

Product Information

Anti-polyHistidine–Peroxidase antibody, Mouse monoclonal

clone HIS-1, purified from hybridoma cell culture

A7058

Product Description

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 double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Cat. No. ISO2). Monoclonal anti-polyHistidine, Peroxidase conjugate is prepared by conjugation of horseradish peroxidase to mouse monoclonal anti-polyHistidine purified from ascites fluid of the HIS-1 hybridoma.

Monoclonal anti-polyHistidine, Peroxidase conjugate, recognizes native as well as reduced, denatured forms of synthetic polyhistidine or polyhistidine-tagged fusion proteins. The product is reactive with fusion proteins expressed by prokaryotic pET, pRSET, and pTrc expression vectors. The antibody is reactive in immunoblotting, dot blot, and 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. The 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 antibody reacting specifically with poly-histidine 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

Reconstitute the contents of the vial with 0.5 mL of water. This results in a solution of antibody conjugate in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 0.05% MIT.

Antibody concentration: 5–11 mg/mL

Molar ratio Ab/Enzyme: 0.7–1.4

Preparation Instructions

Reconstitute the contents of the vial with 0.5 mL of water. This results in a solution of antibody conjugate in 0.01 M sodium phosphate buffered saline containing 1% BSA and 0.05% MIT.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the lyophilized product at 2–8 °C.

For extended storage after reconstitution with water, keep at –20 °C in working aliquots. Avoid repeated freeze-thaw cycles. Do not store in frost-free freezers. For continuous use after reconstitution, keep at 2–8 °C for up to 1 month. 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 by using bacteria lysates expressing a recombinant histidine-tagged fusion protein.

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 2.5–20 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 using a solution of 5% non-fat dry milk in phosphate buffered saline (PBS, Cat. No. D8537) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20 (Cat. No. P3563).
5. Incubate the membrane with Anti-polyHistidine, Peroxidase conjugate using an optimized concentration in PBS containing 0.05% TWEEN® 20 and 1% bovine serum albumin (BSA, Cat. No. A9647) for two hours.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20.
7. Treat the membrane with a peroxidase substrate.

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