



Product Information

ANTI-FLAG® BioM2 Monoclonal Antibody

Product No. **F 9291**

Store at 0 to -20 °C

Synonym: ANTI-FLAG® Biotinylated M2

Product Description

Note: The ANTI-FLAG® BioM2 monoclonal antibody is now formulated in 50% glycerol for added stability.

ANTI-FLAG® BioM2 monoclonal antibody is a purified murine IgG₁ monoclonal antibody that is covalently attached to biotin by hydrazide linkage. ANTI-FLAG BioM2 antibody 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. ANTI-FLAG BioM2 monoclonal antibody is useful for Western blotting, microscopy applications, and formation of avidin-biotin complexes (ABC). ANTI-FLAG BioM2 antibody 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 ANTI-FLAG BioM2 monoclonal antibody is not calcium dependent. The antigenic binding site is N-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-C

The ANTI-FLAG BioM2 Monoclonal Antibody is supplied in 50% glycerol, 10 mM sodium phosphate, pH 7.25, 150 mM NaCl containing 0.02% sodium azide.

Storage

Store undiluted antibody at 0 to -20 °C.

Preparation Instructions

Dilute the ANTI-FLAG BioM2 monoclonal antibody solution to 1 - 10 µg/ml in Tris Buffered Saline (TBS). Adjust the antibody concentration to maximize detection sensitivity and to minimize background.

Tris Buffered Saline (TBS):

0.05 M Tris, pH 7.4, with 0.15 M NaCl

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 ANTI-FLAG BioM2 antibody 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 (Product Code A 3151 or S 5512) 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 (Product Code CPS-1-60) 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 ANTI-FLAG BioM2 monoclonal antibody 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 (Product Code D 9154). Monitor staining by microscopy. Stop reaction by washing with distilled water.

Product Profile

Specificity: ANTI-FLAG BioM2 monoclonal antibody detects a single band of protein on a Western blot from a mammalian or *E. coli* crude cell lysate containing a FLAG•BAP fusion protein.

Sensitivity: ANTI-FLAG BioM2 monoclonal antibody at the recommended concentration detects at least 2 ng of FLAG•BAP fusion protein on a dot blot using streptavidin-peroxidase.

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