

Product Information

Monoclonal ANTI-FLAG® M2–Alkaline Phosphatase Clone M2

antibody produced in mouse, purified immunoglobulin

Catalog No. **A9469**

Store at –20 °C

Product Description

Monoclonal ANTI-FLAG M2–Alkaline Phosphatase is a purified IgG 1 monoclonal antibody isolated from a murine cell culture¹, covalently conjugated to calf intestinal alkaline phosphatase (AP). This antibody conjugate binds to FLAG® fusion proteins and will recognize the FLAG epitope at any position in the fusion protein (N-terminal, Met-N-terminal, C-terminal or internal FLAG peptides). This conjugate is useful for detection of FLAG fusion proteins by common immunological procedures such as Western blots, dot blots, and ELISA.

Reagent

Supplied as a solution in 50 mM Tris, pH 8.0, 150 mM NaCl, 1 mM MgCl₂, and 15 mM sodium azide containing 50% glycerol. The conjugate protein concentration is ~1 mg/mL.

Storage

Store undiluted antibody at –20 °C. Once diluted, repeated freezing and thawing is not recommended.

Preparation Instructions

Dilute the antibody conjugate solution to the recommended working dilution in Tris Buffered Saline (TBS; 0.05 M Tris, pH 7.4, with 0.15 M NaCl).

Suggested working dilutions: An antibody titer of 1:1,000 may be used for dot blot and Western blot. Minimum ELISA titer is 1:20,000.

Adjust the antibody concentration to maximize detection sensitivity and to minimize background.

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

Procedure

Procedure for Western Blot

1. Carry out SDS-PAGE of FLAG fusion protein and transfer the protein to a PVDF membrane (e.g. Immobilon®-P).
2. Block the membrane using 5% Nonfat Dried Milk, Catalog Number M7409, in TBS with 0.05% TWEEN® 20, Catalog Number P9416, at room temperature for 1 hour.
3. Wash the membrane in TBS with 0.05% TWEEN 20 twice for 5 minutes each.
4. Incubate the membrane with Monoclonal ANTI-FLAG M2–Alkaline Phosphatase titered at 1:1000 in TBS with 0.05% TWEEN 20 at room temperature for 1 hour.
5. Wash the membrane in TBS with 0.05% TWEEN 20 six times for 5 minutes each.
6. Treat the membrane with CDP *Star*® Chemiluminescent Substrate, Catalog Number C0712, or other alkaline phosphatase substrate at pH 9.8-10.4 to detect the FLAG fusion protein.

Procedure for ELISA

Note: This procedure is based on direct adsorption of target protein onto an ELISA plate. In some cases, target proteins may not adsorb efficiently and a primary antibody directed against the target protein may first be adsorbed to provide for subsequent immobilization of target protein.

1. Prepare FLAG fusion protein sample at 1-10 µg/mL in 0.1 M NaHCO₃, pH 9.5. Use higher concentrations for crude preparations and lower concentrations for purified proteins.
2. Coat the plate with 100-200 µL of the sample solutions at 2-8 °C overnight.
3. Rinse the plate using TBS with 0.05% TWEEN 20 three times.

4. Block the plate with 100-200 μ L of 1% Nonfat Dried Milk, Catalog No. M7409, at room temperature for one hour.
5. Rinse the plate using TBS with 0.05% TWEEN 20 twice.
6. Incubate the plate with 100-200 μ L of Monoclonal ANTI-FLAG M2-Alkaline Phosphatase titered at a minimum of 1:20,000 at room temperature for one hour.
7. Rinse the plate using TBS with 0.05% TWEEN 20 five times.
8. Incubate the plate with 100-200 μ L of pNPP substrate, Catalog Number N2765 or equivalent, at room temperature for 30 minutes.
9. Read the plate at 405 nm.

