Data Sheet

Monoclonal Anti-HA Agarose, Clone HA-7

Produced in mouse, purified immunoglobulin

A2095

Product Description

Recombinant DNA technology enables the insertion of specific sequences to a target gene. These sequences can provide "affinity handles" (tags), which enable 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, called the HA tag, that corresponds to amino acids 98-106. The HA-tag facilitates the detection, isolation and purification of such proteins.[4-6]

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.

Monoclonal Anti-HA Agarose Conjugate recognizes native as well as denatured-reduced forms of HA (hemagglutinin)-tagged proteins. This product is reactive with N- or C-terminal HA-tagged fusion proteins expressed in *E. coli* or in mammalian cells. It may be used for immunoprecipitation assays and immunoaffinity purification of HA-tagged fusion proteins from bacterial lysates or in transfected cells. Several theses and dissertations cite use of this product in their research protocols.[8-20]

Reagent

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

Storage/Stability

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

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.

Reagents Recommended but not included

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

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.
- Perform all steps at room temperature.
- To prevent clogging the column, highly viscous samples that contain 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 protocols may be performed at 2-8 °C.

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. Rinse the column with PBS, pH 7.4. Allow the buffer to drain from the column. Leave residual PBS in the column to aid in packing the Anti-HA Agarose.

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*.

Washing the Column

- Wash the resin with three sequential 5 mL aliquots of glycine-HCl, pH 2.5 (or 3 M sodium thiocyanate, Cat. 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.

Binding the HA Fusion Protein to the Column

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. Collect the "flow through" of unbound protein. Wash the column with PBS until the $A_{280} \leq 0.01$.

Elution of the HA Fusion Protein

Select one of the following elution procedures:

a. Elution by Glycine-HCl, pH 2.5

Elute the bound HA-tagged fusion protein from the column with 10 × 1 mL aliquots of 0.1 M glycine-HCl (pH 2.5) into vials which contain 30-50 µL of 1 M Tris buffer (pH 8.0) for neutralization.

Notes:

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

b. Elution by HA Peptide

This is a milder elution method. Elute the bound HA-tagged fusion protein by adding 5 × 1 column volume aliquots of a solution containing 100 µg/mL HA peptide (Cat. 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.

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 depend on variables such as sample condition.
- Do not leave the column in glycine-HCl for longer than 20 minutes.

Storing the Column

- Wash the column with three column volumes of PBS.
- 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 seconds). Discard the liquid.
3. Wash the resin with PBS or RIPA buffer, 5 × 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 × 1 mL each. After the final wash, aspirate the supernatant, and leave ~10 µL above the beads.
7. Add 20–50 µL 2× 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 (Cat. 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 (Cat. No. H9658) or Rabbit Anti-HA (Cat. 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.

