Product Information

Carboxy-terminal FLAG-BAP™ Fusion Protein

P7457

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.

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 the 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 (Cat. No. A2220).

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

Reagent

This reagent 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, 0.15 M NaCl, pH 7.4
- Non-fat dry milk
- Monoclonal ANTI-FLAG® M2, Cat. No. F3165
- Anti-Mouse IgG-Peroxidase
- Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), Cat. No. A4685, or another peroxidase substrate

Procedure

Note: 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 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 another peroxidase substrate.

References

1. Terpe, K., *Appl. Microbiol. Biotechnol.*, **60(5)**, 523-533 (2003).
2. Widmaier, Daniel Matthew, "Engineering the *Salmonella* Type III Secretion System". University of California San Francisco, Ph.D. dissertation, p. 26 (2010).
3. Graeff, Maria Carolina Durán, "Analysis and improvement of the in vitro transfection efficiency of plasmid-DNA encoding for equine IL-12 used for melanoma therapy in horses". University of Veterinary Medicine Hannover, Ph.D. dissertation, p. 55 (September 2012).

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