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A Geno Technology, Inc. (USA) brand name

Streptavidin Resin Kit

(Cat. # 786-555)



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INTRODUCTION

The Streptavidin Resin is designed for the single-step affinity purification of proteins and antibodies with a biotin tag. The resin consists of streptavidin coupled to 4% cross-linked agarose, via a 15 carbon spacer arm. 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 solubility of streptavidin (*isoelectric pH 5*) in aqueous buffer is much lower than avidin, but the binding to biotin is similar. The advantage of streptavidin is that the lack of carbohydrates significantly reduces the amount of non-specific binding.

Description	Size
Caps, Screw	5
Spin Column, 1ml	5
Empty disposable columns-1-5ml Medi	5
Streptavidin Resin*	5ml resin
Streptavidin Binding Buffer	100ml
Streptavidin Elution Buffer	100ml

ITEM(S) SUPPLIED (Cat. # 786-555)

*Streptavidin resin is supplied 10ml as 50% slurry in 20% ethanol.

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store resin & Elution Buffer refrigerated at 4°C, <u>DO NOT FREEZE</u> and all other kit components at room temperature. The kit components stable for 1 year when stored and used as recommended.

SPECIFICATIONS

- Biotin Binding Capacity: ≥100 nmoles/ml resin (measured as binding of biotin-4-fluorescein)
- Streptavidin Density: >1mg/ml/ml packed resin
- Bead Structure: 4% cross-linked agarose
- Bead Size: 75-300 microns

BINDING PROPERTIES:

Binding of biotinylated material is rapid and essentially irreversible. Material modified with 2-iminobiotin may be bound to streptavidin at high pH (>9.5) and eluted at low pH (<4).

ADDITIONAL ITEMS REQUIRED

Biotinylated sample in solution (1-3mg biotinylated protein/ml packed resin)

PROTOCOL

A. Gravity Flow Column

- 1. Allow the resin and sample to equilibrate to room temperature.
- 2. Pack an appropriate volume of Streptavidin Resin into an appropriate size column.
- 3. Equilibrate the column with 3 volumes of Streptavidin Binding/Wash buffer.
- 4. 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.
- Incubate the column at room temperature for 10 minutes.
 <u>Note</u>: If the volume of the sample is too large, add appropriate amount, incubate for 10 minutes, drain column and repeat steps 4 and 5.
- 6. Wash the column with 10 column volumes of Streptavidin Binding/Wash buffer.
- Elute the protein with 5-10 volumes of Streptavidin Elution buffer. Collect in 0.5-1ml fractions and monitor protein collection with a suitable protein assay or reading absorbance at 280nm.
- Immediately, desalt or dialyze the fractions of interest and inhibit protein precipitation by neutralizing the pH with 1M Tris, pH9.0.

B. Spin Column Protocol

- 1. Allow the resin and sample to equilibrate to room temperature.
- 2. Pack an appropriate volume of streptavidin resin into a column.
- 3. Centrifuge at 500x *g* for 1 minute to remove storage buffer.
- 4. Add 1 column volume of Streptavidin Binding/Wash buffer and centrifuge at 500 x *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.
- Incubate the column at room temperature for 10 minutes.
 <u>Note</u>: 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.
- Wash the column with 1 column volume of Streptavidin Binding/Wash buffer. Centrifuge at 500x g for 1 minute. Repeat wash step four additional times.
- 8. Elute the protein with 5-10 volumes of Streptavidin Elution buffer. Collect in 0.5-1ml fractions. Monitor protein collection with a suitable protein assay or absorbance at 280nm.

<u>Note</u>: Elution can be also be performed by boiling the beads in SDS-PAGE loading buffer. Alternatively, use a thiol cleavable biotinylation reagent, such as $HOOK^{\stackrel{\frown}{\sim}}$ NHS-S-S-Biotin (Cat. # BG-04).

9. Immediately, desalt or dialyze the fractions of interest and inhibit protein precipitation by neutralizing the pH with 1M Tris, pH9.0.

RELATED PRODUCTS

Download our Protein Purification Handbook.



http://info2.gbiosciences.com/complete-protein-labeling-conjugation-handbook For other related products, visit our website at <u>www.GBiosciences.com</u> or contact us.

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