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Streptavidin Agarose Resin

User's Guide

DESCRIPTION

UBPBio Streptavidin Agarose Resin is an affinity chromatography medium designed for easy, one-step purification of biotinylated peptides, antibodies, lectins, etc from samples. The purified recombinant streptavidin is covalently coupling to 4% highly cross-linked agarose. The coupling is optimized to give high binding capacity for biotinylated molecules. The total binding capacity of Streptavidin Resin is more than 120 nmol of D-Biotin/ml settled resin.

Streptavidin is a biotin-binding protein found in the culture broth of the bacterium Streptomyces avidinii. Streptavidin binds 4 moles of biotin per mole of protein with an extremely high affinity. Streptavidin lacks carbohydrate side chains present on avidin and has an isoelectric point of 6.5 (vs 10 for avidin) close to where most useful biological interactions occur. As a result, streptavidin frequently exhibits much lower non-specific binding than avidin does.

Table 1. Characteristics of Streptavidin Agarose Resin

Resin Volume	5 ml settled resin (10 ml 50% slurry)
Ligand	Streptavidin
Number of biotin binding sites per ligand	4
M.W. of ligand	Approximately 54 kDa
PI of ligand	6.52
Degree of substitution	Approximately 2 mg streptavidin/ml settled resin
Total binding capacity	> 120 nmol of D-Biotin/ml settled resin
Matrix spherical	agarose, 4% cross-linked
Average particle size	90 µm (45 - 165 µm)
Storage solution	1X PBS containing 20% ethanol
Storage conditions	2 - 8°C

INSTRUCTIONS

I. Method for Purifying Antigens

1.- Additional Materials Required

a) Biotinylated antibody: Use approximately 2-3 mg of biotinylated antibody/ml settled Streptavidin Resin
b) Binding/Wash Buffer: 0.1 M phosphate, 0.15 M NaCl, pH 7.2
c) Elution Buffer: 0.1 M glycine•HCl, pH 2.5 - 2.8
d) Disposable Empty Columns

2.- Procedure

This procedure is optimized for a column of 0.5 ml bed volume. The volumes of the reagents can be scaled up or down according to the size of the column.

a) Completely resuspend the resin and transfer 1 ml slurry to a new column, in which 1 ml Binding Buffer was added in advance.
b) Wash the column with 5 bed volumes of Binding/Wash Buffer.

c) Add biotinylated antibody solution to the column and allow solution to enter the resin bed. Cap the bottom and top sequentially and incubate at room temperature for 10 minutes.

d) Wash column with 10 bed volumes of Binding/Wash Buffer.

e) Add antigen sample to the column and allow the solution to enter the resin bed. Cap the bottom and top sequentially and incubate at room temperature for 30 minutes or overnight at 4°C.

f) Wash the column with 10 bed volumes of Binding/Wash Buffer.

g) Elute the antigen with 5 -10 bed volumes of Elution Buffer. Collect the eluate in 0.5 -1 ml fractions. Monitor protein content by measuring the absorbance of each fraction at 280 nm.

h) Desalt or dialyze the eluted fractions into a buffer suitable for the downstream application.

i) Wash the immobilized biotinylated-antibody column with 10 bed volumes of Binding/Wash Buffer before reuse.

j) For long-term storage, store the resin in 1X PBS containing 20% ethanol at 2 - 8°C.

II. Method for Purifying Biotinylated Molecules

1.- Additional Materials Required

a) Biotinylated sample in solution: Use approximately 2-3 mg of biotinylated sample/ml settled Streptavidin Resin

b) Binding/Wash Buffer: 0.1 M phosphate, 0.15 M sodium chloride, pH 7.2

c) Elution Buffer: 8 M guanidine•HCl, pH 1.5

d) Disposable Empty Columns

2.- Procedure

a) Completely Resuspend the resin and transfer 1 ml slurry to a new column, in which 1 ml Binding/Wash Buffer was added in advance.

b) Wash the column with 3 bed volumes of Binding/Wash Buffer.

c) Add biotinylated sample to the column and allow sample to enter the resin bed. Sequentially cap the bottom and top and incubate at room temperature for 10 minutes.

d) Wash column with 10 bed volumes of Binding/Wash Buffer.

e) Elute the bound biotinylated sample with 5-10 bed volumes of Elution Buffer. Collect the eluate in 0.5-1 ml fractions. Monitor protein content by measuring the absorbance of each fraction at 280 nm.

f) 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 1 M Tris, pH 9.0.