INSTRUCTIONS

Pierce® Monomeric Avidin Agarose

20228 20267

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Number	Description
20228	**Pierce Monomeric Avidin Agarose,** 5mL of settled resin
20267	**Pierce Monomeric Avidin Agarose,** 10mL of settled resin
	Support: 4% beaded agarose supplied as 50% slurry (e.g., 5mL of settled resin is equivalent to 10mL of slurry) in 0.02% sodium azide
	Binding Capacity: ≥ 1.2mg biotinylated BSA/mL settled resin
	Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Monomeric Avidin Agarose is ideal for purifying biotinylated proteins, peptides and other molecules. Avidin monomers immobilized onto beaded agarose results in a support with a much lower biotin-binding affinity than native tetrameric avidin. The lower binding affinity enables recovery of biotinylated molecules using mild elution conditions. During the monomeric avidin immobilization, polymeric forms of avidin with strong binding characteristics also are immobilized. Such binding sites are first blocked with a biotin-containing buffer. A glycine solution is used to elute biotin from monomers revealing only the reversible-binding sites, enabling biotinylated molecule purification. The Pierce Monomeric Avidin Agarose can be regenerated at least 10 times with marginal loss in binding capacity.

Although there are several publications on the efficacy of monomeric avidin as a 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 high binding capacity with minimal nonspecific binding and provides excellent recovery of biotinylated molecules.

Procedure for Affinity Purification of a Biotinylated Molecule

This protocol is for a column packed with 2mL of settled resin (i.e., 4mL of the 50% slurry). Adjust reagent amounts accordingly when using other resin volumes. This procedure may be performed either at room temperature or 4°C.

A. Materials Required

- Disposable column capable of containing at least 2mL resin-bed volume such Pierce Product No. 89897.
- 16 × 125 mm test tubes, 12 each
- Phosphate-buffered Saline (PBS): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7 (e.g., Product No. 28372)
- Biotin Blocking/Elution Buffer: 2 mM D-biotin in PBS (Biotin, Product No. 29129)
- Regeneration Buffer (can also be used for elution): 0.1M 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 2mL (4mL of slurry) of monomeric avidin agarose according to the packing instructions provided with the columns. For 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 8mL of PBS.

3. Add 6mL of Biotin Blocking/Elution Buffer to block non-reversible biotin binding sites.
4. Remove biotin from the reversible binding sites by adding 12mL of Regeneration Buffer.
5. Wash column with 8mL of PBS.
6. Place at least 12 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 0.25mL of PBS to force sample completely into the resin bed. If the sample volume is less than 1.75mL, add an additional volume of PBS to adjust the volume to 2.0mL.
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 2.0mL of PBS. Continue adding PBS until a total of six 2.0mL fractions have been collected. Monitor protein by measuring the absorbance of each fraction at 280nm (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 0.5-2.0mL fractions. Measure the absorbance of each fraction at 280nm (use PBS to obtain a baseline value) and reserve the fractions of interest for further analysis.

    Note: Some molecules might elute more efficiently using the Regeneration Buffer (0.1M glycine, pH 2.8) for elution. If desired, neutralize the pH of the collected fractions with 1/10 volume of 1M Tris•HCl, pH 9.5.
11. Regenerate the column by washing two times with 4mL of Regeneration Buffer.
12. The procedure may be repeated, or the column may be prepared for storage. For storage, wash column with 5mL 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. Store column upright at 4°C.

Related Thermo Scientific Products

21329	**NHS-PEG$_4$-Biotin, No-Weigh™ Microtubes,** 8 × 2mg
21425	**EZ-Link® Sulfo-NHS-LC-Biotinylation Kit**
21126	**Streptavidin, Horseradish Peroxidase Conjugated,** 1mg
66380	**Slide-A-Lyzer® Dialysis Cassette,** 10 K MWCO, 0.5-3mL, 10/pkg
20227	**Monomeric Avidin Agarose Kit**

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.