

Product Information

Anti-polyHistidine-Alkaline Phosphatase antibody, Mouse monoclonal

Clone HIS-1, purified from hybridoma cell culture

A5588

Product Description

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes that can provide 'affinity handles' (tags) designed to enable the selective identification of the protein of interest. These sequences of tails or tags are genetically engineered away from the protein active site, by insertion at the N- or C-terminus.

One widely used protein tag is the polyHistidine tag ('His-tag'). Several publications have studied the potential effects of the His-tag on properties of His-tagged proteins.¹⁻²

Anti-polyHistidine (mouse IgG2a isotype) is derived from the HIS-1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with a polyHistidine-tagged fusion protein. The isotype is determined by a double diffusion assay with Mouse Monoclonal Antibody Isotyping Reagents (Cat. No. ISO2). Monoclonal Anti-polyHistidine-Alkaline Phosphatase is prepared by conjugation of calf intestinal alkaline phosphatase to Monoclonal Anti-polyHistidine that has been purified from ascites fluid of the HIS-1 hybridoma.

Anti-polyHistidine-Alkaline Phosphatase recognizes native as well as denatured / reduced forms of synthetic polyHistidine or polyHistidine-tagged fusion proteins. The product is reactive with fusion protein expressed by prokaryotic pET, pRSET and pTrc expression vectors. The antibody is reactive in immunoblotting, dot blotting, or ELISA.

Monoclonal antibodies that specifically recognize polyHistidine may be useful in various immunotechniques to identify the expression of a polyHistidine fusion protein in bacteria, bacterial lysates or cells and tissues transfected with a polyHistidine-tagged fusion protein expressing vectors. Several publications cite use of this product in their studies.³⁻⁴

Reagent

This product is supplied as a solution in 0.05 M Tris buffer (pH 8.0), containing 1% BSA, 1mM MgCl₂, 50% glycerol and 15 mM sodium azide as a preservative.

Storage/Stability

For continuous use and extended storage, store at 2-8 °C. Do not freeze. Solutions at working dilution should be discarded if not used within 12 hours.

Product Profile

Immunoblotting

A minimum working dilution of 1:2,000 is determined using bacteria lysates which express a recombinant histidine-tagged fusion protein.

Note: To obtain the best results using various techniques and preparations, we recommend determining optimal working dilutions by titration.

Procedure

Procedure for Immunoblotting

Note: All incubation steps should be performed at room temperature.

1. Separate the proteins present in sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5 to 20 µg of total lysate protein per lane. The amount of lysate to be loaded per lane depends on the level of protein expression and may vary between experiments.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane for at least 60 minutes using a solution of 5% non-fat dry milk in Dulbecco's Phosphate Buffered Saline (such as Cat. No. D8537).
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 (such as Cat. No. P3563).
5. Incubate the membrane for two hours with Monoclonal Anti-polyHistidine-Alkaline Phosphatase as the primary antibody, using an optimized concentration in PBS containing 1% bovine serum albumin (such as Cat. No. A9647).
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20.
7. Treat the membrane with an alkaline phosphatase substrate.

References

1. Thigeles, M.C. *et al.*, *Biochemistry*, **50(25)**, 5799-5805 (2011).
2. Booth, W.T. *et al.*, *ACS Omega*, **3(1)**, 760-768 (2018).
3. Dong, S. *et al.*, *Science*, **343(6170)**, 552-555 (2014).
4. Yang, M. *et al.*, *Nat. Chem. Biol.*, **14(12)**, 1109-1117 (2018).

Note

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