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

A Geno Technology, Inc. (USA) brand name

Immobilized Biotin Resin

(Cat. #786-598)

INTRODUCTION

Immobilized Biotin Resin is designed for the high affinity chromatography purifications of avidin, streptavidin and Neutravidin protein. The resin consists of biotin coupled to 6% cross-linked agarose via a 9Å spacer arm.

Biotin, a 244 Dalton vitamin (Vitamin H) molecule, exhibits an extraordinary binding affinity for avidin ($Ka=10^{15}M^{-1}$) and streptavidin. Biotin and avidin interaction is rapid and once the bond is established it can survive up to 3M guanidine-hydrochloride and extremes of pH. Biotin-avidin bonds can only be reversed by denaturing the avidin protein molecule with 8M guanidine-hydrochloride at pH1.5 or by boiling in SDS PAGE sample loading buffer.

ITEMS SUPPLIED

Cat. #	Description	Size*
786-598	Biotin, Immobilized	5ml resin

** Immobilized biotin resin is supplied as a 50% slurry with 0.02% 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: 2mg avidin/ml resin
- Bead Structure: 6% cross-linked agarose
- Spacer Arm: 9Å

PROTOCOL: IMMUNOPRECIPITATION OR PULL-DOWN PROCEDURE

The following protocol is a general pull down procedure for proteins interacting with avidin, streptavidin or Neutravidin. This procedure involves gentle elution of these interacting proteins. For the elution of avidin, streptavidin or Neutravidin a strong denaturing buffer (8M guanidine•HCl) is required that results in the denaturation and release of avidin, streptavidin or Neutravidin.

ADDITIONAL ITEMS

- Sample equilibrated by dialysis with Binding/Wash Buffer
- Avidin, streptavidin or Neutravidin labeled antibody or protein
- Binding/Wash Buffer: 1X PBS
- Elution Buffer: 0.1M Glycine•HCl, pH 2.8
- Neutralization Buffer: 1M Tris, pH8.5
- Columns (optional): G-Biosciences offers columns for a large range of resin volumes (Cat. # 786-718 to 786-724)

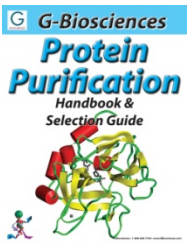
PROCEDURE

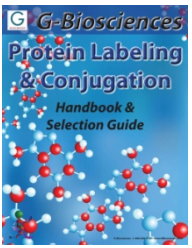
Note: *The amount of antigen, capture antibody/protein, resin volume and incubation times need to be optimized for each specific system.*

1. Incubate the sample with the avidin, streptavidin or Neutravidin labeled antibody or protein for 30 minutes at room temperature or 4°C overnight.
2. Allow the resin and reagents to equilibrate to room temperature.
3. Add an appropriate volume of homogenous biotin 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.*
4. Centrifuge at 1,000g for 2 minutes and remove the supernatant. Retain the supernatant to evaluate binding efficiency.
5. Wash the resin/protein complex with 1 resin volume of Binding/Wash Buffer. Centrifuge at 1,000g for 2 minutes and remove the wash. Repeat the wash step at least four more times.
6. Elute the protein with 1 resin volume of Elution Buffer and immediately neutralize the pH with 10µl Neutralization Buffer for every 100µl Elution Buffer. Repeat the elution 3-4 times and monitor elutions by absorbance at 280nm.
7. The samples can be used directly for SDS PAGE, or alternatively, can be dialyzed for specific downstream applications.
8. Immediately after elution, equilibrate the resin with 5 resin volumes of Binding/Wash Buffer. The resin can be reused at least 10 times. Store at 4°C in Binding/Wash Buffer supplemented with 0.02% sodium azide.

RELATED PRODUCTS

Download our Protein Purification and Protein Labeling & Conjugation Handbooks.





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