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

References

1. Narayanan, S.R., *J. Chromatogr. A*, **658(2)**, 237-258 (1994).
2. Olins, P.O., and Lee, S.C., *Curr. Opin. Biotechnol*., **4(5)**, 520-525 (1993).
3. Uhlén, M., and Moks, T., *Methods Enzymol.*, **185**, 129-143 (1990).
4. Kolodziej, P.A., and Young, R.A., *Methods Enzymol*., **194**, 508-519 (1991).
5. Pines, J., and Hunter, T., *J. Cell Biol*., **115(1)**, 1-17 (1991).
6. Antebi, A., and Fink, G.R., *Mol. Biol. Cell*, **3(6)**, 633-654 (1992).
7. Wilson, I.A. *et al*., *Cell*, **37(3)**, 767-778 (1984).
8. Howell, Jessica Jean, "Mechanistic and Physiological Studies of the Insulin-Dependent Regulation of FOXA2". Rockefeller University, Ph.D. dissertation, p. 98 (June 2009).
9. Pavlova, Natalya Nickolayevna, "A Role for PVRL4-Driven Cell-Cell Interactions in Tumorigenesis. Harvard University, Ph.D. dissertation, pp. 120, 121 (2012).
10. Maas, Nancy L., "A Chemical-Genetic Screen for Identifying Substrates of the ER Kinase Perk". University of Pennsylvania, Ph.D. dissertation, p. 51 (2014).
11. Chen, Yu-tsung Shane, "Structural Dynamics and Novel Biological Function of Topoisomerase 2". Duke University, Ph.D. dissertation, pp. 82, 84 (2015).
12. Fritz, Sarah Elizabeth, "Molecular basis of the DExH-box RNA helicase RNA helicase A (RHA/DHX9) in eukaryotic protein synthesis". The Ohio State University, Ph.D. dissertation, pp. 70, 163 (2015).
13. Schmidt, Marvin Lee, Jr., "Coordinate regulation of the WNT and Hippopathways by the novel Ras effector NORE1A". University of Louisville, Ph.D. dissertation, p. 58 (August 2015).
14. Shi, Yuji, "Functional interaction between PTEN and NEDD4 in IGF signaling". Weill Cornell Graduate School of Medical Sciences, Ph.D. dissertation, p. 91 (2015).
15. Collins, Mahlon Angus, "Identification of amyotrophic lateral sclerosis disease mechanisms by cerebrospinal fluid proteomic profiling". University of Pittsburgh, Ph.D. dissertation, p. 162 (2016).
16. Tayou, Junior, "Homo-oligomerization of the Gβ5-RGS7 Complex and its Regulation by R7BP and G Protein Activation". University of Miami, Ph.D. dissertation, p. 22 (2016).
17. Srivastava, Shashank, "Regulation of Alteration/Deficiency in Activation 3 (ADA3) by Acetylation and its Role in Cell Cycle Regulation and Oncogenesis". University of Nebraska Medical Center, Ph.D. dissertation, p. 123 (December 2016).
18. Huang, Ting-Hsiang, "Investigation of the roles of ASF1 and CAF-1 mediated chromatin assembly in the human DNA damage response". University of Texas MD Anderson Cancer Center and University of Texas Graduate School of Biomedical Sciences, Ph.D. dissertation, p. 41 (May 2017).
19. Remsberg, Jarrett Renn, "Biochemical And Functional Studies Of Histone Deacetylase 3 In Metabolic Tissues". University of Pennsylvania, Ph.D. dissertation, pp. 39, 40, 85, 86 (2017).

20. Ali, Ibraheem Irfan, "Role of Post-Translational Protein Modifications in Regulating HIV-1 and Mammalian Transcription". University of California San Francisco, Ph.D. dissertation, p. 186 (2018).
21. Head, PamelaSara Elbaz, "Sirtuin 2 is a Human Tumor Suppressor and a Novel Regulator of the Non-Homologous End Joining Repair Pathway". Emory University, Ph.D. dissertation, pp. 42, 44, 80 (2018).
22. Gan, Ninghai, "Modulation of the host ubiquitin machinery by *Legionella pneumophila* effectors". Purdue University, Ph.D. dissertation, p. 100 (2019).
23. Tien, Chih-Feng, "Understanding regulation of HIV-1 Protease precursor autoprocessing". Colorado State University, Ph.D. dissertation, p. 28 (2019).
24. Schlütermann, David, "Autophagy: Molecular insights into its role and therapeutic potential in bladder cancer and neurodegeneration". Heinrich-Heine-Universität Düsseldorf, Ph.D. dissertation, p. 72 (December 2020).
25. Steiner, Andrea, "NMR studies on the disease-linked single-pass membrane proteins TREM2 and TNFα". Technischen Universität München, Dr. rer. nat. dissertation, p. 40 (2020).
26. Wolf, Lucie Magdalena, "Ubiquitin-dependent regulation of the WNT cargo protein EVI/WLS". Ruperto-Carola University, Dr. rer. nat. dissertation, p. 35 (2021).

Related Products

- Anti-HA Immunoprecipitation Kit (Cat. No. IP0010)
- EZview™ Red Anti-HA Affinity Gel (Cat. No. E6779)
- RIPA buffer (Cat. No. R0278)
- Monoclonal Anti-HA–Peroxidase (Cat. No. H6533)
- Monoclonal Anti-HA–Alkaline Phosphatase (Cat. No. A5477)
- Monoclonal Anti-HA–FITC (Cat. No. H7411)
- Monoclonal Anti-HA–TRITC (Cat. No. H9037)
- Monoclonal Anti-HA–Biotin (Cat. No. B9183)
- Protease inhibitor cocktails, for general, bacterial, mammalian, fungal & yeast, plant, and tissue culture (such as Cat. Nos. P2714, P8465, P8340, P8215, P9599, P1860)
- Phosphatase inhibitor cocktails (Cat. Nos. P0044, P2850, P5726)
- BCA protein assay kits: Standard (Cat. No. BCA1) and QuantiPro™ (Cat. No. QPBCA)
- EZview™ Red Protein A Affinity Gel (Cat. No. P6486)

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.