Product Information

Anti-HA Magnetic Beads

Magnetic agarose, suspension

SAE0197

Product Description

Human influenza hemagglutinin (HA) is a surface glycoprotein that is required for infectivity of the human virus.[1] Many recombinant proteins have been engineered to express a short sequence derived from the HA molecule, known as the HA-tag, that corresponds to amino acids 98-106:

N-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-C

This HA-tag facilitates the detection, isolation and purification of proteins of interest with this specific tag.[2-4]

The anti-HA antibody used for this magnetic agarose bead conjugate is a high-affinity mouse IgG1 monoclonal antibody (Clone HA-7) that recognizes the HA-epitope tag (YPYDVPDYA) which is derived from the human influenza hemagglutinin (HA) protein. The antibody is conjugated to cyanogen bromide-activated magnetic agarose beads at a ~ 4 mg/mL protein-to-beads ratio.

Anti HA-Magnetic Beads provide high affinity with high specificity binding to HA-tagged proteins. This product may be used in various immunological techniques,[5] including immunoprecipitation (IP) and immunoaffinity purification.

Reagent

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

Storage/Stability

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

Product Profile

Binding capacity: 30 nmoles of HA-tagged fusion protein per 1 mL of settled resin

Elution capacity: 10 nmol/mL settled resin

Precautions and Disclaimer

This product is 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.

Procedure

General Procedure for Purification of HA-tagged proteins

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-HA magnetic agarose beads, and an estimated bead capacity of 50 µg of HA-tagged protein.
- Our suggestion is to add a sufficient amount of cell lysate where 50 µg of the HA-tagged protein is expected to be present.
- Each sample will differ, depending on the individual researcher's system, and also determination or estimation of the degree of HA-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 Procedure

1. Pre-washing: add 20 µL of Anti HA-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 PBS. Repeat the PBS wash 3-5 times.
4. Apply the sample which contains the estimated amount of 50 µg HA-tagged protein. Incubate for 60 minutes at 37 °C using a rotating platform.
5. Wash with PBS until the absorbance at 280 nm in minimal. Save the flowthrough for calculating the binding capacity.
6. Elution of HA-tagged proteins may be done with one of two incubation methods:
   (a) Incubation with solution containing 100 µg HA peptide (Cat. No. I2149) for 30 minutes at 37 °C using a rotating platform.

   Note: HA peptide has detectable absorbance at 280 nm, and thus interferes in other protein determination assays that are based on peptide bonds. Therefore, it is recommended to determine the eluted amount by Coomassie staining of SDS-PAGE relative to a known standard.

   (b) Incubation with 0.1 M Glycine-HCl (pH 2.5) for 10 minutes at room temperature. If required for sample stability, immediately neutralize the eluted samples.

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 HA-tagged fusion proteins. For optimal results, it is recommended to try different elution buffers.

References

1. Wilson, I.A. *et al*., *Cell*, **37(3)**, 767-778 (1984)
2. Kolodziej, P.A., and Young, R.A., *Methods Enzymol*., **194**, 508-519 (1991).
3. Moon, J.-M. *et al*., *Biotechnol. Lett*., **34(10)**, 1841-1846 (2012).
4. Zhao, X. *et al*., *J. Anal. Methods Chem*., 2013:581093 (2013).
5. Lu, Z.-H. *et al*., *Kaohsiung J. Med. Sci*., **38(12)**, 1190-1202 (2022).

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