

Streptavidin Agarose 6FF

1 Contents

Components	HY-K0218A-5 mL	HY-K0218A-10 mL	HY-K0218A-50 mL
Streptavidin Agarose 6FF (Settled Resin)	5 mL	10 mL	50 mL

2 Introduction

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. Streptavidin has a strong affinity with biotin and needs to be eluted under denaturing conditions when purified. However, streptavidin has a relatively weak affinity for iminobiotin, which binds at pH 9.5-11.0 and elutes at pH 4.0 without the use of denaturing agents.

MCE Streptavidin Agarose 6FF, a 6% 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 6FF is more than 200 nmol of D-Biotin/mL settled resin.

3 Characteristics

Matrix Spherical	6% cross-linked agarose
Ligand	Streptavidin
Bead Diameter	45-165 μm
Binding Capacity	>200 nmol of D-Biotin/mL Settled Resin
Maximum Pressure	0.3 MPa, 3 bar
pH	4.0-9.0
Storage Solution	50% slurry in 1 \times PBS containing 20% ethanol

3 Protocol

1. Buffer Preparation

Purification of biotin or biotinylated substances	Binding/Wash Buffer	20 mM NaH_2PO_4 , 0.15 M NaCl, pH 7.4
	Elution Buffer	8 M guanidine-HCl, pH 1.5
Purification of iminobiotin labeled substances	Binding/Wash Buffer	50 mM Ammonium Carbonate, 0.5 M NaCl, pH 10.0
	Elution Buffer	50 mM Ammonium Carbonate, 0.5 M NaCl, pH 4.0

Note: Buffer filtration with 0.22 μm or 0.45 μm filter is recommended.

2. Sample Preparation

(1) Sample filtration with 0.22 µm or 0.45 µm filter is recommended before loading to the column.

(2) It is necessary to ensure that the proper ionic strength and pH are maintained for optimal binding. For serum samples, ascites fluid or tissue culture supernatant, it is necessary to dilute them at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight with Binding/Wash Buffer.

3. Load of Streptavidin Agarose 6FF

Completely suspend the agarose by gently inverting the bottle several times before using.

3.1 Load the gravity-flow packed column

Rinse the upper and lower gaskets before using the gravity column

1) Select the appropriate-size of gravity column, load the lower gasket, rinse the column tube and gasket with enough ddH₂O, and close the lower outlet.

Note: Avoid the bubble retained after rinsing.

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

Note: a) Pay attention to the timely supplement of the slurry.

b) Avoid the gaps in the resin.

3) Load the upper gaskets rinsed to the column.

Note: Avoid the gaps between the gaskets and the resin.

4) Add appropriate Binding/Wash Buffer to balance the column.

Note: When not in use, add protective solution timely and store at 2-8°C.

3.2 Load the FPLC packed column

1) Follow the formula to calculate the volume of the Streptavidin Agarose 6FF required.

$$V=1.15 \pi r^2 h$$

V (mL): the volume of the resin required

1.15: coefficient of compressibility

r (cm): radius of the column

h (cm): the height loaded of the column

2) Wash the column and the connector with enough ddH₂O, retain the ddH₂O about 1-2 cm at the bottom of the column. Close the lower outlet.

Note: Avoid the bubble retained after washing.

3) Transfer an appropriate-sized slurry to the column.

4) Connect the column to the pump, open the bottom outlet of the column, and start the pump. Allow the slurry to settle down at the set flow rate.

5) When the height of the column bed is stable, Wash the resin with ≥3× bed column of ddH₂O under the final loading flow rate. Mark the height of the column bed.

6) Close the pump and the column outlet.

7) Push the dispenser to the marked height of the column bed. Allow slurry to enter the distributor, then lock the distributor connector.

8) Connect the column to the pump, add appropriate Binding/Wash Buffer to balance the column.

4. Sample Purification

4.1 Incubation method

1) Gently invert the bottle several times to completely suspend the agarose.

2) Transfer an appropriate-sized slurry to a new cube, centrifuge at 1,000 rpm for 1 min, discard the supernatant.

3) Equilibrate the tube with 5× bed slurry of Binding/Wash Buffer, centrifuge at 1,000 rpm for 1 min, discard the supernatant. Repeat twice.

