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Product Information

Monoclonal Anti-HA-Agarose, clone HA-7
produced in mouse, purified immunoglobulin

Catalog Number **A2095**
Storage Temperature 2–8 °C

Product Description

Monoclonal Anti-HA-Agarose is the immunoglobulin fraction of Monoclonal Anti-HA (mouse IgG1 isotype) covalently linked to agarose. The monoclonal antibody is purified from ascites fluid of the hybridoma HA-7 using Protein A affinity chromatography, and then immobilized on agarose at 2.0–2.4 mg antibody per ml bed volume.

Recombinant DNA technology enables the insertion of specific sequences to a target gene. These sequences can provide "affinity handles" (tags), which enable the selective identification and purification of the protein of interest.[1-6] The addition of a tag to a given gene, 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.

Human influenza hemagglutinin (HA) is a surface glycoprotein required for infectivity of the human virus.[7] Many recombinant proteins have been engineered to express a short sequence derived from the HA molecule corresponding to a.a. 98–106, known as HA-tag. This tag facilitates the detection, isolation and purification of these proteins.[4-6]

Monoclonal Anti-HA Agarose Conjugate recognizes native as well as denatured-reduced forms of HA (hemagglutinin)-tagged proteins and is reactive with N- or C-terminal HA-tagged fusion proteins expressed in *E.coli* or in mammalian cells. The product may be used for immunoprecipitation assays and immunoaffinity purification of HA-tagged fusion proteins from bacterial lysates or in transfected cells.

Reagent

Supplied as a 1:1 suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Reagents Recommended but Not Provided

- HA peptide, Catalog No. I2149
- Sodium thiocyanate, Catalog No. S7757
- Glycine HCl, Catalog No. G2879
- Protease Inhibitor Cocktails (for general, bacterial, mammalian, fungal & yeast, plant, and tissue culture) Catalog Nos. P2714, P8465, P8340, P8215, P9599, and P1860

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.**

Product Profile

Binding capacity: 30–50 nmoles of HA-tagged fusion protein per 1 ml of settled resin.
Elution capacity: 20-50 nmol/mL elution capacity (HA-tagged fusion protein)

Note: Binding capacity and elution capacity may vary, depending on the characteristics of the HA-fusion proteins. For best results it is recommended to try different elution buffers.

Procedures

Column Purification of HA-tagged fusion proteins

Pre-equilibrate the column and all buffers and perform all steps at room temperature. To prevent clogging the column, highly viscous samples containing chromosomal DNA or RNA should be sonicated or treated with nuclease to reduce the viscosity, and cellular debris and particulate matter must be removed by centrifugation or filtration.

In cases where the stability of the protein is temperature sensitive, the following steps may be performed at 2–8 °C.

A. Column Set Up

1. Place the empty chromatography column on a firm support.
2. Attach a drainage tube to the column to control the flow rate. Limit the length of tubing to 25 cm.
3. Remove the top and bottom tabs and rinse the column with PBS, pH 7.4. Allow the buffer to drain from the column and leave residual PBS in the column to aid in packing the Anti-HA Agarose.

B. Packing the Column

1. Thoroughly suspend the vial of Anti-HA Agarose to make a uniform suspension of the resin.
2. Immediately transfer the suspension to the column.
3. Allow the agarose bed to drain and rinse the vial with PBS.
4. Add PBS to the column and allow the column to drain again. Do not let the resin bed dry.

C. Washing the Column

Wash the resin with three sequential 5 ml aliquots of glycine-HCl, pH 2.5 (or 3 M sodium thiocyanate, Catalog No. S7757) followed by three sequential 5 ml aliquots of PBS. Avoid disturbing the agarose bed while loading. Let each aliquot drain completely before adding the next. Do not leave the column in glycine-HCl for more than 20 minutes.

D. Binding the HA Fusion Protein to the Column

1. Load the sample (neutralized to pH 7–8) onto the column under gravity flow, or use a peristaltic pump at a flow rate of 0.5 ml/min.
   Note: Depending upon the protein and flow rate, not all of the protein may bind. Multiple passes over the column or closing the loaded column and incubating it on a rotator for about one hour may improve the binding efficiency.
2. Collect the "flow through" of unbound protein.
3. Wash the column with PBS until the $A_{280} \leq 0.01$.

E. Elution of the HA Fusion Protein

Select one of the following elution procedures:

E1. Elution by Glycine-HCl, pH 2.5

Elute the bound HA-tagged fusion protein from the column with 10 x 1 ml aliquots of 0.1 M glycine-HCl, pH 2.5 into vials containing 30–50 µl of 1 M Tris buffer, pH 8.0 for neutralization.

Notes:

a. Occasionally, low pH may cause the eluted protein to aggregate. In such cases choose an alternative buffer for elution, for example 3 M sodium thiocyanate.
b. The column may lose activity after prolonged exposure to low pH.

E2. Elution by HA Peptide

This is a milder elution method. Elute the bound HA-tagged fusion protein by adding 5 x 1 column volume aliquots of a solution containing 100 µg/ml HA peptide (Catalog No. I2149) in PBS.
Note: HA peptide has a detectable absorbency at 280 nm and also 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.

F. Recycling the Column

It is recommended that the column be regenerated immediately after use by washing with three column volumes of glycine-HCI, pH 2.5. The column should be immediately re-equilibrated in PBS until the effluent is at neutral pH. The number of cycles observed will be dependent on variables such as sample condition.
Note: Do not leave the column in glycine-HCl for longer than 20 minutes.

G. Storing the Column

Wash the column with three column volumes of PBS and store the column at 2–8 °C in PBS containing 15 mM sodium azide.

