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Product Information

Carboxy-terminal FLAG–BAP™ Fusion Protein

Catalog Number **P7457**
Store at –20 °C

Product Description
Carboxy-terminal FLAG-BAP Fusion Protein is a 466 amino acid C-terminal FLAG® fusion protein of *E. coli* bacterial alkaline phosphatase (BAP) with a calculated molecular weight of 49.1 kDa.

The Carboxy-terminal FLAG-BAP fusion protein migrates as a 45-55 kDa band by SDS-PAGE depending on electrophoresis conditions.

Carboxy-terminal FLAG-BAP Fusion Protein has been found to be useful for assurance of the functional integrity of ANTI-FLAG® M2 monoclonal antibody in immunological procedures such as Western blotting, ELISA, immunoprecipitation, fluorescence microscopy, light microscopy and FACS. It is also useful as a functional control in immunoaffinity chromatography with the ANTI-FLAG M2 Affinity Gel, Catalog Number A2220.

Reagent
Supplied in 10 mM Tris, 120 mM NaCl, 0.05 mM $ZnCl_2$ in 50% glycerol, pH 8.0.

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.

Reagents Required but Not Provided

- Tris buffered saline (TBS), 0.05 M Tris, 0.15 M NaCl, pH 7.4
- Non-fat dry milk
- Monoclonal ANTI-FLAG M2, Catalog No. F3165
- Anti-Mouse IgG-Peroxidase
- Luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione) Catalog No. A4685, or other peroxidase substrate

Preparation Instructions
Dilute the ANTI-FLAG M2 antibody solution to 10 µg/ml in TBS. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.

Procedure
Procedure for Western Blot

1. Transfer the Carboxy-terminal FLAG-BAP Fusion Protein 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 M2 antibody as the primary antibody 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 or other peroxidase substrate.

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