Technical Bulletin

Monoclonal ANTI-FLAG® M2-Alkaline Phosphatase antibody produced in mouse

Clone M2, purified immunoglobulin, buffered aqueous glycerol solution

A9469

Product Description

Monoclonal ANTI-FLAG® M2-Alkaline Phosphatase is a covalent conjugate of a purified IgG1 monoclonal antibody, which has been isolated from a murine cell culture,[1] with calf intestinal alkaline phosphatase. 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.

Several theses[2,3] and dissertations[4-15] cite use of this product in their research protocols.

Reagent

This product is supplied as a solution in 50 mM Tris buffer (pH 8.0), 150 mM NaCl, 1 mM $MgCl_2$, and 15 mM sodium azide containing 50% glycerol.

Conjugate protein concentration: ~1 mg/mL (exact value on lot-specific Certificate of Analysis)

Storage/Stability

The undiluted antibody should be stored at –20 °C. Once diluted, repeated freezing and thawing is **not** recommended.

Precautions and Disclaimer

For Research use only. Not for drug, household, or other uses. Because of the sodium azide content, a Safety Data Sheet for this product has been sent to the attention of the safety officer of your institution. Consult the Safety Data Sheet for information regarding hazardous and safe handling practices.

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:

- Dot blot, Western blot: an antibody titer of 1:1,000 may be used.
- ELISA: the minimum titer is 1:20,000.

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

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

Procedure

Procedure for Western Blot

1. Carry out SDS-PAGE of the FLAG® fusion protein. Transfer the protein to a PVDF membrane (such as Immobilon®-P).
2. Block the membrane using 5% Nonfat Dried Milk (Cat. No. M7409) in TBS-T [Tris-Buffered Saline (TBS) with 0.05% TWEEN® 20 (Cat. No. P9416)], at room temperature for 1 hour.
3. Wash the membrane in TBS-T twice for 5 minutes each.
4. Incubate the membrane with Monoclonal ANTI-FLAG® M2-Alkaline Phosphatase titered at 1:1000 in TBS-T at room temperature for 1 hour.
5. Wash the membrane in TBS-T six times for 5 minutes each.
6. Treat the membrane with CDP-*Star*® Chemiluminescent Substrate (Cat. No. C0712), or another 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. A primary antibody directed against the target protein may first be adsorbed to provide for subsequent immobilization of target protein.

1. Prepare the FLAG® fusion protein sample at 1-10 µg/mL in 0.1 M $NaHCO_3$, 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-T three times.
4. Block the plate with 100-200 µL of 1% Nonfat Dried Milk (Cat. No. M7409), at room temperature for one hour.
5. Rinse the plate using TBS-T twice.
6. Incubate the plate with 100-200 µL 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-T five times.
8. Incubate the plate with 100-200 µL of pNPP substrate (Cat. No. N2765 or equivalent) at room temperature for 30 minutes.
9. Read the plate at 405 nm.

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General Western Blot References

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Western Blot Troubleshooting Guide[16-19]

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 substrates: • 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 0.1% sodium azide 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 × *g* for 10 minutes and use the supernatant.

Western Blot Troubleshooting Guide (continued)[16-19]

Problem	Possible Cause	Solution
No signal	FLAG® not expressed on fusion protein.	Verify the nucleic acid sequence of FLAG® in the vector construct.
	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 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 (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
	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 (Cat. 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, such as 5 minutes, 10 minutes, or other times. If excess signal is seen, try as short an exposure as practical (down to 1 second) without using a cassette.
	Antibody concentration is 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-*Star*® (Cat. No. C0712) for chemiluminescent detection SIGMA*FAST*™ BCIP®/NBT (5-Bromo-4-Chloro-3-Indolyl Phosphate/Nitro Blue Tetrazolium, Cat. No. B5655), for blue-purple colorimetric end products SIGMA*FAST* Fast Red TR/Naphthol AS-MX Tablets (Cat. Nos. F4648 or F4523), for red colorimetric end products
	Enzyme conjugate may have lost enzymatic activity if old or improperly stored.	Determine if the enzyme conjugate is active.

Notice

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