

## Product Information

**Monoclonal ANTI-FLAG® M1, Clone M1**  
produced in mouse, purified immunoglobulin

Catalog Number **F3040**

### Product Description

Monoclonal ANTI-FLAG M1 antibody is a purified immunoglobulin, IgG<sub>2b</sub>, monoclonal antibody isolated from mouse 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. The 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.

### Reagent

Supplied in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, containing 0.02% 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.

### Storage

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

### Preparation Instructions

Dilute the antibody to 10 µg/mL in 0.05 M Tris buffered saline (TBS), pH 7.4, containing 1 mM CaCl<sub>2</sub>. Adjust the antibody concentration to maximize detection sensitivity.

### 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 Monoclonal ANTI-FLAG M1 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 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 sodium salt (5-amino-2,3-dihydro-1,4-phthalazinedione sodium salt), Catalog Number A4685, or other peroxidase substrate.

### Product Profile

Protein concentration (E<sub>280</sub>): 2.0 – 5.0 mg/mL

Antigenic binding site:

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

Specificity: The 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: The antibody detects 1 ng of FLAG-BAP fusion protein on a dot blot using chemiluminescent detection.

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