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Product Information

Monoclonal Anti-polyHistidine, Clone HIS-1
produced in mouse, ascites fluid

Catalog Number **H1029**

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 BALB/c mouse immunized with a recombinant His-tagged fusion protein. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Monoclonal anti-polyHistidine 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 preferentially recognizes N-terminal tagged fusion proteins. The antibody is reactive in immunoblotting, dot blot, immunofluorescent staining of cultured cells 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. 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.

A 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
Supplied as ascites fluid with 15 mM sodium azide.

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, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile
Immunoblotting: a minimum working dilution of 1:3,000 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 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 using a solution of 5% non-fat dry milk in Dulbecco's Phosphate Buffered Saline, Catalog Number D8537, for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20, Catalog Number P3563.

5. Incubate the membrane with Monoclonal Anti-polyHistidine as the primary antibody using an optimized concentration in PBS containing 1% bovine serum albumin, Catalog Number A9647, for 120 minutes.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
7. Incubate the membrane with Anti-Mouse IgG-Peroxidase, e.g., Catalog Numbers A9917, A3682, or A2304, or with Anti-Mouse IgG-Alkaline Phosphatase, e.g., Catalog Numbers A1293, A2179, or A1682, as the secondary antibody at the recommended concentration in PBS containing 0.05% TWEEN 20. Incubate for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
9. Treat the membrane with either a peroxidase or an alkaline-phosphatase substrate as appropriate.

Indirect Immunofluorescent Staining of Cultured Cells

All incubation steps should be performed at room temperature (except step 3).

1. Grow transfected cultured cells expressing histidine-tagged protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in Dulbecco's PBS, Catalog Number D8537.
3. Fix the cells with −20 °C methanol (10 minutes) and then with −20 °C acetone (1 minute).
4. Wash coverslips twice in PBS (5 minutes each wash).
   Note: Blocking with PBS containing 1% BSA for 10 minutes at room temperature followed by draining prior to step 5 may minimize non-specific adsorption of the antibody.
5. Incubate coverslips cell-side-up with Monoclonal Anti-polyHistidine in PBS containing 1% BSA, Catalog Number A9647. Incubate for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate coverslips cell-side-up with Anti-Mouse IgG-FITC, e.g., Catalog Numbers F4018 or F8771, as the secondary antibody, at the recommended dilution, in PBS containing 1% BSA, for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles.
10. Examine using a fluorescence microscope with appropriate filters.

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