



## Product Information

### ANTI-FLAG<sup>®</sup> M1 Monoclonal Antibody

Product Code **F 3040**

Store at 0 to -20 °C

#### Product Description

ANTI-FLAG<sup>®</sup> M1 monoclonal antibody is a purified IgG<sub>2B</sub> monoclonal antibody isolated from Murine ascites fluid that binds to proteins with a FLAG marker at the free N-terminus. It is useful for identification of proteins with a FLAG marker at the free N-terminus by common immunological applications in yeast, animal and *E. coli* cells. ANTI-FLAG M1 monoclonal antibody is also useful for calcium mediated affinity purification of proteins with a FLAG marker at the free N-terminus when bound to a solid support.

M1 antibody/antigen binding is calcium dependent.

ANTI-FLAG M1 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 M1 monoclonal antibody solution to 10 µg/ml in Tris Buffered Saline (TBS). Adjust the antibody concentration to maximize detection sensitivity.

Tris Buffered Saline (TBS) containing 1 mM CaCl<sub>2</sub>, 0.05 M Tris, 0.15 M NaCl, pH 7.4

#### Procedure

Procedure for Western Blot

1. Transfer the FLAG•BAP (bacterial alkaline phosphatase) control protein or 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 ANTI-FLAG M1 antibody as the primary antibody at 10 µg/ml in TBS containing 1 mM CaCl<sub>2</sub> at room temperature for 30 minutes.
5. Wash the membrane three times for 1-2 minutes each in TBS containing 1 mM CaCl<sub>2</sub> at room temperature.
6. Incubate the membrane with anti-mouse IgG peroxidase conjugate as the secondary antibody at the manufacturer's recommended concentration in TBS containing 1 mM CaCl<sub>2</sub>. 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 containing 1 mM CaCl<sub>2</sub> at room temperature.
8. Treat the membrane with luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Product Code A 4685) or other peroxidase substrate.

**Product Profile**

Antigenic binding site:

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

Specificity: ANTI-FLAG M1 monoclonal antibody

detects a single band of protein on a Western blot from an *E. coli* crude cell lysate or two bands from a *BJ3505* yeast crude extract expressing a FLAG•BAP fusion protein with minimal cross reactivity. The two bands from the yeast extract represent two different glycosylated forms of FLAG•BAP.

Sensitivity: ANTI-FLAG M1 monoclonal antibody detects 1 ng of FLAG•BAP fusion protein on a dot blot using chemiluminescent detection.

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