4) Add the appropriate-sized sample to the tube and incubate at 4°C for 2-4 h (or 37°C for 0.5-2 h).

5) Centrifuge at 1,000 rpm for 1 min and discard the supernatant. The supernatant can be saved for SDS-PAGE analysis.

6) Wash the resin with 5× bed slurry of Binding/Wash Buffer, centrifuge at 1,000 rpm for 1 min, discard the supernatant. Repeat 3-5 times.

7) Elute the resin with 3-5× bed slurry of Elution Buffer, incubate at room temperature for 5 min, and then centrifuge at 1,000 rpm for 1 min. Repeat 2-3 times.

8) Wash the resin with 5-10× bed slurry of Binding/Wash Buffer, then wash with 5-10× bed slurry of ddH₂O, Finally, wash with 2× bed slurry of 20% ethanol and store at 2-8°C.

4.2 Gravity-flow Column Method

1) Equilibrate the column with 5× bed volumes of Binding/Wash Buffer. Repeat 2-3 times.

2) Add the appropriate-sized sample to the column. Collect and save the flow-through for analysis.

Note: Binding capacity is flow rate and protein-dependent. Higher flow rates will decrease production time, but may result in losing a small portion of the target antibody.

- 3) Wash the column with 10-15× bed volumes of Binding/Wash Buffer. If desired, save the flow-through for downstream analysis.
- 4) Elute with approximately 5-10× bed volumes of Elution Buffer. Collect the eluate with new tubes.
- 5) Wash the resin with 5-10× bed volumes of Binding/Wash Buffer, then wash with 5-10× bed volumes of ddH₂O. Finally, wash with 2× bed volumes of 20% ethanol and store at 2-8°C.

4.3 FPLC Method

- 1) Fill the pump with ddH₂O. Connect the column to the chromatographic system. Wash the column with approximately 3-5× bed volumes of Binding/Wash Buffer.
- 2) Equilibrate the column with 5× bed volumes of Binding/Wash Buffer at a flow rate of 0.5-1 mL/minute for a 1 mL column or 1-5 mL/minute for a 5 mL column.
- 3) Add the sample to the column by pump or sample ring. Use a 0.5-1 mL/minute flow rate for a 1 mL column or 1-5 mL/minute for a 5 mL column.
- 4) Wash the column with 10-15× bed volumes of Binding/Wash Buffer with a flow speed of about 1 mL/min until the absorbance at 280 nm is stable. If desired, save the flow-through for downstream analysis.
- 5) Elute with approximately 5-10× bed volumes of Elution Buffer with a flow speed of about 1 mL/min. Collect the eluate with new tubes.
- 6) Wash the resin with 5-10× bed volumes of Binding/Wash Buffer, then wash with 5-10× bed volumes of ddH₂O. Finally, wash with 2× bed volumes of 20% ethanol and store at 2-8°C.

5. Detection

Analyze the target protein by SDS-PAGE, along with fractions collected from different steps if necessary.

Note: a) Guanidine hydrochloride has strong punctability and neutralizes the charge of SDS, so it will affect the charged property of protein in loading buffer, and precipitate will be generated during electrophoresis, so the sample can be dialysis or salted out. During dialysis, use the dialysis bags or PBS to dialyze for twice, 1 h/time. It is recommended that the volume of dialysate should be 100 times that of the sample.

b) To minimize protein precipitation caused by rapid pH change, neutralize the fractions by slowly adding 1 M Tris (pH 9.0).

6. Resin washing

Remove the precipitate and deteriorate	Wash the resin with 2× bed volumes of 0.1 M NaOH (or 6 M guanidine•HCl, or 8 M urea), then wash the resin with 5× bed volumes of PBS (pH 7.4) immediately.
Remove the nonspecific adsorption	Wash the resin with 3-4× bed volumes of 70% ethanol (or 2× bed volumes of 1% Triton X-100), then wash the resin with 5× bed volumes of PBS (pH 7.4) immediately.

4 Storage

2-8°C, 2 years.

Do not dry or freeze.

5 Precautions

1. Do not dry or freeze the agarose beads.
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.