G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Immobilized Streptavidin Resin

(Cat. # 786-390, 786-590, 786-591, 786-592)

INTRODUCTION

Immobilized Streptavidin 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 streptavidin coupled to 6% cross-linked agarose.

Streptavidin is a tetrameric protein and in many respects is similar to avidin except that it has no carbohydrate and has a slightly lower molecular weight of about 60kDa. The advantage of streptavidin is that the lack of carbohydrates significantly reduces the amount of non-specific binding.The solubility of streptavidin (isoelectric pH5) in aqueous buffer is much lower than avidin, but the binding to biotin is similar.

ITEMS SUPPLIED

Cat. #	Description	Size*
786-590	Streptavidin, Immobilized	2ml resin
786-390	Streptavidin, Immobilized	5ml resin
786-591	Streptavidin, Immobilized	10ml resin
786-592	Streptavidin, Immobilized	5 x 1ml resin[1]

** Immobilized streptavidin resin is supplied as a 50% slurry in 20% ethanol as a preservative.*

[1] *Supplied in a spin column format*

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: ≥15-30µg biotin/ml resin
- Streptavidin Density: >1mg/ml/ml packed 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 streptavidin 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 streptavidin 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)

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 streptavidin 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 and inhibit protein precipitation by neutralizing the pH with 1M Tris, pH9.0.

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 streptavidin 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 and inhibit protein precipitation by neutralizing the pH with 1M Tris, pH9.0.

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 streptavidin 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 into a buffer compatible for downstream applications.
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 streptavidin 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)
- 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

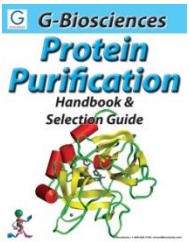
PROCEDURE

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 streptavidin 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

Download our Protein Purification Handbook.



http://info.gbiosciences.com/complete-protein-purification-handbook

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