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Streptavidin Agarose

1 Contents

Components	HY-K0218-5 mL	HY-K0218-10 mL	HY-K0218-50 mL
Streptavidin Agarose (Settled Resin)	5 mL	10 mL	50 mL

2 General Information

Streptavidin, isolated from *Streptomyces avidinii*, binds 4 moles of biotin per mole of protein with an extremely high affinity. With no carbohydrate group and an isoelectric point of 6.5 (different from avidin), streptavidin has less nonspecific binding.

MCE Streptavidin Agarose, a 4% highly cross-linked agarose reagent coupled with recombinant streptavidin, is an affinity chromatography medium for separation and purification of biotinylated peptides, antibodies, lectins, etc. The total binding capacity of Streptavidin Agarose is more than 30 μg of D-Biotin/mL settled resin.

3 Characteristics

Matrix Spherical	4% cross-linked agarose
Bead Diameter	45-165 μm
Binding Capacity	>30 μg of D-Biotin/mL settled resin
Storage Solution	50% slurry in 1× PBS containing 20% ethanol

4 General Protocol for Purifying Antigens

1. Buffer Preparation

Binding/Wash Buffer	0.1 M phosphate, 0.15 M NaCl, pH 7.2
Elution Buffer	0.1 M glycine•HCl, pH 2.5 - 2.8

Buffer filtration with 0.45 μm filter is recommended.

2. Load of Streptavidin Agarose

(1) Completely suspend the agarose by gently inverting the bottle several times.

(2) Transfer an appropriate-sized agarose to the column and allow the agarose to settle down and the storage buffer to drain from the column.

3. Antigens Purification

(1) Equilibrate the column with 5× bed volumes of Binding/Wash Buffer.

(2) Add biotinylated antibody to the column and allow it to enter the agarose bed. Sequentially cap the bottom and top and incubate at room temperature for 10 minutes.

(3) Wash the column with approximately 10× bed volumes of Binding/Wash Buffer.

(4) Add antigen sample to the column and allow the solution to enter the agarose bed. Sequentially cap the bottom and top and then incubate at room temperature for 30 minutes or overnight at 4°C.

(5) Wash the column with approximately 10× bed volumes of Binding/Wash Buffer.

(6) Elute with approximately 5-10× bed volumes of Elution Buffer. Collect the eluate. Monitor protein content by measuring the absorbance of each fraction at 280 nm.

(7) Immediately desalt or dialyze the eluted fractions of interest.

(8) Wash the immobilized biotinylated-antibody column with 10× bed volumes of Binding/Wash Buffer before reuse. For long-term storage, store the agarose in 1× PBS containing 20% ethanol at 2 - 8°C.

5 General Protocol for Purifying Biotinylated Molecules

1. Buffer Preparation

Binding/Wash Buffer	0.1 M phosphate, 0.15 M NaCl, pH 7.2
Elution Buffer	8 M guanidine•HCl, pH 1.5

Buffer filtration with 0.45 μm filter is recommended.

2. Load of Streptavidin Agarose

(1) Completely suspend the agarose by gently inverting the bottle several times.

(2) Transfer an appropriate-sized slurry to the column and allow the agarose to settle down and the storage buffer to drain from the column.

3. Biotinylated Molecules Purification

(1) Equilibrate the column with 5× bed volumes of Binding/Wash Buffer.

(2) Add biotinylated molecules to the column and allow sample to enter the agarose bed. Sequentially cap the bottom and top and then incubate 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 (2).

(3) Wash the column with approximately 10× bed volumes of Binding/Wash Buffer.

(4) Elute with approximately 5-10× bed volumes of Elution Buffer, collect the eluate. Monitor protein content by measuring the absorbance of each fraction at 280 nm.

(5) Immediately, desalt or dialyze the eluted fractions into a buffer suitable for the downstream application. To minimize protein precipitation caused by rapid pH change, neutralize the fractions by slowly adding 1 M Tris (pH 9.0).

6 Storage

Store at 2-8ºC, and is stable for at least 2 years.

Do not dry or freeze.

7 Precautions

1. Do not dry or freeze the Streptavidin Agarose.

2. This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

3. For your safety and health, please wear a lab coat and disposable gloves to operate.