45 minutes at 37 °C.
10. Wash with PBS for 5 minutes. Repeat twice.

11. Mount coverslips with cells side down on glass slides using a small drop of mounting medium such as polyvinyl alcohol for semi-permanent mounting. The inclusion of an anti-fading agent like DABCO® in the mounting medium (25–100 mg/mL, for example Cat. No. 10981) is strongly recommended. Seal coverslips to glass slides (with nail polish, for instance).
12. Examine by fluorescence microscopy. FITC has an absorption maximum at 492 nm with an emission maximum at 520 nm.

Immunoprecipitation (IP)

Monoclonal ANTI-FLAG® M2 may be used in IP procedures when used in conjunction with an insoluble carrier matrix, such as a Protein G resin. Alternatively, EZview™ Red Protein G Affinity Gel (Cat. No. E3403) or the Protein G Immunoprecipitation Kit (Cat. No. IP50) may be used.

EZview Red ANTI-FLAG® M2 Affinity Gel (Cat. No. F2426) or ANTI-FLAG® M2 Affinity Gel (Cat. No. A2220) may be utilized directly for IP. See reference 5 for general protocols.

Enzyme Immunoassay (EIA)

Monoclonal ANTI-FLAG® M2 may be used in EIA procedures. Typically, a fusion protein containing a FLAG® peptide sequence is directly adsorbed (or otherwise presented) within the wells of a multiwell polystyrene plate. The Monoclonal ANTI-FLAG® M2 antibody may be diluted up to 1:50,000 for subsequent incubation within the plate wells. Detection may be accomplished using Anti-Mouse IgG-Peroxidase (Cat. No. A9044) or equivalent, diluted 1:10,000, followed by an appropriate substrate for visualization.

We also offer the ANTI-FLAG® High Sensitivity, M2 coated 96-well plate (Cat. No. P2983) for EIA-based screening applications.

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(4)**: 730-735 (1994).
2. Bjerrum, O.J., and Heegaard. N.H.H., *CRC Handbook of Immunoblotting of Proteins*, Volume I, Technical Descriptions. CRC Press (Boca Raton, FL), pp. 229-236 (1988).
3. Dunbar, B.S. (ed.), *Protein Blotting: A Practical Approach.* IRL Press (New York, NY), pp. 67-70 (1994).
4. 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(5-6)**: 239-243 (1994).
5. Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY) (1988).
6. Ciaccia, A.V., and Price, E.M., IBI FLAG Epitope, **1**: 4-5 (1992).

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Troubleshooting Guide (Western Blot Immunostaining Procedure)

Problem	Possible Cause	Solution
Fusion protein is not detected.	Protein is not expressed.	Verify nucleic acid sequence and reading frame of the FLAG® fusion protein in vector construct. If sequence is present, attempt to optimize expression.
	Target protein poorly represented in sample.	Positive controls (10 ng/lane recommended) 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. Immuno-precipitation with ANTI-FLAG® M2 Affinity Gel (Cat. No. A2220) may be required for low concentrations of FLAG fusion proteins. Positive controls we have available: • 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 fresh lot of secondary antibody-HRP conjugate, along with freshly prepared reagents.
	Inadequate exposure time using chemiluminescent system.	If no signal is observed on the film, expose for longer times. It is recommended to try exposure times ranging from about 5 seconds to as long as 10 minutes.
	Inappropriate film	Switch to film designated for chemiluminescent detection such as BioMax® Light.
	No target protein present on membrane.	Verify transfer onto the membrane by visualizing proteins using Ponceau S solution (Cat. No. P7170). If possible, a positive control should always be run to ensure that the detection system components are functioning normally. Pre-stained protein markers (for example, Cat. No. C1992 or C4861) may also be used to verify complete transfer of proteins from gel to membrane.
	Antigen is covered by blocking reagent, due to overblocking.	Masking of a signal can occur if the blocking reagent (such as casein or gelatin containing blocking buffers) is used at an excessively high concentration. A dilution ranging from 1:1 to 1:3 may be performed to decrease the concentration of blocking reagent. If the problem persists, use TBS with 3% nonfat milk (Cat. No. T8793).
	Antibody concentration is not optimal.	Determine the optimal working dilution for the Monoclonal ANTI-FLAG® M2 antibody via titration. Consider using a higher concentration of antibody, if no signal or a weak signal is detected. Also, antibody used at an excessively high concentration can cause signal inhibition, especially in chemiluminescent detection systems.

Problem	Possible Cause	Solution
High non-specific background	Cellular extract concentration is too high.	2.5–10 µg of total lysate protein per lane is usually enough to obtain a good signal. Load less cellular extract or serially dilute the cellular extract to determine the optimal signal to noise ratio.
	Monoclonal ANTI-FLAG® M2 antibody concentration is too high.	Dilute the Monoclonal ANTI-FLAG® M2 antibody to a concentration ranging from 0.1–0.5 µg/mL. Use TBS with 3% nonfat milk as the diluent.
	Secondary antibody cross-reactivity.	For the secondary antibody, it is recommended that users initially employ dilutions of 1:30,000. Higher dilutions may be necessary, or a more specific secondary antibody should be used.
	Monoclonal 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 lacking insert DNA) will help distinguish the FLAG®-fusion proteins from other cross-reacting proteins.

Notice

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