Technical Bulletin

ANTI-FLAG® M2-FITC Antibody, Mouse Monoclonal

Clone M2, purified immunoglobulin, buffered aqueous glycerol solution

F4049

Product Description

Monoclonal ANTI-FLAG® M2-FITC is a purified IgG_1 monoclonal antibody isolated from a mouse cell culture,[1] covalently conjugated to fluorescein isothiocyanate (FITC). The antibody conjugate binds to FLAG® fusion proteins. It 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.

Monoclonal ANTI-FLAG® M2-FITC may be used for the detection of FLAG® fusion proteins. It can be used in fluorescent immunocytochemistry and immunohistochemistry. The conjugate can also be used in Western blot and flow cytometry applications. Several theses[5] and dissertations[6-21] cite use of this product in their research.

Reagent

This product is supplied as a solution in 10 mM sodium phosphate (pH 7.4), 150 mM NaCl, 1% bovine serum albumin, and 0.1% sodium azide.

F/P molar ratio: 3.0-6.0

Specificity: The antibody has been found to detect FLAG-BAP™ fusion protein in transfected COS cells.

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

Dilute the antibody to the recommended working dilution in Tris-Buffered Saline (TBS): 0.05 M Tris, 0.15 M NaCl, pH 7.4.

Storage/Stability

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

Product Profile

Suggested working dilutions

Immunocytochemistry, Immunohistochemistry and Western blotting: an antibody concentration of 1-10 µg/mL may be used.

Flow cytometry: an antibody concentration of ≤ 5 µg/mL may be used.

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

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 Monoclonal 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.[2-4]

References

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General Immunofluorescence References

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Immunofluorescence Troubleshooting Guide[22-28]

Problem	Possible Cause	Solution
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 (such as goat serum, Cat. No. G9023) to occupy Fc receptors prior to applying antibody conjugate.
	Wash steps not adequate	Increase the number, or the length, of washes.
	Antibody concentration not optimal	Determine optimal working dilution for the 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.[22]	If not sufficiently blocked by serum alone, one or more of the following can be tried:[22] (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.
Tissue Autofluore-scence	Glutaraldehyde may induce autofluorescence in tissue and decrease antibody penetration into tissues.[23]	• Avoid aldehyde fixatives. • Consider preparing acetone-fixed frozen sections.
	Usually tissue autofluorescence is greenish-yellow when viewed using a fluorescein filter set-up. It may even show through using a rhodamine filter set-up.	Consider masking the autofluorescence with a fluorescent background dye. Chicago Sky Blue 6B (Pontamine Sky Blue), Cat. No. C8679, 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.[22]

Immunofluorescence Troubleshooting Guide (continued)[22-28]

Problem	Possible Cause	Solution
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: • Excitation maximum for FITC: 492 nm • Emission maximum for FITC: 520 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: • Propyl gallate[24] (Cat. No. P3130) • *p*-phenylenediamine dihydrochloride[25] (Cat. No. 78460) • 1,4-Diazabicyclo[2.2.2]octane (DABCO®)[26-28] (Cat. No. D27802)
	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 °C for 10 minutes, followed by 1 minute in acetone at –20 °C. • Alternatively, try fixing cells in 3% paraformaldehyde containing 0.5% Triton® X-100 detergent 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 (Cat. No. P7012) in 0.01 N HCl • 0.1% protease (Cat. No. P5380) in PBS • 0.1% trypsin (Cat. No. T8003) in water containing 10 mM $CaCl_2$ at 37 °C for 2-30 minutes. Optimal conditions must be determined empirically.

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