



## Product Information

### ANTI-FLAG® M2 Monoclonal Antibody-FITC CONJUGATE

Product Code **F 4049**

Store at 0 to -20 °C

#### Product Description

ANTI-FLAG® M2 monoclonal antibody-FITC conjugate is a purified IgG<sub>1</sub> monoclonal antibody isolated from a murine cell culture,<sup>1</sup> covalently conjugated to fluorescein isothiocyanate (FITC). The antibody conjugate binds to FLAG fusion proteins, and will recognize the FLAG sequence at the N-terminus, Met-N-terminus or C-terminus of FLAG fusion proteins. This conjugate is useful for identification of FLAG fusion proteins by common immunological procedures.

ANTI-FLAG M2-FITC may be used for the detection of FLAG fusion protein. It can be used in fluorescent immunocytochemistry and immunohistochemistry. The conjugate can also be used in Western blot and flow cytometry applications.

#### Storage

Store undiluted antibody at 0 to -20 °C in working aliquots. Repeated freezing and thawing is **not** recommended.

#### Preparation Instructions

Dilute the ANTI-FLAG M2 monoclonal antibody-FITC solution to the recommended working dilution in Tris Buffered Saline (TBS).

Suggested working dilutions: An antibody concentration of 1–10 µg/ml may be used for immunocytochemistry, immunohistochemistry and Western blot. An antibody concentration of 5 µg/ml or less may be used for flow cytometry. In order to obtain best results, it is recommended that each individual user determine working dilution by titration assay.

Tris Buffered Saline (TBS):  
0.05 M Tris, 0.15 M NaCl, pH 7.4

#### Procedure

##### Procedure for Direct Immunofluorescent Staining of Mammalian Cells

1. Wash cells grown in a culture dish or on a slide with TBS twice.
2. Fix cells with a freshly prepared mixture of methanol:acetone (1:1) for 1 minute at room temperature.
3. Wash cells with TBS four times.
4. Incubate cells with ANTI-FLAG M2-FITC at 10 µg/ml in TBS at room temperature for 1 hour.
5. Wash cells with TBS twice.
6. Examine using a fluorescence microscope with appropriate configuration for fluorescein. FITC has an absorption maximum at approximately 492 nm with an emission maximum at 520 nm. Note: The fluorescence properties of FITC and FITC conjugates are pH dependent.<sup>2,3,4</sup>

#### Product Profile

ANTI-FLAG M2 monoclonal antibody-FITC conjugate is supplied as a solution in 10 mM sodium phosphate, 150 mM NaCl, 1% bovine serum albumin, 0.1% sodium azide, pH 7.4.

F/P molar ratio: 3.0-6.0

Specificity: ANTI-FLAG M2-FITC has been found to detect FLAG-BAP fusion protein in transfected COS cells.

#### References

1. Brizzard, B.L., *et al.*, *BioTechniques*, **16**, 730–734 (1994)
2. Chen, R.F., *Archives Biochem. Biophys.*, **133**, 263 (1969)
3. Klugerman, M.R., *J. Immunol.*, **95**, 1165 (1966)
4. Emmart, E. W., *Archives Biochem. Biophys.*, **73**, 1 (1958)

