

Product Information

Monoclonal Anti-polyHistidine-Alkaline Phosphatase, Clone HIS-1

produced in mouse, purified immunoglobulin

Catalog Number **A5588**

Product Description

Monoclonal 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 using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. Monoclonal Anti-polyHistidine-Alkaline Phosphatase is prepared by conjugation of calf intestinal alkaline phosphatase to Monoclonal Anti-polyHistidine purified from ascites fluid of the HIS-1 hybridoma.

Monoclonal 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.

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. It has been reported that the addition of a consecutive histidine amino acid residue tail creates a stable fusion product that does not appear to interfere with the bioactivity of the protein or with the biodistribution of the histidine tagged product.

Monoclonal antibodies reacting specifically with 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.

Reagent

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. The specific antibody concentration is at least 1 mg/ml.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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 expressing a recombinant histidine-tagged fusion protein and BCIP/NBT substrate.

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

Procedure

Immunoblotting

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, Catalog Number D8537.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween[®] 20, Catalog Number 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, Catalog Number 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, e.g., BCIP/NBT, Catalog Number B1911.

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