Product Information

Anti-HIS Magnetic Beads

Magnetic agarose, suspension

SAE0222

Storage Temperature: 2-8 °C

Synonyms: Monoclonal 6 His epitope tag, Monoclonal 6xHis-tag, Monoclonal HHHHHH epitope tag, Monoclonal Hexa His tag, His-tag, His6 tag, Histidine tagged, Poly-His-tag

Product Description

In situations where protein-specific antibodies are unavailable, epitope tags enable researchers to study protein topology, characterize and identify new proteins, protein complexes, and associated proteins, even if the proteins of interest are in low abundance or are poorly immunogenic.[1-4]

Monoclonal anti-HIS antibody, derived from the monoclonal HIS-1 produced by the fusion of mouse myeloma cells and splenocytes from mouse immunized with a polyhistidine-tagged fusion protein. The isotype is determined using a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents. Monoclonal anti-polyHistidine recognizes native as well as denatured, reduced forms of synthetic polyhistidine or polyhistidine-tagged fusion proteins. The antibody preferentially recognizes N-terminal tagged fusion proteins. Monoclonal anti-HIS antibody reacts specifically with HIS-tagged fusion proteins and may be used for the immunoprecipitation or immunoaffinity purification.

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 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 tagged product.[5-7] His-tag has almost no effect on the characteristics of the target protein itself and will not change the solubility and biological functions of the target protein.

The use of Polyhistidine antibody is effective to identify the expression of a polyhistidine tagged protein in bacteria, bacterial lysates, or cells and tissues transfected with a polyhistidine-tagged fusion protein expressing vectors.[8]

Anti-HIS magnetic bead allows to purify HIS-tagged proteins expressed in bacterial and mammalian cell lysates in a faster and more efficient way.

Anti-HIS Magnetic beads are prepared with an affinity purified anti-HIS antibody, produced in mouse (SAB4200620). The antibody is conjugated to activate Magnetic beads at a 4:1 protein to beads ratio.

Anti-HIS magnetic beads are useful in the purification of a HIS fusion protein in bacteria or in transfected mammalian cells with HIS fusion protein expressing vectors.

Reagent

The Anti-HIS Magnetic Beads product is provided as suspension containing 50% Beads, in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

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.

Product Profile

Binding capacity: 600-5000 µg of HIS-tagged fusion protein per 1 mL of settled resin

Elution capacity: 300-5000 µg/mL settled resin

Storage/Stability

For continuous use and extended storage, store at 2 °C to 8 °C. Do not freeze.

General Notes

- To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing, or using a rotating platform.
- The following General Procedure is written for use of 20 µL of the anti-HIS magnetic agarose beads, and an estimated bead capacity of 50 µg of HIS tagged protein.
- Our suggestion is to add enough cell lysate where 50 µg of the HIS-tagged protein is expected to be present.
- Each sample will differ, depending on the individual researcher's system, and determination or estimation of the degree of HIS tagged protein in the lysate sample.
- A method like SDS-PAGE analysis can be performed on an aliquot of the lysate, with estimated quantitation of lysate protein bands against known amounts of defined protein standards in a separate gel lane.

General Procedures

To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.

1. Prewashing: Add 20 µL of Anti HIS-Magnetic beads into a 1.5 mL microcentrifuge tube.
2. Place the tube into a magnetic stand to collect the beads against the side of the tube.
3. Remove and discard the supernatant. Wash with 200 µL 0.01 M PBS, repeat washing 3-5 times.
4. Apply the sample which contains the estimated amount of 50 µg HIS tagged protein. Gently pipette mix. Incubate for 60 min at 37 °C using a rotating platform.
5. Save the flowthrough for calculating the binding capacity.
6. Wash with 0.01 M PBS until the absorbance at 280 nm is minimal.
7. Elution of HIS tagged proteins may be done by incubation with 80 µL sample buffer X1 for 5 min at 95 °C.

General Notes

- To obtain the best results in different techniques and preparations, we recommend on determining optimal working concentration by titration test.
- Binding capacity and elution capacity may vary, depending on the characteristics of the HIS-tagged fusion proteins. For optimal results, it is recommended to try different elution buffers.

References

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6. Cassey, J.L., J.Immunol. Meth., 179, 105-116 (1995).
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