Product Information

Monoclonal ANTI-FLAG® BioM2 antibody produced in mouse

Clone M2, purified immunoglobulin, buffered aqueous glycerol solution

F9291

Product Description

The FLAG® peptide sequence, known also as DYKDDDDK, is one of the most widely used protein tags in recombinant protein expression and purification.[1] Protein tagging with the FLAG® tag may be done at the *N*-terminus, the *N*-terminus preceded by a methionine residue, the *C*-terminus, or at internal positions of the target protein. The small size of the FLAG® tag or sequence and its high hydrophilicity tend to decrease the possibility of interference with the protein expression, proteolytic maturation, antigenicity, and function. The *N*-terminal FLAG® peptide sequence contains a unique enterokinase cleavage site which allows it to be completely removed from the purified fusion proteins.

Monoclonal ANTI-FLAG® BioM2 is a purified mouse IgG_1 monoclonal antibody that is covalently attached to biotin by a hydrazide linkage. ANTI-FLAG® BioM2 will recognize the FLAG® sequence at the *N*-terminus, Met-*N*-terminus or *C*-terminus of FLAG® fusion proteins. The antibody 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 a 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.

Several theses[2-5] and dissertations[6-20] cite use of product F9291 in their protocols.

Product Profile

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

Antigenic binding site:
N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C

Protein concentration: ~1 mg/mL (exact value on Certificate of Analysis for particular lot)

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

Reagent

This product is supplied in buffered aqueous solution that contains 50% glycerol, 10 mM sodium phosphate (pH 7.25), and 150 mM NaCl, and also with 0.02% sodium azide present.

Storage/Stability

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. Incubate the membrane either with avidin-peroxidase conjugate (Cat. No. A3151), or with streptavidin-peroxidase conjugate (Cat. No. S5512) in TBS. For S5512, 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.
6. Wash the membrane ten times for a total time of 10 minutes in TBS at room temperature.
7. Treat the membrane with a chemiluminescent peroxidase substrate.

Procedure for immunostaining of cultured mammalian cells

1. Wash cells grown in a 9 cm^2 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-peroxidase 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 such as *o*–dianisidine dihydrochloride (Cat. No. D9154). Monitor the staining by microscopy. Stop the reaction by washing with distilled water.

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