INSTRUCTIONS

Pierce® Avidin Agarose

20219 20225

0260.3

Number	Description
20219	**Pierce Avidin Agarose,** 5mL of settled gel (10mL total slurry volume)
20225	**Pierce Avidin Agarose,** 25mL of settled gel (50mL total slurry volume)

Support: Crosslinked 6% beaded agarose

Binding Capacity: ≥ 20µg biotin/mL of settled resin

Supplied: 50% slurry in 0.02% sodium azide solution

Storage: Upon receipt store at 4°C. Do not freeze agarose. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Avidin Agarose can be used for affinity chromatography purifications, assay development and immunoprecipitation.[1-3] Pierce Avidin Agarose can also be used in the physical separation of two DNA strands produced in a polymerase chain reaction by incorporating biotin into one of the amplification polymers.[4]

Avidin is a protein present in chicken egg white and tissues of birds. Avidin is a glycoprotein rich in tryptophan and highly resistant to denaturation by acids or proteolytic enzymes. Pierce Avidin Agarose is purified avidin that has been immobilized on crosslinked 6% beaded agarose. The resulting affinity support is leak-resistant, stable at pH ranging from 2 to 11, and ideal for gravity-flow and low-speed centrifugation applications.

Important Product Information

- Biotinylated molecules can be eluted from the Pierce Avidin Agarose with 8M guanidine•HCl, pH 1.5 (Product No. 24115) or by boiling with SDS-PAGE sample buffer. These harsh elution conditions may cause leaching of avidin subunits into the sample. Additionally, the avidin may lose considerable binding capacity from the loss of these subunits.

  Note: These elution conditions may irreversibly damage the protein of interest. If the biotinylated molecule needs to be eluted using non-denaturing conditions, use Thermo Scientific Pierce Monomeric Avidin Agarose (Product No. 20228), which allows for mild elution conditions to recover biotinylated molecules without contamination from avidin subunits and without reducing the resin's binding capacity. Alternatively, biotinylate the protein using NHS-Iminobiotin (Product No. 21117), which binds to avidin at pH 9.5 and is easily dissociated at pH 4. Reversible biotinylation may be achieved using a cleavable reagent such as NHS-SS-Biotin (Product No. 21331).

- The protocols included in this instruction booklet are examples of applications for this product. Specific applications and systems will require optimization.

Procedure for Batch Format Immunoprecipitation Using Avidin Agarose

A. Additional Materials Required

- Binding Buffer: Phosphate Buffered Saline (e.g., 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372). To reduce possible nonspecific binding, add 0.1% SDS, 1% NP-40 or 0.5% sodium deoxycholate.
- Biotinylated Antibody: Use approximately 3mg of biotinylated protein/mL of settled gel (2mL of the 50% slurry is equivalent to 1mL of settled gel). Prepare 0.1-1.2mg of biotinylated antibody at a concentration of 0.2-10mg/mL in Binding Buffer.
- Elution Buffer: IgG Elution Buffer (Product No. 21004) or Gentle Ag/Ab Elution Buffer (Product No. 21027); alternatively, prepare 0.1M glycine•HCl, pH 2.8
- Microcentrifuge tube(s)
- SDS-PAGE sample buffer (optional): 2% SDS, 62.5mM Tris base, 10% glycerol, 2.5% 2-mercaptoethanol, pH 6.8

B. Procedure

Note: The amount of antigen needed and the incubation time are dependent upon the antibody-antigen system used and will have to be optimized for each specific system. To allow for proper mixing, make sure the total reaction volume does not completely fill the microcentrifuge tube.

1. Equilibrate the Avidin Agarose and reagents to room temperature.
2. To form the immune complex, add biotinylated antibody to the sample or lysate and incubate for at least 30 minutes at room temperature or overnight at 4ºC.
3. Gently swirl the bottle of Avidin Agarose to obtain an even suspension. Pipette the appropriate amount of resin slurry into a microcentrifuge tube. Centrifuge the tube for 1 minute at medium speed (i.e., 3000-5000 × *g*) and discard supernatant.
4. Wash resin twice by adding Binding Buffer and centrifuging for 1 minute at medium speed. Discard the supernatant.
5. Add the immune complex to the resin and incubate with mixing for 1 hour at room temperature or 4°C.
6. Wash the avidin-bound complex with Binding Buffer and centrifuge for 1 minute at medium speed. Discard the supernatant. Repeat this wash procedure at least four times.
7. The sample may be boiled in SDS-PAGE sample buffer and electrophoresed for analysis. Alternatively, add Elution Buffer to the resin to recover the antigen. If using IgG Elution Buffer or 0.1M glycine•HCl, pH 2.8, remove liquid and immediately adjust the pH of the recovered fraction by adding a suitable concentrated buffer such as 1M Tris, pH 7.5 (100µL of this buffer to 1mL of the sample is sufficient).

