

## Product Information

### Monoclonal ANTI-FLAG® M5, Clone M5 Produced in mouse, purified immunoglobulin

Catalog Number **F4042**

Store at -20 °C

#### Product Description

Monoclonal ANTI-FLAG M5 is a purified IgG<sub>1</sub> monoclonal antibody isolated from a mouse cell culture that binds to N-terminal Met-FLAG fusion proteins. It is useful for detection of N-terminal Met-FLAG fusion proteins expressed in mammalian and *Drosophila* cells.

M5 binding is not calcium dependent.

Monoclonal ANTI-FLAG M5 is **not** recommended for detection of FLAG® fusion proteins expressed in *E. coli*.

#### Reagent

Supplied in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% (w/v) 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.

#### Preparation Instructions

Dilute the antibody to 10 µg/mL in Tris buffered saline (TBS): 0.05 M Tris, 0.15 M NaCl, pH 7.4

Adjust the antibody concentration to maximize detection sensitivity.

#### Procedure

Procedure for Western Blot

1. Transfer the N-terminal Met-FLAG-BAP (bacterial alkaline phosphatase) or 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 1 hour.

3. Wash the membrane twice for 1-2 minutes each in TBS at room temperature.
4. Incubate the membrane with Monoclonal ANTI-FLAG M5 as the primary antibody at 10 µg/ml in TBS at room temperature for 30 minutes.
5. Wash the membrane three times for 1-2 minutes each in TBS at room temperature.
6. Incubate the membrane with Anti-Mouse IgG-Peroxidase as the secondary antibody at the manufacturer's recommended concentration in TBS. Incubate at room temperature for 30 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
7. Wash the membrane three times for 15 minutes each in TBS at room temperature.
8. Treat the membrane with luminol sodium salt (5-Amino-2,3-dihydro-1,4-phthalazinedione sodium salt), Catalog. No. A4685, or other peroxidase substrate.

#### Product Profile

Protein concentration: 2.0-5.0 mg/ml

Antigenic binding site:

N-Met-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-C

Specificity: Anti-FLAG M5 monoclonal antibody detects an N-terminal Met-FLAG fusion protein in a crude extract of mammalian cells with minimal cross reactivity.

Sensitivity: Anti-FLAG M5 monoclonal antibody detects less than 1 ng of Met-FLAG-BAP™ fusion protein on a dot blot using chemiluminescent detection.

FLAG and ANTI-FLAG are registered trademarks and FLAG-BAP is a trademark of Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co..

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