Technical Bulletin

Monoclonal ANTI-FLAG® M2-Cy3™ antibody produced in mouse

Clone M2, purified immunoglobulin, buffered aqueous solution

A9594

Product Description

Monoclonal ANTI-FLAG® M2-Cy3™ is a covalent conjugate of a purified IgG1 monoclonal ANTI-FLAG® M2 antibody, which has been isolated from a mouse cell culture,[1] to the cyanine dye Cy3™. 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-Cy3™ may be used for the detection of FLAG® fusion protein in applications such as fluorescent immunocytochemistry. Cy3™ conjugates are well suited for dual-labeling experiments.[2,3] Several theses[4-10] and dissertations[11-16] cite use of this product in their research protocols.

Reagent

This product is supplied as a solution in 10 mM sodium phosphate, pH 7.4, with 150 mM NaCl, 1% bovine serum albumin, and 15 mM sodium azide.

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

F/P molar ratio: 3.0-6.0

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

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.

Storage/Stability

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

Preparation Instructions

Dilute the antibody 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:

Immunocytochemistry: an antibody concentration of 1–10 µg/mL may be used.

Note: To obtain best results, it is recommended that each individual user determine the optimal 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 the antibody 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 Cy3™. Cy3™ has an absorption maximum at approximately 550 nm with an emission maximum at 570 nm.

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Immunofluorescence Troubleshooting Guide

Problem	Possible Cause	Solution
No staining	FLAG® is not expressed on fusion protein.	Verify expression of FLAG® tag on fusion protein by immunoblotting or other method.
	Antibody concentration is not optimal.	• Determine optimal working dilution for Cy3™ conjugate by titration. • Consider using more antibody if no signal or weak signal is detected.
	Inappropriate filter for fluorescent microscopy	Use the filter recommended for visualizing Cy3™: • Excitation maximum for Cy3™: 550 nm • Emission maximum for Cy3™: 570 nm
	Incubation time with antibody is not adequate.	Increase incubation time.
	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 for 10 minutes at room temperature.
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 the antibody conjugate.
	Wash steps are not adequate.	Increase number or length of washes.
	Antibody concentration is not optimal.	• Determine optimal working dilution for the Cy3™ conjugate by titration. • Consider using less antibody if background is too high.

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