

Product Information

Monoclonal ANTI-FLAG® M2–Cy3™, Clone M2
produced in mouse, purified immunoglobulin

Catalog Number **A9594**
Store at –20 °C

Product Description

Monoclonal ANTI-FLAG M2–Cy3 is a purified IgG1 monoclonal antibody isolated from a mouse cell culture,¹ covalently conjugated to cyanine dye Cy3. 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.

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}

Reagent

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.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

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: an antibody concentration of 1–10 µg/ml may be used for immunocytochemistry.

Note: In order to obtain best results, it is recommended that each individual user determine 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.

Product Profile

Protein concentration: ~1 mg per ml.

F/P molar ratio: 3.0-6.0

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

Immunofluorescence Troubleshooting Guide

Problem	Possible Cause	Possible 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 filter recommended for visualizing Cy3. The excitation maximum for Cy3 is 550 nm and the emission maximum is 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 $-20\text{ }^{\circ}\text{C}$ 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.
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, Catalog No. G9023, to occupy Fc receptors prior to applying antibody conjugate.
	Wash steps are not adequate.	Increase number or length of washes.
	Antibody concentration is not optimal.	Determine optimal working dilution for Cy3 conjugate by titration. Consider using less antibody if background is too high.

References

1. Brizzard, B.L., et al., *BioTechniques*, **16**, 730–735 (1994).
2. Sargent, P.B., *Neuroimage*, **4**, 288-295 (1994).
3. Southwick, P.L., et al., *Cytometry*, **11**, 418-430 (1990).

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