G-Biosciences, St Louis, MO. USA ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Immobilized Avidin Resin

INTRODUCTION

Immobilized Avidin Resin is designed for the affinity chromatography purifications, assay development and immunopreciptations of proteins, antibodies and other molecules with a biotin tag. The resin consists of recombinant avidin coupled to 6% cross-linked agarose.

Avidin is a glycoprotein with approximately 10% of its total mass coming from carbohydrates. Avidin has a molecular weight of 67kDa and contains four identical 128 amino acid subunits that each has a single biotin binding domain. Avidin is a basic protein with an isoelectric pH of 10-10.5 and is readily soluble in aqueous buffers containing a wide range of salt, pH (2-11), temperature and other laboratory agents. This wide range of tolerance makes avidin suitable for a wide variety of analytical applications. Avidin has extraordinary binding affinity for biotin ($Ka=10^{15}M^{-1}$).

ITEMS SUPPLIED

Cat. #	Description	Size*
786-593	Avidin, Immobilized	5ml resin
786-594	Avidin, Immobilized	25ml resin

** Immobilized avidin resin is supplied as a 50% slurry with 0.05% sodium azide as a preservative.*

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store refrigerated at 4°C, **DO NOT FREEZE**. This product is stable for 1 year at 4°C.

SPECIFICATIONS

- Biotin Binding Capacity: ≥15-20µg biotin/ml resin
- Bead Structure: 6% cross-linked agarose

PRODUCT INFORMATION

- **Elution**
  - Elute with 8M Guanidine•HCl, pH 1.5, or
    Note: *Guanidine.HCl is a strong denaturing agent that can damage protein or molecule of interest and remove avidin from the resin, resulting in lower binding capacity. Consider the following options as an alternative.*
  - Boil the beads in SDS-PAGE loading buffer, or
  - Use a thiol cleavable biotinylation reagent, such as HOOK™ NHS-S-S-Biotin (Cat. # BG-04) and elute with DTT, or
  - Label target molecules with 2-iminobiotin, which binds to avidin at high pH (>9.5) and elutes at low pH (<4).
  - Use Immobilized Monomeric Avidin (Cat. # 786-595) for gentle elution conditions.

PROTOCOL 1: BIOTINYLATED MOLECULE PURIFICATION (GRAVITY FLOW METHOD)

ADDITIONAL ITEMS

- Biotinylated protein, antibody or other molecules in solution (1-3mg biotinylated protein/ml packed resin)
- Columns (optional): G-Biosciences offers columns for a large range of resin volumes (Cat. # 786-718 to 786-724)
- Binding buffer: 1X PBS
- Elution buffer: 8M Guanidine•HCl, pH 1.5

PROCEDURE

1. Allow the resin and reagents to equilibrate to room temperature.
2. Pack an appropriate volume of avidin resin into a column.
3. Equilibrate the column with 5 column volumes of binding buffer.
4. Add the biotinylated antibody/protein/molecule to the column and allow it to enter the resin. Place a stopper on the bottom of the column and then apply a cap to the top of the column.
5. Incubate the column at room temperature for 10 minutes.

Note: *If the volume of the sample is too large, then add appropriate amount, incubate for 10 minutes, drain column and repeat steps 4 and 5. Do not exceed resin's binding capacity.*

6. Wash the column with 10 column volumes of binding buffer.
7. Elute the protein with 5-10 volumes of elution buffer. Collect in 0.5-1ml fractions. Monitor protein collection with a suitable protein assay or absorbance at 280nm.
8. Immediately, desalt or dialyze the fractions of interest.

PROTOCOL 2: BIOTINYLATED MOLECULE PURIFICATION (SPIN METHOD)

ADDITIONAL ITEMS

- Biotinylated protein, antibody or other molecules in solution (1-3mg biotinylated protein/ml packed resin)
- Columns (optional): G-Biosciences offers spin columns for a large range of resin volumes (Cat. # 786-718 to 786-724)
- Binding buffer: 1X PBS
- Elution buffer: 8M Guanidine•HCl, pH 1.5

PROCEDURE

1. Allow the resin and reagents to equilibrate to room temperature.
2. Pack an appropriate volume of avidin resin into a column.
3. Centrifuge at 500*g* for 1 minute to remove storage buffer.
4. Add 1 column volume of binding buffer and centrifuge at 500*g* for 1 minute. Repeat twice more for a total of three washes.
5. Place the column in a new collection vial and add the sample to the column and allow it to enter the resin. Place a stopper on the bottom of the column and then apply a cap to the top of the column.
6. Incubate the column at room temperature for 10 minutes.

Note: *If the volume of the sample is too large, then add appropriate amount, incubate for 10 minutes, drain column and repeat step 5.*

7. Wash the column with 1 column volume of binding buffer. Centrifuge at 500*g* for 1 minute. Repeat wash step four additional times.
8. Elute the protein with 5-10 volumes of elution buffer. Collect in 0.5-1ml fractions. Monitor protein collection with a suitable protein assay or absorbance at 280nm.
9. Immediately, desalt or dialyze the fractions of interest.

