

## Product Information

### Monoclonal ANTI-FLAG® M2-Peroxidase (HRP) Clone M2

produced in mouse, purified immunoglobulin

Catalog Number **A8592**

Store at -20 °C

#### Product Description

Monoclonal ANTI-FLAG M2-Peroxidase is a purified IgG<sub>1</sub> monoclonal antibody isolated from a mouse cell culture,<sup>1</sup> covalently conjugated to horseradish peroxidase (HRP). 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 identification of FLAG fusion proteins by common immunological procedures.

Monoclonal ANTI-FLAG M2-Peroxidase may be used for the detection of FLAG fusion proteins. Applications for the conjugate include Western blots, dot blots, ELISA, and immunocytochemistry.

#### Reagent

Supplied as a solution in 10 mM sodium phosphate, pH 7.4, containing 150 mM NaCl and 50% glycerol plus stabilizer and preservative.

Protein concentration: ~1 mg/mL.

#### Storage

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

#### Preparation Instructions

Dilute ANTI-FLAG M2-Peroxidase solution to the recommended working dilution in Tris Buffered Saline (TBS): 0.05 M Tris, pH 7.4, with 0.15 M NaCl.

#### Product Profile

Suggested working dilutions: An antibody titer of 1:100 to 1:1000 may be used for immunocytochemistry and Western blot. Minimum antibody titer for ELISA is 1:20,000. Adjust the antibody concentration to maximize detection sensitivity and to minimize background. 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 with 5% nonfat 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 ANTI-FLAG M2-HRP titrated at 1:100 to 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 luminol, sodium salt, Cat. No. A4685, or other peroxidase substrate to detect the FLAG fusion protein.

##### Procedure for ELISA

This procedure is based on direct adsorption of the 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 the FLAG fusion protein sample at 1-10 µg/ml in 0.1 M NaHCO<sub>3</sub>, pH 9.5. Use higher concentrations for crude preparations and lower concentrations for purified proteins.
2. Coat plate overnight with 100-200 µL of the FLAG fusion protein sample at 2-8 °C.

3. Rinse plate with TBS with 0.05% TWEEN 20 three times.
4. Block plate with 100-200  $\mu$ L of 1% non-fat dried milk, Cat. No. M7409, at room temperature for one hour.
5. Incubate the plate with 100-200  $\mu$ L of ANTI-FLAG M2-HRP titered at a minimum of 1:20,000 at room temperature for one hour.  
**Note:** In order to obtain best results, it is recommended that each individual user determine working dilution by titration assay.
6. Rinse plate with TBS with 0.05% TWEEN 20 five times.
7. Incubate the plate with 100-200  $\mu$ L of SIGMAFAST™ OPD (o-phenylenediamine dihydrochloride) substrate, Cat. No. P9187 or equivalent, at room temperature for 30 minutes.
8. Stop reaction with 50  $\mu$ L of 1 N HCl.
9. Read plate at 450 nm.

#### References

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

#### Western Blot Troubleshooting Guide

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 horseradish peroxidase 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 x g for 10 minutes and use the supernatant.

**Western Blot Troubleshooting Guide (continued)**

<b>Problem</b>	<b>Possible Cause</b>	<b>Remedy</b>
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 Number P7170. If possible, a positive control should always be run to ensure 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 (Product Code A 1205) may be required for low FLAG fusion protein concentrations. Positive controls available from Sigma: 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. It is recommended to try 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 is not optimal.	Determine optimal working dilution for ANTI-FLAG peroxidase conjugate 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 horseradish peroxidase.	Choose substrate recommended for use with horseradish peroxidase such as luminol for chemiluminescent detection or SIGMAFAST 3,3'-Diaminobenzidine (DAB, Catalog No. D4418), 3-Amino-9-Ethylcarbazole (AEC, Catalog No. A6926), or 4-Chloro-1-Naphthol (4C1N, Catalog No. C6788, tablet, or C8302, solution) for brown, red, or blue colorimetric end products, respectively.
	Enzyme conjugate may have lost enzymatic activity if old or improperly stored.	Determine if the enzyme conjugate is active.

**General Western Blot References**

1. Bjerrum, O. J., and Heegaard, N. H. H., *CRC Handbook of Immunoblotting of Proteins, Volume I, Technical Descriptions*, pp. 229-236 (CRC Press, 1988).
2. Dunbar, B. S., (ed.) *Protein Blotting: A Practical Approach*, pp. 67-70 (IRL Press, 1994).
3. 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**, 239-43 (1994).
4. Harlow, E., and Lane, D., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory, 1988), Catalog No. A2926
5. 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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