References

1. Brizzard, B.L., *et al.*, BioTechniques, **16**, 730-734 (1994)

Western Blot Troubleshooting Guide

Problem	Possible Cause	Solution
No Signal	FLAG not expressed on fusion protein	Verify nucleic acid sequence of FLAG in vector construct.
	No target protein present on membrane	Verify transfer by visualizing proteins on the membrane using a Ponceau S solution, Catalog No. P7170. If possible, a positive control should always be run to insure components are functioning.
	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 ANTI-FLAG M2 Affinity Gel, Catalog No. A2220, may be required for low FLAG fusion protein concentrations. Positive controls available from Sigma: <ul style="list-style-type: none"> • Amino-terminal FLAG-BAP Fusion Protein, Catalog No. P7582 • Carboxy-terminal FLAG-BAP Fusion Protein, Catalog No. P7457 • Amino-terminal Met-FLAG-BAP Fusion Protein, Catalog No. P5975
	Overblocking such that antigen is covered by blocking reagent	Masking of a signal can occur if the blocking reagent, such as the casein or gelatin blocking buffers, Catalog Nos. 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, different blocking reagents should be tried.
	Inadequate exposure time using chemiluminescence system	First exposure should be 1 minute. If no signal is seen, expose for longer times. Sigma recommends trying 5 minutes, 10 minutes, etc. If excess signal is seen, try as short an exposure as practical (down to 1 second) without using a cassette.
	Antibody concentration not optimal	Determine optimal working dilution for Monoclonal ANTI-FLAG M2 ⁺ Alkaline Phosphatase 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.
	Substrate solution is inappropriate for alkaline phosphatase	Choose substrate recommended for use with alkaline phosphatase such as CDP- <i>Star</i> , Catalog No. C0712, for chemiluminescent detection, or SIGMAFAST TM BCIP [®] /NBT (5-Bromo-4-Chloro-3-Indolyl Phosphate/Nitro Blue Tetrazolium), Catalog No. B5655, or SIGMAFAST Fast Red TR/Naphthol AS-MX Tablets, Catalog Nos. F4648 or F4523, for blue-purple, or red colorimetric end products, respectively.
	Enzyme conjugate may have lost enzymatic activity if old or improperly stored	Determine if the enzyme conjugate is active.

Western Blot Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
High Background	Too much conjugated antibody	Perform a titer of the conjugated antibody until an acceptable signal to noise ratio is obtained.
	Inappropriate blocking reagent	Increase the concentration of the blocking reagent by preparing the reagent with one-half the recommended volume of water. In addition, some antibodies may cross-react with certain blocking reagents. To test for this possibility, prepare a "blank" membrane that does not contain the primary antibody.
	Inappropriate blocking protocol	Increase the blocking time and/or increase the blocking temperature to 37 °C.
	Inappropriate wash protocol	Increase the number of washes. Consider using more stringent washes. For example, include 0.05% TWEEN 20 or 0.1% TRITON® X-100 in the wash buffer.
	Overincubation in colorimetric substrate solution	Decrease the staining time. The membrane should be exposed to the colorimetric substrate until a positive signal is seen, but as the background begins to develop, the reaction should be stopped. For colorimetric substrate: Incubate for 5-10 minutes or whenever bands are visible. The time required may be increased or decreased, but should not be longer than 60 minutes. For alkaline phosphatase substrates, wash the membrane with 1% SDS in either TBS (Tris buffered saline) or PBS (phosphate buffered saline) to stop the reaction.
	Inappropriate film	Switch to film designated for chemiluminescent detection such as Biomax® Light, MS, and MR.
Extraneous spots	Aggregated protein or antibody conjugate	Centrifuge the conjugate solution at 10,000 x g for 10 minutes and use the supernatant.

General Western Blot References

1. Bjerrum, O.J. and Heegaard. N.H.H. *CRC Handbook of Immunoblotting of Proteins, Volume I, Technical Descriptions*, CRC Press, (1988) p. 229-236
2. Dunbar, B.S. (ed.) *Protein Blotting: A Practical Approach*, IRL Press, NY, p. 67-70 (1994)
3. Harlow, E. and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988, Product No. A 2926
4. Pampori, N.A. *et al.*, "Dilution of the chemiluminescence reagents reduces the background noise on Western Blots." *BioTechniques*, **18**, 589-590 (1995)

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AH,RS,PHC 08/10-1

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