INSTRUCTIONS

NeutrAvidin® Agarose Resins

29200 29201 29202 29204

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Number	Description
29200	**NeutrAvidin Agarose Resin,** 5mL of settled resin (10mL total volume)
29201	**NeutrAvidin Agarose Resin,** 10mL of settled resin (20mL total volume)
	Support: 6% crosslinked beaded agarose
	Binding Capacity: ≥ 20µg biotinylated p-NPE/mL of settled resin
	Supplied: 50% aqueous slurry with 0.02% sodium azide
29202	**High Capacity NeutrAvidin Agarose Resin,** 5mL of settled resin (10mL total volume)
29204	**High Capacity NeutrAvidin Agarose Resin,** 10mL of settled resin (20mL total volume)
	Support: 6% crosslinked beaded agarose
	Binding Capacity: ≥ 75µg biotinylated p-NPE/mL of settled resin
	≥ 8mg biotinylated BSA/mL of settled resin
	Supplied: 50% aqueous slurry with 0.02% sodium azide

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

Introduction

The Thermo Scientific NeutrAvidin Protein is a chemically modified version of avidin with a molecular weight of approximately 60,000. Unlike avidin, NeutrAvidin Protein has no carbohydrate portion and a neutral isoelectric point (pI = 6.3), resulting in minimal nonspecific binding.[1] NeutrAvidin Protein immobilized onto 6% crosslinked beaded agarose is leak resistant, stable at pH 2-11 and suitable for gravity flow, spin and FPLC methods. This resin can be used to separate biotinylated products from non-biotinylated products and to affinity purify antigens when used with biotinylated antibodies.

Important Product Information

- To elute biotinylated molecules from the NeutrAvidin Agarose Resins, use 8M guanidine•HCl, pH 1.5 (Product No. 24115) or boil the beads in SDS-PAGE sample buffer. For non-denaturing elution conditions, biotinylate the protein using NHS-Iminobiotin (Product No. 21117), which binds to NeutrAvidin Protein at pH 9.5 and dissociates at pH 4. Alternatively, use a thiol-cleavable biotinylation reagent such as NHS-SS-Biotin (Product No. 21331).
- Guanidine•HCl may irreversibly damage the protein of interest. Furthermore, this harsh elution condition may result in leaching of NeutrAvidin Protein subunits and a considerable reduction in resin binding capacity from the loss of these subunits. Monomeric Avidin (Product No. 20228) allows for gentle elution conditions to recover biotinylated molecules without protein subunit contamination or reducing the column's binding capacity.
- The protocols included in these instructions are examples of applications for this product. Using specific applications and systems requires optimization.
- When using 1mL of resin in a 5mL or larger column, incubate the resin with the biotinylated molecule for at least 10 minutes. Omitting incubation may result in decreased binding capacity.

Gravity-flow Column Method for Purifying Antigens

A. Materials Required

- Biotinylated antibody: Use approximately 3mg of biotinylated antibody/mL of the settled NeutrAvidin Agarose Resin or 8mg of biotinylated antibody/mL of settled High Capacity NeutrAvidin Agarose Resin (2mL of the 50% slurry is equivalent to 1mL of settled resin). Prepare biotinylated antibody at 0.2-10mg/mL in Binding Buffer.
- Sample containing antigen of interest
- Binding Buffer: Phosphate-buffered saline (e.g., 0.1M phosphate, 0.15M sodium chloride; pH 7.2; Product No. 28372)
- Elution Buffer: Thermo Scientific Pierce IgG Elution Buffer (Product No. 21004), Gentle Ag/Ab Elution Buffer (Product No. 21027) or 0.1M glycine•HCl, pH 2.8
- Empty columns: Disposable Polystyrene Columns (Product No. 29920 for ≤ 2mL of resin or Product No. 29924 for 2-10mL of resin)

B. Procedure

1. Pack NeutrAvidin Agarose Resin into the column.

   Note: When using packed columns, remove the top cap first, empty the storage solution and then remove the bottom cap. This procedure prevents air bubbles from being drawn into the resin.

2. Equilibrate column with 3-5 column volumes of Binding Buffer.
3. Add biotinylated antibody solution to the column and allow solution to enter the resin bed. Replace the bottom and top caps sequentially and incubate at room temperature for 10 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. Then add more antibody solution and incubate. Do not exceed the resin's binding capacity.

4. Wash column with 5-10 column volumes of Binding Buffer.
5. Add antigen sample in 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 4°C.
6. Wash the column with 5-10 column volumes of Binding Buffer.

   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.

