

Product Information

Amino-terminal Met-FLAG-BAP™ Fusion Protein

Catalog Number **P5975**

Store at -20 °C

Product Description

Amino-terminal Met-FLAG-BAP control protein is a 468 amino acid N-terminal Met-FLAG fusion protein of *E. coli* bacterial alkaline phosphatase (BAP) with a calculated molecular weight of 49.4 kDa.

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

Amino-terminal Met-FLAG-BAP control protein has been found to be useful for assurance of the functional integrity of ANTI-FLAG® M2 and M5 monoclonal antibodies 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.

The N-terminal Met-FLAG-BAP control protein is supplied in 10 mM Tris, 120 mM NaCl, 0.05 mM ZnCl₂ in 50% glycerol, pH 8.0.

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, or Monoclonal ANTI-FLAG M5, Catalog No. F4042.
- Anti-mouse IgG peroxidase conjugate
- Luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione), Catalog No. A4685, or other peroxidase substrate

Preparation Instructions

Dilute the ANTI-FLAG M2 or ANTI-FLAG M5 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 Amino-terminal Met-FLAG-BAP control 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 or ANTI-FLAG M5 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 and ANTI-FLAG are registered trademarks of Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co.
pFLAG, p3XFLAG, pFLAG-1, pFLAG-2, pFLAGSHIFT, pFLAG-CTS, pFLAG-ATS, pFLAG-MAC, pFLAG-CMV, YEpFLAG, and FLAG-BAP are trademarks of Sigma-Aldrich Biotechnology LP and Sigma-Aldrich Co.

AH,RS,PHC 08/10-1