INSTRUCTIONS

Thermo SCIENTIFIC

Pierce® Monomeric Avidin Kit

20227

0340.5

Number	Description
20227	**Pierce Monomeric Avidin Kit**
	Kit Contents:
	Monomeric Avidin Column, 2mL prepacked column containing monomeric avidin immobilized to 4% beaded agarose supplied as a 50% slurry (i.e., 4mL of slurry equals 2mL of settled resin); Binding Capacity: ≥ 1.2mg biotinylated BSA/ml settled resin
	BupH™ Phosphate Buffered Saline Pack, 1 pack, results in 0.1M sodium phosphate, 0.15M NaCl, pH 7.2 when reconstituted with 500mL of ultrapure water
	Biotin Blocking and Elution Buffer, 200mL, contains 2 mM D-biotin in PBS
	Regeneration Buffer, 250mL, contains 0.1M glycine, pH 2.8
	Column Accessories, white tips (2) and column extender
	Storage: Upon receipt store kit at 4°C. Kit is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Monomeric Avidin Kit enables mild affinity purification of biotinylated proteins, peptides and other molecules. Avidin monomers have a much lower biotin-binding affinity than native tetrameric avidin enabling dissociation of biotinylated molecules using mild elution conditions.

During monomeric avidin immobilization, polymeric forms of avidin with strong binding characteristics also are immobilized. These high affinity biotin-binding sites must first be blocked with a biotin-containing buffer. Biotin molecules are eluted from monomers with a glycine solution revealing only the reversible binding sites. The biotinylated molecule of interest may then be applied to the support for purification and then eluted by ligand competition using a biotin-containing solution. The monomeric avidin resin 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, the monomeric avidin agarose resin in this kit is produced using a procedure that results in a high-binding capacity support with minimal nonspecific binding and excellent recovery of biotinylated molecules.

Procedure for Affinity Purification of a Biotinylated Molecule

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

1. Equilibrate kit components to room temperature.
2. Remove top cap and twist off bottom tab from the column. Allow storage solution to drain.

   Note: The column contains two porous discs that produce a stop-flow action to prevent resin from drying when left unattended for brief periods. Solutions applied to the column will automatically stop flowing when the liquid level reaches the top disc. Always remove top column cap before bottom cap to prevent air bubbles from entering resin.
3. Add 500mL of ultrapure water to the BupH Phosphate Buffered Saline (PBS) Pack. Store buffer at 4°C. If storing buffer for > 1 week, sterile filter solution and add a preservative such as 0.02% sodium azide.
4. Wash column with 8mL of PBS.
5. Add 6mL Biotin Blocking and Elution Buffer to block any non-reversible biotin binding sites.
6. Remove biotin from the reversible binding sites by adding 12mL Regeneration Buffer.

7. Wash column with 8mL of PBS.
8. Place at least 12 test tubes (16 × 125mm) 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.
9. When the entire sample has passed through the disc, add 0.25mL 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.
10. Remove nonbound material (Step 11) or allow the biotinylated sample to incubate. For incubation, cap the bottom and then the top of the column and incubate at room temperature for 1 hour. After incubation, remove caps in reverse order.

    Note: Binding is only slightly increased by incubation.
11. 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 approaches baseline, nonbound protein has been removed.
12. To elute the bound biotinylated molecule, add Biotin Blocking and Elution Buffer to the column and collect at least six 2.0mL fractions. Measure the absorbance of each fraction at 280nm (use PBS to obtain a baseline value) and save 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.
13. Regenerate column by washing two times with 4mL of Regeneration Buffer.
14. 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 (a supplied white tip) on the column and add additional preservative-containing PBS above the top disc before replacing the top cap. Store column at 4°C.

Additional Information Available from our Website

- Tech Tip #4: Batch and spin cup methods for affinity purification of proteins
- Tech Tip #43: Protein stability and storage
- Tech Tip #7: Remove air bubbles from columns to restore flow rate
- Tech Tip #29: Degas buffers for use in affinity and gel filtration columns

Related Thermo Scientific Products

20228	**Pierce Monomeric Avidin Agarose,** 5mL
21329	**EZ-Link® NHS-PEO$_4$-Biotin, No-Weigh™ Format,** 8 × 2mg microtubes
21126	**Pierce Streptavidin, Horseradish Peroxidase Conjugated,** 1mg
28372	**BupH Phosphate Buffered Saline Packs,** 40 packs

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.

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

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