Product Information

Amino-terminal FLAG-BAP™ Fusion Protein

P7582

Product Description

The FLAG® peptide sequence, known also as DYKDDDDK, is one of the most widely used protein tags in recombinant protein expression and purification.[1] Protein tagging with the FLAG® tag may be done at the N-terminus, the N-terminus preceded by a methionine residue, the C-terminus, or at internal positions of the target protein. The small size of the FLAG® tag or sequence and its high hydrophilicity tend to decrease the possibility of interference with the protein expression, proteolytic maturation, antigenicity, and function.

The 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 the electrophoresis conditions.

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

Several theses[2] and dissertations[3-6] cite of use of this product in their protocols.

Reagent

The product is supplied as a buffered aqueous glycerol solution in 10 mM Tris, 120 mM NaCl, and 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 (Cat. No. F3040), or Anti-FLAG® M2 monoclonal antibody (Cat. No. F3165)
- Anti-mouse IgG peroxidase conjugate
- Luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione, Cat. No. A4685) or another peroxidase substrate

Procedure

Note: 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.

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 another peroxidase substrate.

References

1. Terpe, K., *Appl. Microbiol. Biotechnol.*, **60(5)**, 523-533 (2003).
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3. Scietti, Luigi A.D., "Exploring Host-Pathogen Interactions Through Protein Microarray: Large-scale protein microarray analysis revealed novel human receptors for the staphylococcal immune evasion protein FLIPr and for the neisserial adhesin NadA". Università di Bologna, Ph.D. dissertation, p. 23 (2015).
4. Al-Dieri, Ali, "Inflammation in Obesity and Molecular Engineering of a Transgenic Mouse Model of Diabetes". University of Toledo, Ph.D. dissertation, p. 54 (December 2016).
5. Ravi, Ayswarya, "Evaluation of Mixed-Mode Chromatography Resins for Isolation of Recombinant Therapeutic Proteins". Texas A&M University, Ph.D. dissertation, p. 46 (August 2019).
6. Campaña, Maria Belén, "Structural and Functional Insights into Ghrelin Acylation by Ghrelin O-Acyltransferase". Syracuse University, Ph.D. dissertation, p. 73 (August 2020).

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