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ProductInformation

Avidin (Monomeric) HC Agarose

Product Number **A 1979**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Synonym: Avidin (Monomeric) High Capacity Agarose

Product Description
Avidin (Monomeric) HC Agarose is an ideal purification reagent for biotinylated proteins, peptides, and other biomolecules. The advantage of monomeric avidin is the lower biotin-binding affinity (K_d ~10^{-8} M) compared to native tetrameric avidin (K_d ~10^{-15} M). Due to this great difference in affinity, recovery of the biotinylated molecule from the agarose can be accomplished under mild conditions. Specifically, elution of the biotinylated molecule may be accomplished via the addition of 2 mM biotin at neutral pH, or 0.1 M glycine at low pH. Avidin (Monomeric) HC Agarose can be used for the purification of biotinylated proteins,[1] peptides,[2, 3] antibodies,[4] and nucleic acids.[5, 6]

Avidin (Monomeric) HC Agarose is composed of monomeric avidin attached to 6% cross-linked beaded agarose. The resulting linkage has an effective spacer length of 27 atoms. The protein has been immobilized on the agarose in optimal form for maximum exchangeable biotin binding capacity. All non-exchangeable, high affinity, biotin binding sites have been pre-blocked with biotin. This formulation facilitates optimal recovery of biotinylated target molecules; however, it is recommended that re-blocking be undertaken immediately prior to use. This measure will block any non-exchangeable sites that may have formed during storage. Avidin (Monomeric) HC Agarose can be regenerated for up to 10 additional uses, with minimal loss of binding activity following each cycle.

Binding capacity:
- 140–200 nmole of biotin per ml of resin
- minimum 1.7 mg of biotinylated BSA per ml of resin

Specificity:
Avidin (Monomeric) HC Agarose has been tested for binding specificity of biotinylated proteins. Less than 3% non-specific binding is observed.

Reagent
Avidin (Monomeric) HC Agarose is supplied as a 50% suspension in 0.01 M sodium phosphate, pH 6.8, containing 0.15 M NaCl, 50% glycerol, and 15 ppm Kathon® CG/ICP II (as a preservative).

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.

Preparation Instructions
Prior to use, the Avidin (Monomeric) HC Agarose should be equilibrated with a neutral buffer such as Phosphate Buffered Saline, pH 7.4 (PBS, Product Code P 3813). The beads may be equilibrated using either a column or batch technique, depending on the nature of the research. Carefully mix the Avidin (Monomeric) HC Agarose beads until uniformly suspended and proceed with the appropriate equilibration procedure.

Equilibration for Column Format - For the procedure described in this bulletin, 1 ml of Avidin (Monomeric) HC Agarose is utilized. Transfer the required amount of resin to an appropriately sized column. Wash the resin with 4 column volumes of PBS. Do not let the column run dry.

Equilibration for Batch Format - For the procedure described in this bulletin, 100 µl of Avidin (Monomeric) HC Agarose is utilized. Transfer the required amount of resin slurry to an appropriately sized centrifuge tube. When pipetting, use a wide orifice tip or cut ~1 mm off the end of a regular pipette tip to allow unrestricted flow of the bead suspension. To equilibrate the resin, add 5 volumes of PBS, mix, and centrifuge for 30 seconds at 2,000 x *g* (e.g., 5,000 rpm in a Eppendorf® 5415 C microcentrifuge). Carefully remove the supernatant using a micropipette. Repeat the equilibration step two more times.

Storage/Stability
The beads as supplied should be stored at –20 °C.

For storage of the resin following use, wash with 4 column volumes of PBS. Store at 2–8 °C with a preservative (e.g. 15 ppm of Kathon CG/ICP II, Product Code 48178U) for up to 6 weeks. For long-term storage, it is recommended to mix the resin with an equal volume of glycerol (Product Code G 5516) and store at –20 °C. Freezing the agarose in the absence of 50% glycerol will irreversibly damage the bead structure.

Procedures
Purification of biotinylated proteins in a column format

1. Prepare a column of equilibrated resin (see Preparation Instructions). For this procedure, 1 ml of Avidin (Monomeric) HC Agarose is utilized.

2. To block non-exchangeable biotin binding sites, wash the resin with 4 ml of a 1 mg/ml biotin (Product Code B 4501) solution prepared in PBS.

