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Product Information

Monoclonal Anti-FLAG® BioM5−Biotin, Clone M5
produced in mouse, purified immunoglobulin

Catalog Number **F2922**
Store at 0 to –20 °C

Product Description

Anti-FLAG BioM5 monoclonal antibody is a purified murine IgG_1 monoclonal antibody that is covalently attached to biotin by hydrazide linkage. Anti-FLAG BioM5 antibody will recognize the FLAG sequence at the Met-N-terminus of FLAG fusion proteins. It can be detected by avidin or streptadivin conjugates Anti-FLAG BioM5 monoclonal antibody is useful for Western blotting, microscopy applications and formation of avidin-biotin complexes (ABC) in mammalian and *Drosophila* cells. Anti-FLAG BioM5 antibody in combination with an avidin or streptavidin conjugate is the preferred anti-FLAG antibody for detection of FLAG fusion proteins expressed in mouse cells.

Binding of anti-FLAG BioM5 monoclonal antibody is not calcium dependent.

Anti-FLAG BioM5 is **not** recommended for detection of FLAG fusion proteins in *E. coli*.

Anti-FLAG BioM5 monoclonal antibody is supplied in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% sodium azide.

Storage

Store undiluted antibody at 0 to –20 °C in working aliquots. Repeated freezing and thawing is not recommended.

Preparation Instructions

Dilute the Anti-FLAG BioM5 monoclonal antibody solution to 2 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, 0.15 M NaCl, pH 7.4

Procedure

Procedure for Western Blot

1. Transfer the N-terminal Met-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 BioM5 antibody at 2 μg/ml in TBS at room temperature for 30 minutes.
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 at the manufacturer's recommended concentration in TBS. 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 of 10 minutes in TBS at room temperature.
8. Treat the membrane with luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Product No. A4685) or other peroxidase substrate.

Procedure for immunostaining of cultured mammalian cells using avidin-biotin complexes (ABC)

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 mix of acetone:methanol.

3. Wash four times with 2.5 ml of TBS/Ca.

4. Incubate with 2 μg/ml of Anti-FLAG BioM5 monoclonal antibody in TBS/Ca for 1 hour.

5. Wash five times with 2 ml of TBS/Ca.

6. Add preformed ABC complexes prepared according to the manufacturer's instructions, e.g. Vectastain® ABC kit with horseradish peroxidase conjugate. Incubate 30 minutes at room temperature

7. Wash five times with 2 ml of TBS/Ca.

8. Stain with peroxidase substrate e.g. o-dianisidine (Catalog No. D9154). Monitor staining by microscopy. Stop reaction by washing with distilled water.

Product Profile

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

Specificity: Anti-FLAG BioM5 monoclonal antibody detects a single band of protein on a Western blot from a mammalian crude cell lysate containing a FLAG•BAP fusion protein.

Sensitivity: Anti-FLAG BioM5 monoclonal antibody at the recommended concentration detects 1 ng of N-terminal Met-FLAG•BAP fusion protein on a dot blot using streptavidin-peroxidase.

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