

Product Information

Monoclonal ANTI-FLAG® BioM2-Biotin, Clone M2
produced in mouse, purified immunoglobulin

Catalog Number **F9291**

Synonym: ANTI-FLAG Biotinylated M2

Product Description

Note: Monoclonal ANTI-FLAG BioM2-Biotin is now formulated in 50% glycerol for added stability.

Monoclonal ANTI-FLAG BioM2 is a purified mouse IgG₁ monoclonal antibody that is covalently attached to biotin by hydrazide linkage. ANTI-FLAG BioM2 will recognize the FLAG® sequence at the N-terminus, Met-N-terminus or C-terminus of FLAG fusion proteins. It can be detected by avidin or streptavidin conjugates. Monoclonal ANTI-FLAG BioM2-Biotin is useful for Western blotting, microscopy applications, and formation of avidin-biotin complexes (ABC). Monoclonal ANTI-FLAG BioM2-Biotin, in combination with an avidin or streptavidin conjugate, is the preferred ANTI-FLAG antibody for detection of FLAG fusion proteins expressed in mammalian cells.

Binding of the monoclonal antibody is not calcium dependent.

The antigenic binding site is
N-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-C

Reagent

Supplied in 50% glycerol, 10 mM sodium phosphate, pH 7.25, 150 mM NaCl containing 0.02% 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.

Preparation Instructions

Dilute the monoclonal antibody solution to 1-10 µg/mL in Tris Buffered Saline (TBS): 0.05 M Tris, pH 7.4, with 0.15 M NaCl.

Adjust the antibody concentration to maximize detection sensitivity and to minimize background.

Procedure

Procedure for Western Blot

1. Transfer the FLAG fusion protein of interest to a nitrocellulose membrane.
2. Block the membrane using a solution of 5% non-fat dry milk in TBS at 37 °C for 30 minutes.
3. Wash the membrane twice for 5 minutes each in TBS at room temperature.
4. Incubate the membrane with Monoclonal ANTI-FLAG BioM2-Biotin at 1-10 µg/ml in TBS for 30 minutes at room temperature.
5. Wash the membrane ten times for a total time of 10 minutes in TBS at room temperature.
6. Incubate the membrane with avidin- or streptavidin-peroxidase conjugate (Cat. No. A3151 or S5512) in TBS. For S 5512, a concentration of 1 µg/ml is appropriate. Incubate at room temperature for 30 minutes. Adjust the conjugate concentration to maximize detection sensitivity and to minimize background.
7. Wash the membrane ten times for a total time of 10 minutes in TBS at room temperature.
8. Treat the membrane with Chemiluminescent Peroxidase Substrate 1 (Cat. No. CPS160) or other peroxidase substrate.

Procedure for immunostaining of cultured mammalian cells

1. Wash cells grown in a 9 cm² culture dish with 5 ml of TBS containing 1 mM calcium chloride (TBS/Ca).
2. Fix with 2 ml of a freshly prepared 1:1 mixture of acetone:methanol.
3. Wash four times with 2.5 ml of TBS/Ca.
4. Incubate with 10 µg/ml of Monoclonal ANTI-FLAG BioM2-Biotin in TBS/Ca for 1 hour.
5. Wash five times with 2 ml of TBS/Ca.
6. Add avidin- or streptavidin-peroxidase at a concentration of 1 µg/ml in TBS/Ca. Incubate 30 minutes at room temperature
7. Wash five times with 2 ml of TBS/Ca.
8. Stain with a peroxidase substrate, e.g. o-dianisidine dihydrochloride (Cat. No. D9154). Monitor staining by microscopy. Stop reaction by washing with distilled water.

Product Profile

Protein concentration: ~1 mg/ml

Dot blot: the monoclonal antibody at the recommended concentration detects at least 2 ng of FLAG-BAP™ fusion protein using chemiluminescent detection

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