Immunoprecipitation

This procedure is recommended for work with small volumes of resin (20–50 µl). The work can be performed in 1.5 ml microcentrifuge tubes or in spin columns.

1. Add 40–100 µl of 1:1 suspension of the Anti-HA Agarose.
2. Pellet the resin by a short spin (12,000 x *g*, 30 sec.). Discard the liquid.
3. Wash the resin with PBS or RIPA buffer, 5 x 1 ml each. Aspirate traces of final wash.
4. Add clarified bacterial lysate or cell extract to the settled resin. Bring the volume to at least 200 µl with PBS or RIPA if needed.
5. Incubate for 1 hour to overnight on an orbital shaker at 4 °C. Shaking must be vigorous enough to suspend the resin.
6. Wash the resin with PBS or RIPA, 4 x 1 ml each. After the final wash aspirate the supernatant and leave ~10 µl above the beads.
7. Add 20–50 µl 2x SDS sample buffer. Denature the proteins by heating at 95–100 °C for 3 minutes. Note: Alternatively, the immunoprecipitated HA-tagged proteins can be specifically eluted with HA peptide (Catalog No. I2149). In this case, after the last wash (step 6), incubate the agarose resin with HA peptide, 100 µg/ml in PBS or RIPA buffer, for 5 minutes. Spin and collect the supernatant for further analysis.
8. Vortex, then centrifuge for 5 seconds. Transfer the supernatant to a fresh tube. Load the supernatant into a gel lane and analyze by SDS-PAGE.
9. Detection of the HA-tagged fusion protein is determined by immunoblotting, using Monoclonal Anti-HA (Product Code H9658) or Rabbit Anti-HA (Catalog No. H6908).

Note: The HA peptide has detectable absorbance at 280 nm and also 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.

Note: The Anti-HA Agarose is resistant to RIPA buffer (1% sodium deoxycholate, 0.1% SDS, 1% Triton™ X-100, 0.01M Tris-HCl, pH 8, 0.14 M NaCl).

Related Products

- Anti-HA Immunoprecipitation Kit, Catalog No. IP0010
- EZview™ Red Anti-HA Affinity Gel, Catalog No. E6779
- RIPA buffer, Catalog No. R0278
- Monoclonal Anti-HA–Peroxidase, Catalog No. H6533
- Monoclonal Anti-HA–Alkaline Phosphatase, Catalog No. A5477
- Monoclonal Anti-HA–FITC, Catalog No. H7411
- Monoclonal Anti-HA–TRITC , Catalog No. H9037
- Monoclonal Anti-HA–Biotin, Catalog No. B9183
- Protease inhibitor cocktails (for general, bacterial, mammalian, fungal & yeast, plant, and tissue culture) (Catalog Nos. P2714, P8465, P8340, P8215, P9599, and P1860)
- Phosphatase inhibitor cocktails (Catalog Nos. P2850 and P5726)
- BCA protein assay kits: Standard, Catalog No. BCA1 and QuantiPro™, Catalog No. QPBCA
- EZview™ Red Protein A Affinity Gel, Catalog No. P6486

References

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3. Uhlen, M., and Moks, T., Gene fusions for purpose of expression: an introduction. *Methods Enzymol.*, **185**, 129-143 (1990).
4. Kolodziej, P.A., and Young, R.A., Epitope tagging and protein surveillance. *Methods Enzymol.*, **194**, 508 (1991).
5. Pines, J. and Hunter, T., Human cyclins A and B1 are differentially located in the cell and undergo cell cycle-dependent nuclear transport. *J. Cell Biol.*, **115**, 1 (1991).
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7. Wilson, I.A., et al., The structure of an antigenic determinant in a protein. *Cell*, **37**, 767-778 (1984).

Troubleshooting Guide

Problem	Possible Cause	Solution
No signal is observed	HA-tagged protein is not present in the sample.	• Make sure that the protein of interest contains the HA-tag by immunoblot or dot blot analysis. • Prepare fresh lysates. Avoid using frozen lysates. • Use appropriate protease inhibitors in the lysate or increase their concentrations to prevent degradation of the HA-tagged protein.
	Washes are too stringent.	• Reduce the number of washes • Avoid adding high concentrations of NaCl to the mixture. • Use solutions that contain less or no detergent.
	Incubation times are inadequate.	• Increase the incubation times with the affinity resin (from several hours to overnight).
	Interfering substance is present in sample.	• Lysates containing high concentrations of dithiothreitol (DTT), 2-mercaptoethanol, or other reducing agents may destroy antibody function, and must be avoided. • Excessive detergent concentrations may also interfere with the protein binding interactions.
	Detection system is inadequate.	If Western blotting detection is used: • Check primary and secondary antibodies with proper controls to confirm binding and reactivity in detection system. • Verify that the transfer was adequate. • Use fresh detection substrate or try a different detection system.
Background is too high.	Proteins bind non-specifically to the anti-HA monoclonal antibody, the resin beads, or the microcentrifuge tubes.	• Pre-clear lysate with Mouse IgG-Agarose (A0919) to remove non-specific binding proteins. • After suspending beads for the final wash, transfer entire sample to a clean microcentrifuge tube before centrifugation.
	High protein concentration in the extract	• Calibrate the system with different amounts of agarose using a fixed amount of protein, and/or different amounts of protein using a fixed amount of agarose beads.
	Washes are insufficient.	• Increase the number of washes • Prolong duration of the washes, incubating each wash for at least 15 minutes. • Increase the salt and/or detergent concentrations in washing solutions. • Centrifuge at lower speed to avoid non-specific trapping of denatured proteins from the lysate during the initial centrifugation of the affinity resin complexes.