7. Elute the antigen with 5-10 column volumes of Elution Buffer. Collect the eluate in 0.5-1mL fractions. If using IgG Elution Buffer or 0.1M glycine•HCl, pH 2.8, immediately adjust the pH by the adding 100µL of 1M Tris, pH 7.5-9.0 per 1mL of sample. Monitor protein content by measuring the absorbance of each fraction at 280nm.
8. Desalt or dialyze the eluted fractions into a buffer suitable for the downstream application.

   Note: Wash the immobilized biotinylated-antibody column with 10 column volumes of Binding Buffer before using it to purify more antigen. To store column, add a final concentration of 0.02% sodium azide and store at 4°C.

Gravity-flow Column Method for Purifying Biotinylated Molecules

A. Materials Required

- Biotinylated sample in solution: Use approximately 3mg of biotinylated protein/mL of settled NeutrAvidin Agarose Resin or 8mg of biotinylated antibody/mL of settled High Capacity NeutrAvidin Agarose 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 sodium chloride; pH 7.2; Product No. 28372)
- Elution Buffer: 8M guanidine•HCl, pH 1.5 (Product No. 24115)
- Empty columns: Disposable Polystyrene Columns (Product No. 29920 for ≤ 2mL of resin or Product No. 29924 for 2-10mL of resin)

B. Procedure

1. Pack the NeutrAvidin Agarose Resin into the column.

   Note: When using packed columns, remove the top cap first, empty the storage solution and then remove the bottom cap. This procedure prevents air bubbles from being drawn into the resin.

2. Equilibrate the column with three column volumes of Binding Buffer.
3. Add biotinylated sample to the column and allow sample to enter the resin bed. Sequentially replace the bottom and top caps and incubate at room temperature for 10 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. Then add more antibody solution and incubate. Do not exceed the resin's binding capacity.

4. Wash the column with 5-10 column volumes of Binding Buffer.
5. Elute the bound biotinylated sample with 5-10 column volumes of Elution Buffer. Collect eluate in 0.5-1mL fractions. Monitor protein content by measuring the absorbance of each fraction at 280nm.
6. Immediately desalt or dialyze the eluted fractions of interest. To minimize protein precipitation caused by rapid pH change, neutralize the fractions by slowly adding a high-ionic strength alkaline buffer, such as 1M Tris, pH 9.0.

Spin Method for Purifying Biotinylated Molecules

A. Additional Materials Required

- Biotinylated sample in solution: Use approximately 3mg of biotinylated protein/ml of settled Immobilized NeutrAvidin Protein or 8mg of biotinylated protein/ml of settled High Capacity NeutrAvidin Agarose 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 sodium chloride; pH 7.2; Product No. 28372)
- Elution Buffer: 8 M guanidine•HCl, pH 1.5 (Product No. 24115)
- Pierce® Centrifuge Columns (Product No. 89896 for ≤ 2mL of resin or Product No. 89897 for ≤ 5mL of resin)
- Collection tubes

B. Procedure

1. Equilibrate the NeutrAvidin Agarose Resin and reagents to room temperature.
2. Pack resin into a column. Place column into a collection tube.
3. Centrifuge at 500 × *g* for 1 minute to remove storage solution.
4. Add one column volume of Binding Buffer on top of the resin bed. Centrifuge at 500 × *g* for 1 minute to remove buffer.
5. Repeat Step 4 two additional times, discarding buffer from collection tube.
6. Place column in a new collection tube, add biotinylated sample to the column and allow sample to enter the resin bed. Sequentially replace the bottom and top caps and incubate at room temperature for 10 minutes.

   Note: If the solution volume is such that the entire sample cannot be added at once, incubate for 10-15 minutes. Centrifuge the column to allow some of the solution to pass through. Then add more protein solution and incubate. Do not exceed the resin's binding capacity.

7. Wash the column with one column volume of Binding Buffer. Centrifuge at 500 × *g* for 1 minute.
8. Repeat Step 7 four additional times, discarding buffer from collection tube.
9. Elute the bound biotinylated sample with 5-10 column volumes of Elution Buffer. Centrifuge at 500 × *g* for 1 minute and collect the eluate for each fraction. Monitor protein content by measuring the absorbance of each fraction at 280nm.
10. Immediately desalt or dialyze the eluted fractions of interest.