### Immunofluorescence Troubleshooting Guide

Problem	Possible Cause	Possible Remedy
No staining	FLAG not expressed on fusion protein	Verify expression of FLAG tag on fusion protein by immunoblotting or other method.
	Antibody concentration not optimal	Determine optimal working dilution for FITC conjugate by titration. Consider using more antibody if no signal or weak signal is detected.
	Inappropriate filter for fluorescent microscopy	Use filter recommended for visualizing FITC. The excitation maximum for FITC is 495 nm and the emission maximum is 525 nm.
	Incubation time with antibody not adequate	Increase incubation time.
	FITC label quenched or faded	Avoid exposure to light. Consider using anti-fade reagent in mounting medium such as n-propyl gallate (Product Code P 3130), <sup>1</sup> p-phenylenediamine (Product Code P 1519) <sup>2</sup> , or DABCO (Product Code D 2522). <sup>3,4,5</sup>
	Cultured cells: Intracellular expression of FLAG fusion protein in cultured cells not accessible by antibody	Cells need to be permeabilized. Consider fixing cells in methanol at $-20\text{ }^{\circ}\text{C}$ for 10 minutes followed by 1 minute in acetone at $-20\text{ }^{\circ}\text{C}$ . Alternatively, try fixing cells in 3% paraformaldehyde containing 0.5% Triton X-100 for 10 minutes at room temperature.
	Formalin-fixed paraffin sections: Antibody does not have access to FLAG tag due to cross-linking caused by aldehyde fixation.	Consider unmasking with proteases or stain frozen sections. Commonly used proteases are 0.4% pepsin (Product Code P 7012) in 0.01N HCl, 0.1% protease (Product Code P 4789) in PBS, or 0.1% trypsin (Product Code T 8003) in water containing 10 mM $\text{CaCl}_2$ at $37\text{ }^{\circ}\text{C}$ for 2-30 minutes. Optimal conditions must be determined empirically.
High Background	Aggregates	Centrifuge antibody conjugate briefly in microcentrifuge at highest speed to remove antibody aggregates.
	Antibody binding to Fc receptors on cell surface	Incubate sample with 10% irrelevant serum (e.g. goat serum, Product Code G 9023) to occupy Fc receptors prior to applying antibody conjugate
	Wash steps not adequate	Increase number or length of washes.
	Antibody concentration not optimal	Determine optimal working dilution for FITC conjugate by titration. Consider using less antibody if background is too high.
	Aldehydes left in the tissue by fixatives (formaldehyde and particularly glutaraldehyde) provide covalent binding sites for applied immunoreagents via their amino groups. <sup>6</sup>	If not sufficiently blocked by serum alone, one or more of the following can be tried: <sup>6</sup> (a) 0.02-1% sodium or potassium borohydride in 0.1 M phosphate buffer, pH 7.4 for 30 minutes at room temperature. (b) 50-100 mM ammonium chloride added to the blocking serum. (c) 100 mM ethanolamine added to the blocking serum. (d) 0.2 M glycine in PBS for 5 minutes.

### Immunofluorescence Troubleshooting Guide (Continued)

Tissue Autofluorescence	Glutaraldehyde may induce autofluorescence in tissue and decrease antibody penetration into tissues. <sup>7</sup>	Avoid aldehyde fixatives. Consider preparing acetone fixed frozen sections.
	Usually tissue autofluorescence is greenish-yellow when viewed using a fluorescein filter set-up and may even show through using a rhodamine filter set-up.	Consider masking the autofluorescence with a fluorescent background dye. Pontamine Sky Blue (Chicago Sky Blue 6B, Product Code C 8679) (0.05% (w/v) in PBS with 1% (v/v) dimethyl sulfoxide) fluoresces red using a fluorescein filter set-up. Tissue sections should be stained with the masking reagent 30 minutes prior to applying the primary antibody. <sup>6</sup>

#### Immunofluorescence References

1. Giloh H. and Sedat, J.W. Fluorescence microscopy: Reduced photobleaching of rhodamine and fluorescein protein conjugates by n-propyl gallate. *Science (Abstract)* **217**, 1252-1255 (1982)
2. Storz, H. and Jelke, E., Photomicrography of weakly fluorescent objects-employment of p-phenylenediamine as a blocker of fading. *Acta Histochem.* **75**, 133-139 (1984)
3. Harlow, E. and Lane, D. Antibodies: A Laboratory Manual, Cold Spring Harbor Press, NY, 1988 (Product Code A2926)
4. Johnson, G.D. *et al.*, Fading of immunofluorescence during microscopy: A study of the phenomenon and its remedy. *J. Immunol. Methods.* **55**, 231-242 (1982)
5. Longin A., *et al.* Comparison of anti-fading agents used in fluorescence microscopy: image analysis and laser confocal microscopy study. *J. Histochem. Cytochem.* **41**(12), 1833-40 (1993)
6. Beesley, J.E. (ed.) Immunocytochemistry: A Practical Approach, IRL Press, 1993, p. 215, p. 216 (Product Code Z35005-2)
7. Bullock, G.R. and Petrusz, P. (eds.) Techniques in Immunocytochemistry, Volumes 1, 2, 3 and 4, Academic Press, 1982, 1983, 1985, 1989. See Vol 1, p. 186.

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