3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Amino-terminal FLAG-BAP™ Fusion Protein

Catalog Number **P7582**
Storage Temperature –20 °C

Product Description

Amino-terminal FLAG-BAP™ Fusion Protein is a 467 amino acid N-terminal FLAG® fusion protein of *E. coli* bacterial alkaline phosphatase (BAP) with a calculated molecular mass of 49.3 kDa.

The N-terminal FLAG-BAP Fusion Protein migrates as a 45–55 kDa band by SDS-PAGE depending on electrophoresis conditions.

Amino-terminal FLAG-BAP Fusion Protein has been found to be useful for assurance of the functional integrity of anti-FLAG M1 and M2 monoclonal antibodies in immunological procedures such as Western blotting, ELISA, immunoprecipitation, fluorescence microscopy, light microscopy, and FACS.

The product is supplied in 10 mM Tris, 120 mM NaCl, 0.05 mM $ZnCl_2$ in 50% glycerol, pH 8.0.

Reagents Required but Not Provided

- Tris buffered saline (TBS), 0.05 M Tris, 0.015 M NaCl, pH 7.4
- Non-fat dry milk
- Anti-FLAG M1 monoclonal antibody (Catalog No. F3040) or anti-FLAG M2 monoclonal antibody (Catalog No. F3165)
- Anti-mouse IgG peroxidase conjugate
- Luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione, Catalog No. A4685) or other peroxidase substrate

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 anti-FLAG M1 or anti-FLAG M2 antibody solution to 10 µg/ml in TBS. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.

Storage/Stability

The product ships on dry ice and storage at –20 °C is recommended.

Procedure

Procedure for Western Blot

1. Transfer the N-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 M1 or 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 conjugate 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.

FLAG-BAP is a trademark of Sigma-Aldrich® Biotechnology LP and Sigma-Aldrich Co.
FLAG is a registered trademark of Sigma-Aldrich® Biotechnology LP and Sigma-Aldrich Co.

KR,MAM 03/11-1

Sigma brand products are sold through Sigma-Aldrich, Inc.
Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.