FPLC Method for Purifying Biotinylated Molecules

A. Additional Materials Required

- Biotinylated sample in solution: Use approximately 3mg of biotinylated protein/ml of settled Immobilized NeutrAvidin Protein or 8mg of biotinylated protein/ml of settled High Capacity NeutrAvidin Agarose 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 sodium chloride; pH 7.2; Product No. 28372)
- Elution Buffer: 8M guanidine•HCl, pH 1.5 (Product No. 24115)
- Cartridge column (1mL or 5mL)

B. Procedure

1. Equilibrate the NeutrAvidin Agarose Resin and reagents to room temperature. Ensure all solutions are degassed. Pack cartridge column with High Capacity NeutrAvidin Agarose Resin according to cartridge manufacturer's guidelines.
2. Fill the pump tubing with Binding Buffer.
3. Snap off the end-tab at the column outlet.
4. Equilibrate the resin with five column volumes of Binding Buffer at a flow rate of 0.2-1mL/minute for a 1mL column or 1-5mL/minute for a 5mL column.
5. Apply sample to the column. Use a 0.2-1mL/minute flow rate for a 1mL column or 0.5-2mL/minute for a 5mL column.
6. Wash with 5-10 column volumes of Binding Buffer or until the absorbance approaches baseline. Use a 2mL/minute or 5mL/minute flow rate for washing 1mL or 5mL columns, respectively.
7. Elute with 5-10 column volumes of Elution Buffer at a flow rate of 0.2-1mL/minute for a 1mL column or 2-5mL/minute for a 5mL column. Immediately desalt or dialyze the eluted fractions of interest.

Batch Method for Immunoprecipitation

A. Materials Required

- Sample containing antigen of interest
- Biotinylated antibody: Use approximately 3mg of biotinylated antibody/ml of settled NeutrAvidin Agarose Resin or 8mg of biotinylated antibody/mL of settled High Capacity NeutrAvidin Agarose Resin (2mL of the 50% slurry is equivalent to 1mL of settled resin). Prepare biotinylated antibody at 0.2-10mg/mL in Binding Buffer.
- Binding Buffer: Phosphate-buffered saline (e.g., 0.1M phosphate, 0.15M sodium chloride; pH 7.2; Product No. 28372). To reduce nonspecific binding, add 0.1% SDS, 1% NP-40 or 0.5% sodium deoxycholate to the buffer.
- Elution Buffer: For eluting the antigen only, use Pierce IgG Elution Buffer (Product No. 21004), Gentle Ag/Ab Elution Buffer (Product No. 21027) or 0.1 M glycine•HCl, pH 2.8. For eluting the biotinylated molecule, use 8 M guanidine•HCl, pH 1.5 or boil the beads in SDS-PAGE sample buffer.
- Microcentrifuge tube(s)

B. Procedure

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

1. In a microcentrifuge tube, solubilize antigen in 50µL of Binding Buffer and add the biotinylated antibody. Adjust the sample volume to 0.2mL with Binding Buffer. Incubate sample overnight at 4°C.
2. Mix the NeutrAvidin Agarose Resin to ensure an even suspension. Add the appropriate amount of resin to the tube containing the antigen/biotinylated antibody mixture. Incubate the sample with mixing for 1 hour at room temperature or 4°C.
3. Wash the resin-bound complex with 0.5-1.0mL of Binding Buffer. Centrifuge for 1-2 minutes at ~2500 × *g* and remove the supernatant. Repeat this wash procedure at least four times and remove the final wash.

Note: If using Gentle Ag/Ab Elution Buffer, wash resin with Tris-buffered saline before antigen elution. The Gentle Elution Buffer is not compatible with phosphate-based buffers.

4. Add elution buffer to the resin to recover the bound antigen. If using Pierce IgG Elution Buffer or 0.1M glycine•HCl, pH 2.8, remove the liquid and immediately adjust the pH by adding a concentrated buffer such as 1M Tris, pH 7.5-9.0 (add 100µL of this buffer to 1mL of sample). Alternatively, boil the resin-bound complex in SDS-PAGE sample buffer.

Additional Information

Please visit our website for additional information 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**
21440	**EZ-Link NHS-PEO Solid Phase Biotinylation Kit: *pre-packed column***
31000	**NeutrAvidin Biotin-Binding Protein,** 10mg
20227	**Pierce Monomeric Avidin Kit**
66425	**Slide-A-Lyzer® Dialysis Cassette, 10K MWCO, 0.5-3mL capacity,** 10 pack
89868	**Pierce Centrifuge Columns, 0.8mL capacity,** 50 pack
89896	**Pierce Centrifuge Columns, 2mL capacity,** 25 pack
89897	**Pierce Centrifuge Columns, 5mL capacity,** 25 pack
89898	**Pierce Centrifuge Columns, 10mL capacity,** 25 pack
89882	**Zeba™ Spin Desalting Columns, 0.5mL,** 25 columns
89889	**Zeba Spin Desalting Columns, 2mL,** 5 columns, for 200-700µL samples
89891	**Zeba Spin Desalting Columns, 5mL,** 5 columns, for 500-2000µL samples
69702	**Pierce Spin Cups – Cellulose Acetate Filter,** 50 pack

Cited Reference

1. Hiller, Y., *et al.* (1987). Biotin binding to avidin. Oligosaccharide side chain not required for ligand association. *Biochem J* **248:**167-71.