INSTRUCTIONS

Thermo SCIENTIFIC

Pierce® Monomeric Avidin Agarose

20228 20267

0339.6

Number	Description
20228	**Monomeric Avidin Agarose,** 5 ml of settled resin
20267	**Monomeric Avidin Agarose,** 10 ml of settled resin

Support: 4% beaded agarose supplied as 50% slurry (e.g., 5 ml of settled resin is equivalent to 10 ml of slurry) in 0.02% sodium azide

Binding Capacity: ≥ 1.2 mg biotinylated BSA/ml settled resin

Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

Monomeric Avidin Agarose is ideal for affinity purification of biotinylated proteins, peptides and other molecules. Immobilization of avidin monomers to beaded agarose resin results in a support with a much lower biotin-binding affinity than native tetrameric avidin. The lower binding affinity enables recovery of the biotinylated molecule using mild elution conditions. During the monomeric avidin immobilization, polymeric forms of avidin with strong binding characteristics also are immobilized. These high affinity biotin-binding sites are first blocked with a biotin-containing buffer. A glycine solution is then added to elute biotin from monomers revealing only the reversible binding sites. The biotinylated molecule of interest may then be purified and eluted by ligand competition using a biotin solution. Pierce Monomeric Avidin Agarose can be regenerated at least 10 times with marginal loss in binding capacity.

While there are several publications on the efficacy of monomeric avidin as an easily reversible affinity support for biotinylated proteins,[1-3] the published methods suffer from low sample recovery, low biotinylated-protein binding, high nonspecific binding, and poor regeneration characteristics. In contrast, Pierce Monomeric Avidin Agarose is produced using a procedure that results in a high binding capacity support with minimal nonspecific binding and provides excellent recovery of biotinylated molecules.

Procedure for Affinity Purification of a Biotinylated Molecule

The following protocol is for using a column packed with 2 ml of settled resin (i.e., 4 ml of the 50% slurry). When using columns containing other resin volumes, reagent amounts must be adjusted accordingly. This procedure may be performed either at room temperature or 4°C.

A. Materials Required

- Disposable column capable of containing at least 2 ml resin-bed volume such Pierce Product No. 89897.
- 16 × 125 mm test tubes, 12 each
- Phosphate-buffered Saline (PBS): 0.1 M sodium phosphate, 0.15 M sodium chloride; pH 7 (e.g., Product No. 228372)
- Biotin Blocking/Elution Buffer: 2 mM D-biotin in PBS (D-biotin, Product No. 29129)
- Regeneration Buffer (can also be used for elution): 0.1 M glycine, pH 2.8
- Sample containing biotinylated protein, peptide, or other compound

  Note: To obtain optimal binding capacity, remove extraneous sources of biotin by dialysis or resin filtration.

B. Procedure

1. Pack the column with 2 ml (4 ml of slurry) of monomeric avidin agarose according to the packing instructions provided with the columns. For pre-packed columns, remove top cap of the column first to avoid introducing air bubbles into the resin. Remove bottom cap and empty storage solution.
2. Wash column with 8 ml of PBS.
3. Add 6 ml of Biotin Blocking/Elution Buffer to block non-reversible biotin binding sites.
4. Remove biotin from the reversible binding sites by adding 12 ml of Regeneration Buffer.
5. Wash column with 8 ml of PBS.
6. Place at least twelve test tubes in a rack. Place the monomeric avidin column in a tube and add the biotinylated protein by applying solution to the center of the disc.
7. When the entire sample has passed through the disc, add 0.25 ml of PBS to force sample completely into the resin bed. If the sample volume is less than 1.75 ml, add an additional volume of PBS to adjust the volume to 2.0 ml.
8. Proceed directly to the next step or allow biotinylated sample to incubate on the column to maximize binding. Cap the bottom and top of column and incubate at room temperature for 1 hour. After incubation, remove caps in reverse order.

   Note: Binding is only slightly increased by incubation.
9. Place column in a new tube and add 2.0 ml of PBS. Repeat PBS addition until a total of six 2.0 ml fractions have been collected. Monitor protein by measuring the absorbance of each fraction at 280 nm (use PBS to obtain a baseline value). When absorbance value returns to baseline, non-bound protein has been removed.
10. To elute the bound biotinylated molecule, add Biotin Blocking/Elution Buffer to the column and collect 0.5-2.0 ml fractions. Measure the absorbance of each fraction at 280 nm (use PBS to obtain a baseline value) and reserve the fractions of interest for further analysis.
11. Regenerate the column by washing two times with 4 ml of Regeneration Buffer.
12. The procedure may be repeated, or the column may be prepared for storage. For storage, wash column with 5 ml of PBS containing a preservative such as 0.01% sodium azide. Place bottom cap on the column and add additional preservative-containing PBS above the top disc before replacing the top cap.
13. Store column upright at 4°C.

Related Products

21329	**NHS-PEO$_4$-Biotin, No-Weigh™ Microtubes,** 8 × 2 mg
21425	**EZ-Link® Sulfo-NHS-LC-Biotinylation Kit**
21126	**Streptavidin, Horseradish Peroxidase Conjugated,** 1 mg
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
66380	**Slide-A-Lyzer® Dialysis Cassette,** 10 K MWCO, 0.5-3 ml, 10/pkg
20227	**Monomeric Avidin Agarose Kit**
29129	**D-biotin,** 1 g

References

1. Green, N.M. and Toms, E.J. (1973). The properties of subunits of avidin coupled to Sepharose. *Biochem. J.* **133:**687-98.
2. Guchait, R.B., *et al.* (1974). Acetyl coenzyme A carboxylase system of *Escherichia coli*. Purification and properties of the biotin carboxylase, carboxyltransferase, and carboxyl carrier protein components. *J. Biol. Chem.* **249:**6633-45.
3. Henrickson, K.P., *et al.* (1979). An avidin monomer affinity column for the purification of biotin-containing enzymes. *Anal. Biochem.* **94:**366-70.

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Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermo.com/pierce