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 $MgCl_2$, 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

This product is covered by patent DE 19507166 and foreign equivalents exclusively licensed to QIAGEN GmbH, Qiagen Strasse 1, D-40724 Hilden, Germany.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

A5588dat Rev 08/21 DS,KAA,PHC,RC,GCY