PROTOCOL 3: AFFINITY COLUMN GENERATION

ADDITIONAL ITEMS

- Biotinylated protein, antibody or other molecules in solution (1-3mg biotinylated protein/ml packed resin)
- Sample with antigen of interest
- Columns (optional): G-Biosciences offers spin columns for a large range of resin volumes (Cat. # 786-718 to 786-724)
- Binding buffer: 1X PBS
- Elution buffer: 0.1M Glycine•HCl, pH 2.8

PROCEDURE

1. Allow the resin and reagents to equilibrate to room temperature.
2. Pack an appropriate volume of avidin resin into a column.
3. Equilibrate the column with 5 column volumes of binding buffer.
4. Add the biotinylated antibody/protein/molecule to the column and allow it to enter the resin. Place a stopper on the bottom of the column and then apply a cap to the top of the column.
5. Incubate the column at room temperature for 10 minutes.

Note: *If the volume of the sample is too large, then add appropriate amount, incubate for 10 minutes, drain column and repeat steps 4 and 5. Do not exceed resin's binding capacity.*

6. Wash the column with 10 column volumes of binding buffer. The column is now ready to be used as an affinity column.
7. Add the sample with the antigen of interest to the column and allow it to enter the resin. Place a stopper on the bottom of the column and then apply a cap to the top of the column.
8. Incubate the column at room temperature for 30 minutes or overnight at 4°C.
9. Wash the column with 10 column volumes of binding buffer. The column is now ready to be used as an affinity column.
10. Elute the antigen with 5-10 volumes of elution buffer. Collect in 0.5-1ml fractions. Monitor protein collection with a suitable protein assay or absorbance at 280nm.
11. Immediately, desalt or dialyze the fractions of interest or inhibit protein precipitation by neutralizing the pH with 1M Tris, pH7.5-8.5.
12. Wash the column with 10 column volumes of binding buffer before using to purify more antigen. Store in binding buffer supplemented with 0.02% sodium azide at 4°C.

PROTOCOL 4: IMMUNOPRECIPITATION OR PULL-DOWN PROCEDURE

The avidin resin can be used to couple biotinylated antibody or proteins to generate affinity beds for immunoprecipitation or pull down experiments respectively.

ADDITIONAL ITEMS

- Biotinylated protein, antibody or other molecules in solution (1-3mg biotinylated protein/ml packed resin)
- Columns (optional): G-Biosciences offers columns for a large range of resin volumes (Cat. # 786-718 to 786-724)
- Sample with antigen of interest
- Binding buffer: 1X PBS
- Elution buffer: 0.1M Glycine•HCl, pH 2.8 or boil in SDS-PAGE Sample Buffer

PROCEDURE

Note: *The amount of antigen, capture antibody/protein, resin volume and incubation times need to be optimized for each specific system.*

1. Allow the resin and reagents to equilibrate to room temperature.
2. In a 1.5-2ml centrifuge tube solubilized the antigen in 50-100µl binding buffer.
3. Add the biotinylated antibody or biotinylated capture molecule (i.e. protein) and adjust final volume to 200µl.
4. Incubate overnight with mixing at 4°C.
5. Add an appropriate volume of homogenous avidin resin to the tube and incubate with mixing for at least 1 hour at room temperature or 4°C.

Note: *For simpler washing and elution the resin/protein mix can be transferred to a spin column (Cat. # 786-720) at this point.*

6. Centrifuge at 2,000g for 2 minutes and remove the supernatant.
7. Wash the resin/protein complex with 0.5-1ml binding buffer. Centrifuge at 2,000g for 2 minutes and remove the wash. Repeat the wash step at least four more times.
8. Elute the protein with 0.5-1ml elution buffer and immediately neutralize the pH with 100µl 1M Tris pH 7.5-8.5 for every 1ml elution buffer. Alternative boil the resin/protein complex in SDS PAGE Loading Buffer.

RELATED PRODUCTS

1. **HOOK™ Biotin Reagents** (Cat. # BG-01 to BG-20). *A wide selection of biotinylation reagents , also available in a complete kit format (Cat. # BS-01 to BS-20).*
2. **SpinOUT™ Columns** (Cat. # 786-170 to 786-173, 786-703 to 786-708). *The SpinOUT™ GT-600 and GT-1200 columns are versatile, spin-format columns for the desalting and buffer exchange of protein solutions ranging from 5µl through to 4ml sample volumes.*
3. **Empty Columns** (Cat. # 786-718 to 786-727). *A selection of spin and gravity flow columns for small scale purifications.*
4. **Tube-O-DIALYZER™** (Cat. # 786-610 to 786-624) *Allows dialysis of small samples without having to take the sample out of the tube thus eliminates loss (Medi & Micro size available with 1kDa, 4kDa, 8kDa, 15kDa & 50kDa MW cut off limits).*

For related products, visit www.GBiosciences.com.

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