3. Elute the biotin from the exchangeable binding sites with 8 ml of 0.1 M glycine, pH 2.0 (Product Code G 7126).

4. Equilibrate the resin with 6 ml of PBS to prepare for the binding of biotinylated biomolecules.

5. Add 20–50 nmole of biotinylated protein. For optimal binding, load the sample at a flow rate of approximately 0.5 ml/minute.

6. Wash the resin with 8 ml of PBS. If desired, monitor the wash effectiveness by collecting 2 ml fractions and measuring the A_{280} using PBS as the baseline. When A_{280} values for the fractions return to baseline, unbound protein has been removed.

7. To elute the biotinylated protein, wash with 2 mM biotin in PBS or 0.1 M glycine, pH 2.0, and collect at least six 2 ml fractions.

8. Measure the A_{280} of the fractions using PBS as a baseline. Save the fractions of interest for further analysis.

9. To regenerate the resin, wash with 6 ml of 0.1 M glycine, pH 2.0.

10. The procedure can now be repeated or the resin prepared for storage (see the Storage/Stability section for recommendations).

Purification of biotinylated biomolecules in a batch format using a centrifuge tube

1. Transfer the required amount of equilibrated resin (see Preparation Instructions) to an appropriately sized microcentrifuge tube. For this procedure, 100 µl of Avidin (Monomeric) HC Agarose is utilized.

2. To block non-exchangeable biotin binding sites, add 400 µl of a 1 mg/ml biotin solution prepared in PBS to the resin. Incubate with mixing for 15 minutes at room temperature.

3. Centrifuge for 30 seconds at 2,000 x *g* and remove the supernatant.

4. To elute the biotin from the exchangeable binding sites, add 500 µl of 0.1 M glycine, pH 2.0, mix, and centrifuge for 30 seconds at 2,000 x *g*. Remove the supernatant from the resin. Repeat this step five more times.

5. To prepare for the binding of biotinylated biomolecules, equilibrate with PBS. Add 500 µl of PBS, mix, and centrifuge for 30 seconds at 2,000 x *g*. Remove the supernatant. Repeat this step three more times.

6. Add 10–20 nmole of biotinylated sample. Incubate with mixing for a period of at least 15 minutes at room temperature to facilitate optimal binding.

7. Wash away unbound biomolecules with PBS. Add 500 µl of PBS, mix, and centrifuge for 30 seconds at 2,000 x *g*. Remove the supernatant. Repeat this step four more times.

8. To elute the biotinylated biomolecules, add 200 µl of 2 mM biotin in PBS. Incubate with mixing for at least 15 minutes at room temperature.

9. Centrifuge for 30 seconds at 2,000 x *g*, remove and save the supernatant containing the biotinylated biomolecules of interest for further analysis.

10. To regenerate the resin, add 500 µl of 0.1 M glycine, pH 2.0, mix, and centrifuge for 30 seconds at 2,000 x *g*. Remove the supernatant. Repeat this step five more times.

11. The procedure can now be repeated, or the resin prepared for storage (see the Storage/Stability section for recommendations).

Related Products	Product Code
Biotin Polyethyleneoxide Iodoacetamide	B 2059
Biotinamidohexanoic acid N-hydroxysuccinimide ester	B 2643
ImmunoProbe™ Biotinylation Kit	BK-101

References

1. Sabarth, N. *et al*., Identification of Surface Proteins of *Helicobacter pylori* by Selective Biotinylation, Affinity Purification, and Two-dimensional Gel Electrophoresis. JBC Papers, **277(31)**, 27896-901 (2002).
2. Han, D.K. *et al*., Quantitative Profiling of Differentiation-Induced Microsomal Proteins Using Isotope-Coded Affinity Tags and Mass Spectrometry. Nature Biotechnology, **19**, 946-51 (2001).
3. Brady, J., and Robins, S., Structural Characterization of Pyrrolic Cross-links in Collagen Using a Biotinylated Ehrlich's Reagent. J. Biol. Chem., **276(22)**, 18812-18 (2001).
4. Saviranta, P. *et al*., In Vitro Enzymatic Biotinylation of Recombinant Fab Fragments through a Peptide Acceptor Tail. Bioconjugate Chem., **9**, 725-35 (1998).
5. Parrott, M.B. *et al*., Metabolically Biotinylated Adenovirus for Cell Targeting, Ligand Screening, and Vector Purification. Mol. Ther., **8(4)**, 688-700 (2003).
6. Stojanovic, M., Homogeneous Assays Based on Deoxyribozyme. Nucleic Acids Research, **28(15)**, 2915-18 (2000).

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