Procedure for Column Format Affinity Purification Using Immobilized Avidin

A. Additional Materials Required

- Binding Buffer: Phosphate Buffered Saline (e.g., 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372)
- Biotinylated antibody prepared in Binding Buffer: Use approximately 3mg of biotinylated protein/mL of settled resin (2mL of the 50% slurry is equivalent to 1mL of settled gel).
- Sample containing antigen of interest
- Elution Buffer: IgG Elution Buffer (Product No. 21004) or Gentle Ag/Ab Elution Buffer (Product No. 21027); alternatively, prepare 0.1M glycine•HCl, pH 2.8
- Empty column to pack desired bed volume of Pierce Avidin Agarose (e.g., Pierce Centrifuge Columns, Product No. 89896, 89897 or 89898)

B. Procedure

1. Pack resin into the column according to the column instructions. When uncapping column throughout the procedure, always remove the top cap before the bottom cap to prevent air bubbles from entering resin bed.
2. Equilibrate the column with five resin-bed volumes of Binding Buffer.

   Add biotinylated antibody to the column and allow solution to enter the resin bed. Replace the bottom and top caps sequentially and incubate at room temperature for 30 minutes.

   Note: If the solution volume is such that the entire sample cannot be added at once, incubate for 10-15 minutes and allow some of the solution to pass through the column. Add more antibody solution and incubate.
3. Wash the column with 10 resin-bed volumes of Binding Buffer.
4. Add antigen sample to the column and allow the solution to enter the resin bed. Replace the bottom and top caps sequentially and incubate at room temperature for 30 minutes or overnight at 4ºC.
5. Wash the column with 10 resin bed volumes of Binding Buffer.
6. Elute the antigen with 5-10 column volumes of the Elution Buffer. Collect the eluate in 0.5-1mL fractions. If using IgG Elution Buffer or 0.1M glycine•HCl, pH 2.8, immediately neutralize the recovered fraction by adding 100µL of 1M Tris, pH 7.5 to 1mL of the eluted sample. Monitor protein by measuring the absorbance of each fraction at 280nm.

   Note: If using Gentle Ag/Ab Elution Buffer, wash column with three column volumes of Tris buffered saline before antigen elution. The Gentle Elution Buffer is not compatible with phosphate-based buffers (causes precipitation).
7. Desalt or dialyze the eluted fractions into a buffer suitable for the downstream application.
8. To reuse the immobilized biotinylated antibody to purify more antigen, wash column with 10 resin-bed volumes of Binding Buffer, add a final concentration of 0.02% sodium azide and store at 4°C.

Procedure for Gravity-Flow Affinity Purification of Biotinylated Molecules

A. Additional Materials Required

- Biotinylated sample in solution: Use approximately 3mg of biotinylated protein/mL of settled resin (2mL of the 50% slurry is equivalent to 1mL of settled resin).
- Binding Buffer: Phosphate Buffered Saline (e.g., 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372)
- Elution Buffer: 8M guanidine•HCl, pH 1.5 (Product No. 24115)
- Empty column to pack desired bed volume of Pierce Avidin Agarose (e.g., Pierce Centrifuge Columns, Product No. 89896, 89897 or 89898)

B. Procedure

1. Pack resin into the column according to the column instructions. When uncapping column throughout the procedure, always remove top cap before the bottom cap to prevent air bubbles from entering resin bed.
2. Equilibrate the column with five resin-bed volumes of Binding Buffer.

   Add biotinylated sample to the column and allow solution to enter the resin bed. Replace the bottom and top caps sequentially and incubate at room temperature for 30 minutes.

   Note: If the solution volume is such that the entire sample cannot be added at once, incubate for 10-15 minutes and allow some of the solution to pass through the column. Add more biotinylated sample and incubate.
3. Wash the column with 10 resin-bed volumes of Binding Buffer.
4. Elute the bound biotinylated sample with 5-10 resin-bed volumes of Elution Buffer. Collect the eluate in 0.5-1mL fractions. Monitor protein content by measuring the absorbance of each fraction at 280nm.
5. Immediately desalt or dialyze the eluted fractions of interest.

Additional Information

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

- Tech Tip #27: Optimize elution conditions for immunoaffinity purification
- 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
- Tech Tip #13: Pack beaded affinity resin into columns

Related Thermo Scientific Products

21435	**EZ-Link® Sulfo-NHS-LC-Biotinylation Kit**
20349	**Streptavidin Agarose Resin,** 5mL
29200	**NeutrAvidin® Agarose Resin,** 5mL
20227	**Pierce Monomeric Avidin Kit**

Cited References

1. Bruch, R.C. and White, H.B., III. (1982). Compositional and structural heterogeneity of avidin glycopeptides. *Biochemistry* **21:**5334-41.
2. Swack, J.A., *et al.* (1978). Use of avidin-sepharose to isolate and identify biotin polypeptides from crude extracts. *Anal Biochem* **87:**114-26.
3. Wilchek, M. and Bayer, E.A. (1989). A universal affinity column using avidin-biotin technology. In Protein Recognition of Immobilized Ligands. (Hutchins, T.W., ed.). Alan R. Liss, Inc. 83-90.
4. Pellegrini, M., *et al*. (1977). Application of the avidin-biotin method of gene enrichment to the isolation of long double-stranded DNA containing specific gene sequences. *Nucl Acids Res* **4:**2961-73.

General References

Gitlin, G., *et al.* (1987). Studies of the biotin-binding site of avidin. *Biochem J* **242:**923-6.

Green, N.M. (1975). *Avidin. In Adv. in Protein Chemistry*, Academic Press, New York, **29:**85-133.

Manning, J., *et al.* (1977). A method for gene enrichment based on the avidin-biotin interaction. Application to the Drosophila ribosomal RNA genes. *Biochemistry* **16:**1364-70.