INSTRUCTIONS

Pierce® Monomeric Avidin UltraLink® Resin

53146

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Number	Description
53146	**Pierce Monomeric Avidin UltraLink Resin,** 5mL settled resin Supplied: 50% aqueous slurry (10mL total volume) in 0.02% sodium azide Binding capacity: ≥ 1.2mg biotinylated BSA/mL of settled resin **Storage:** Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Monomeric Avidin UltraLink Resin is ideal for affinity purification of biotinylated proteins, peptides and other molecules. Immobilization of avidin monomers results in a purification resin with a much lower biotin-binding affinity than native tetrameric avidin, enabling recovery 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 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 can then be purified and eluted by ligand competition using a biotin solution or low pH.

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 UltraLink Resin is produced using a procedure that results in a high-binding capacity support with minimal nonspecific binding and provides excellent recovery of biotinylated molecules. Additionally, Pierce Monomeric Avidin UltraLink Resin can be regenerated at least 10 times with marginal loss in binding capacity.

The Thermo Scientific UltraLink Biosupport is an azlactone-activated resin that is hydrophilic, charge-free, high capacity, highly crosslinked, rigid, co-polymeric and porous. This resin is especially useful for medium pressure techniques when using large sample volumes requiring fast-flow techniques (FPLC) and large-scale applications. Agarose supports are useful for gravity-flow procedures; however, more rigid UltraLink® Biosupport is required if flow rates require pressures greater than 25 psi. More specific information regarding this resin is detailed in the Additional Information Section.

Procedure for Affinity Purification of a Biotinylated Molecule

Note: If gravity flow is used, column flow rate will be slow. For best results, attach the column to a peristaltic pump by placing flexible tubing over the bottom tip of the column. The column flow rate can then be accelerated and easily controlled. For information and calculations for determining linear flow rate see the Additional Information Section.

A. Materials Required

- Sample containing biotinylated protein, peptide, or other compound. For optimal binding capacity, remove extraneous biotin by dialysis or gel filtration.
- Phosphate-buffered saline (PBS, Product No. 228372): 0.1M sodium phosphate, 0.15M NaCl; pH 7.0
- Biotin Blocking/Elution Buffer: 2mM biotin in PBS (Biotin, Product No. 29129)
- Regeneration Buffer (also can be used for elution): 0.1M glycine, pH 2.8
- Disposable column such as the Disposable Polypropylene Columns for 1.0-5.0mL resin-bed volumes (Product No. 29922) or the Disposable Column Trial Pack (Product No.29925) that contains two each of three column sizes (i.e., 0.5-2.0mL, 1.0-5.0mL and 2.0-10.0mL resin-bed volumes). Alternatively, use a medium-pressure chromatography column.

  Note: For spin-column formats, use Pierce Spin Columns - Screw Cap (Product No. 69705).

B. Procedure

1. Equilibrate the Pierce Monomeric Avidin UltraLink Resin and reagents to room temperature.
2. Carefully pack a column with the desired amount of resin.
3. Wash column with five resin-bed volumes of PBS.
4. Wash the column with three resin-bed volumes of Biotin Blocking and Elution Buffer to block any non-reversible biotin binding sites on the column.
5. Remove biotin bound to reversible biotin-binding sites by washing with five resin-bed volumes of Regeneration Buffer.
6. Wash column with five resin-bed volumes of PBS to re-equilibrate for binding.
7. Apply the biotinylated sample to the column. Add additional PBS to force the sample into the resin bed. Stop the column flow and incubate for 30 minutes at room temperature.

   Note: Binding is only slightly increased by incubation.
8. Wash column with 10 resin-bed volumes of Binding Buffer. Monitor protein by measuring the absorbance at 280nm (use PBS to obtain a baseline value). When absorbance value returns to baseline, all non-bound proteins have been removed.
9. To elute the bound biotinylated molecule, add five resin-bed volumes of Biotin Blocking/Elution Buffer to the column and collect 0.5-2.0mL 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.

   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.
10. Regenerate the column by washing with five resin-bed volumes of Regeneration Buffer.
11. The procedure may be repeated, or the column may be prepared for storage. For storage, wash column with 3-5 resin-bed volumes of PBS containing a preservative such as 0.01% sodium azide. Store column upright at 4°C.

    Note: Pierce Monomeric Avidin UltraLink Resin can be regenerated at least 10 times with marginal loss in binding capacity.

Additional Information

A. Specific Physical Characteristics of the UltraLink Biosupport

The UltraLink Biosupport is an azlactone-activated resin that is hydrophilic, charge-free, high capacity, highly crosslinked, rigid, copolymeric and porous (Table 1). Resin characteristics are important considerations when using large sample volumes requiring fast-flow techniques and large-scale applications.

Table 1. Characteristics of the Thermo Scientific UltraLink Biosupport.

Resin pH Stability:	1-13
Average Particle Size:	50-80µm
Exclusion Limit:	> 2,000,000 Da
Average Surface Area:	> 250 m^2/g of beads
Average Pore Volume:	> 1.2mL/g of beads (> 60% of bead volume)
Pore Size:	1000Å
Maximum Pressure:	100 p.s.i. (6.9 bar)*
Maximum Linear Velocity:	3000cm/hour

*This value refers to the maximum pressure drop across a column that the resin can withstand. The indicated gauge pressure of a liquid chromatography apparatus may not be measuring the pressure drop across the column.

B. Linear Flow Rate for Medium-pressure Chromatography

An important factor for success when performing medium pressure chromatographic (MPC) applications is limiting the pressure drop across the column, which is critical when attempting to increase scale by using a larger column. The indicated gauge pressure of an MPC apparatus may not actually measure the pressure drop across the column. Therefore, a more reliable criterion for MPC applications is to measure the linear flow rate of buffers through the column, which is a pressure-independent measurement. The linear flow rate is defined as the velocity of the buffer front passing through the resin bed and is usually expressed in cm/hour. UltraLink Biosupport has a maximum linear flow rate of approximately 3000 cm/hour.

The linear flow rate through a cylindrical column can be calculated if the height of the resin bed and the inside diameter (or inside radius) of the column is known, and if column effluent is collected and measured for a given time. The calculations for determining linear velocity are shown below.

Calculations:

- r = Radius (cm)
- πr^2 = Column cross-sectional area
- 1 cm^3 = 1mL of buffer
- cm^3/minute = Measured flow rate per minute (i.e., milliliter of effluent collected in 1 minute)

$$\text{Linear velocity/minute} = \frac{cm^3/\text{minute}}{\pi r^2}$$

$$\text{Linear velocity/hour} = (\text{linear velocity/minute})(60\ \text{min/hr})$$

$$\text{therefore, } \frac{(cm^3/\text{min})(60\ \text{min/hr})}{\pi r^2} = \text{Linear velocity (cm/hr)}$$

C. Information Available from the Web

Please visit our website for additional information relating to this product including the following items:

- Tech Tip: Protein Stability and Storage
- Tech Tip Protocol: Batch and Spin Cup Methods for Affinity Purification of Proteins

Related Thermo Scientific Products

21329	**Pre-Measured NHS-PEO_4-Biotin Microtubes, No-Weigh™ Format,** 8 × 2mg
21430	**EZ-Link® Sulfo-NHS-LC-Biotinylation Kit**
21126	**Streptavidin, Horseradish Peroxidase Conjugated,** 1mg
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
20227	**Pierce Monomeric Avidin Kit**
20002	**Bioconjugate Techniques,** by Greg T. Hermanson, softcover
29129	**Biotin,** 1g

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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