INSTRUCTIONS

Pierce® Iminobiotin Agarose

20221

0222.3

Number	Description
20221	**Pierce Iminobiotin Agarose**, 5mL of settled resin Support: 6% crosslinked beaded agarose resin with a diaminodipropylamine spacer, supplied as 50% slurry (i.e., 10mL total volume) containing 0.02% sodium azide Binding Capacity: ≥ 1mg of avidin/mL of settled resin **Storage:** Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Iminobiotin Agarose is for the purification of avidin, streptavidin or Thermo Scientific NeutrAvidin Conjugates. Iminobiotin is a cyclic guanido analog of biotin and has a lower affinity constant for binding avidin, streptavidin or NeutrAvidin® Protein.

Pierce Iminobiotin Agarose is effective in situations that require mild dissociation of the avidin-biotin complex. Normally, disrupting an avidin-biotin interaction requires 6-8M guanidine•HCl, pH 1.5, an environment that is often too harsh for proteins to maintain native structure or activity. Iminobiotin binds at pH values above 9.5 and elution is achieved at pH 4.0. Relatively gentle elution buffers, such as 0.1M acetic acid or 50mM ammonium acetate buffer, pH 4.0 containing 500mM NaCl can be used to recover bound proteins from Pierce Iminobiotin Agarose.

Additional Materials Required

- Disposable column such as the Disposable Polypropylene Columns (Product No. 29922) or the Column Trial Pack (Product No. 29925)

  Note: For spin-column formats, use Pierce Spin Columns - Screw Cap (Product No. 69705).
- Binding Buffer: 50mM ammonium carbonate buffer, pH 11, containing 500mM NaCl
- Elution Buffer: 50mM ammonium acetate buffer, pH 4.0, containing 500mM NaCl or 0.1M acetic acid

Procedure for Gravity-Flow Purification using Immobilized Iminobiotin

1. Pack an appropriate sized column with the resin slurry according to the instructions provided with the column.
2. Equilibrate the column with 4-5 column volumes of Binding Buffer.
3. Apply the sample to the column, add bottom and top cap and incubate for 30 minutes.

   Note: The binding capacity of the resin is ≥ 1mg of avidin/mL of settled resin.
4. Wash the column with 4-5 column volumes of Binding Buffer.
5. Elute the bound sample by adding 1mL aliquots of the Elution Buffer.
6. Collect 1mL fractions and measure the absorbance of each fraction at 280nm to determine which fractions contain the eluted sample.
7. Dialyze or desalt sample into an appropriate storage buffer to avoid loss of activity resulting from extended exposure to acidic pH.

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Related Thermo Scientific Products

46610	**Fluorescence Biotin Quantitation Kit**
20228	**Pierce Monomeric Avidin Agarose,** 5mL
69574	**Slide-A-Lyzer® MINI Dialysis Device Kit, 10K MWCO, 0.1mL**

General References

1. Chaiet, L. and Wolf, F.J. (1964) The properties of streptavidin, a biotin binding protein produced by streptomyces. *Arch Biochem Biophys* **106:**1-5.
2. Heney, G and Orr, G.A. (1981) The purification of avidin and its derivatives on 2-Iminobiotin-6-aminohexyl-Sepharose 4B. *Anal Biochem* **114:**92-6.
3. Gitlin, G. *et al*. (1987) Studies on the biotin-binding site of avidin. *Biochem J* **242:**923-6.
4. Hoffmann, K *et al*. (1980) Iminobiotin affinity columns and their application to retrieval of streptavidin. *Proc Nat'l Acad Sci USA*, **77:**4666-8.

Product Reference

1. Gao, C. *et al.* (1997) Making chemistry selectable by linking it to infectivity. *Proc Nat'l Acad Sci USA*, **94**:11777-82.

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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