PRODUCT INFORMATION

Streptavidin sepharose Purification kit

For mammalian expression system

v. 230201

Catalog number	C07007-K01 / C07007-K02
Package	5 rxns / 10 rxns
Description	Affinity purified Streptavidin is conjugated to NHS-sepharose. It is an efficient technique for isolating biotinylated proteins. Streptavidin is a 52.8 kDa protein purified from the bacterium *Streptomyces avidinii*. Streptavidin homotetramers have an extraordinarily high affinity for Biotin. The Streptavidin sepharose can be used for affinity chromatography purifications, assay development and immunoprecipitation. Streptavidin resins also can be used in the physical separation of two DNA strands produced in a polymerase chain reaction by incorporating biotin in one of the amplification polymers.

Component

Reagents & Materials	Quantity for 10 rxns (C07007-K02)	Quantity for 5 rxns (C07007-K01)	composition
Streptavidin sepharose	2 mL X 1 vial	1 mL X 1 vial	50% slurry of Streptavidin sepharose in 1X Phosphate Buffered Saline
Binding Buffer (10X concentration)	5.0 mL X 1 vial	2.5 mL X 1 vial	200 mM Na_2HPO4; 1.5 M NaCl, pH 7.5
Elution Buffer	10 mL X 1 vial	5 mL X 1 vial	0.1 M Glycine pH 2.7
spin column	10 pcs	5 pcs	
collection tube	10 tubes	5 tubes	

Note: Sepharose is 1:1 suspension in Phosphate Buffered Saline, pH 7.4, containing 0.05% sodium azide as a preservative.

Product capacity	The binding and elution capacity of 1 mL settled Streptavidin sepharose are commonly more than 5 mg of biotinylated protein. Trying different elution buffers for optimal results is recommended.
Materials Required but Not Provided	• Micro-centrifuge capable of 15,000 x g • 1.5 mL Centrifuge tubes • End-over-end rotator • Co-IP Lysis Buffer (mild reaction): 10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40

	• RIPA (vigorous reaction): 100 mM Tris/Cl pH 7.5; 300 mM NaCl; 0.2% Sodium Deoxycholate (or 0.1% SDS); 2% NP-40 • 6 M guanidine-HCl, pH 1.5 for purification of biotinylated molecules
Storage	For sustainable use and long term storage, store at 2 °C to 8 °C. **DO NOT FREEZE.**
Precautions **Disclaimer**	This product is for R&D use only, not for drug, household, or other uses.
Procedure	<u>General notes</u> The Streptavidin sepharose is stored in Phosphate Buffered Saline containing 0.05% sodium azide. The PBS must be removed before use and the resin should be equilibrated with 1X Binding Buffer. The equilibration can be performed at room temperature or at 2-8 °C. The Binding Buffer is original stock concentration. Dilute Binding Buffer (10X concentration) to 1X working concentration with distilled water. In the case of bulk reaction. Users can make a pre-reaction through mixing sample and sepharose in 15 mL / 50 mL tube, and then transfer the mixture into the column for binding. <u>Suggestions on purification of biotinylated protein</u> 1. Cellular debris and particulate matter must be removed by centrifugation or filtration prior to purification on the column. 2. Highly viscous samples which may containing chromosomal DNA or RNA should be sonicated or treated with nuclease to decrease the viscosity. 3. Perform all steps on ice. A. Sample preparation (Lysis of Mammalian Cells) 1. Detach the cells from the culture dish and collect the cell suspension into the centrifuge tube. 2. Centrifuge the cell suspension at 400 x g for 5 minutes to pellet the cells. Carefully remove and discard the supernatant. 3. Wash cells by re-suspending the cell pellet in ice-cold PBS. 4. Centrifuge the cell suspension at 400 x g for 5 minutes to pellet the cells. Carefully remove and discard the supernatant. 5. Add 200 µL of Lysis Buffer to the cell pellet and vortex. 6. Incubate the sample for 15 minutes on ice.

7. Remove cell debris by centrifugation at 15,000 x g for 5 minutes at 4°C.

B. Column preparation

1. Place an empty spin column on the collection tube.
2. Wash the column with 200 µL Binding Buffer.
3. Allow the buffer to drain from the column and leave residual Binding Buffer in the column to aid in packing the Streptavidin sepharose, then discard the buffer in the collection tube.

C. Packing the column

1. Completely suspend the vial of Streptavidin sepharose.
2. Transfer 200 µL volume to an empty centrifuge tube, and wash the sepharose with 1 mL Binding buffer.
3. Spin down the sepharose with 100 x g 30, seconds' centrifugation and discard supernatant.
4. Immediately transfer the sepharose to the spin column. Allow the sepharose bed to settle. Please prevent the sepharose bed from getting dried.
   (Note: Make sure the column filter is fixed in the correct position before transferring the sepharose).

D. Binding biotinylated protein to the column

1. Dilute the sample with Binding Buffer in 1:3 proportion.

Procedure

2. Load the sample on the column and centrifuge the column at 100 x g for 30 seconds. Users can also perform this binding reaction in a new 1.5 mL Eppendorf tube.
   (Note: Depending upon the biotinylated protein and the flow rate, not all of the protein may bind. Repeat loading the sample to increase binding efficiency).
3. Collect the fractions using empty centrifuge tube.
4. Wash the spin column with 300 µL Binding Buffer more than 6 times.
   (Note: To eliminate the noisy band in sample, more washing step is recommended).

E. Elution of biotinylated protein

1. Add 5 x 100 µL Elution Buffer to elute the bound biotinylated protein from the spin column to the collection tube. This step can be supported by a centrifugation at 100 x g for 30 seconds.

(note: Depending on various characteristic of bound proteins, users can try altering the glycine Elution Buffer with guanidine-HCl Elusion Buffer).

2. Assay sample concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance.
   (Note: Measuring the absorbance after each Elution move can help collecting the sample more accurately).

F. Optional instead of elution step

Resuspended Streptavidin sepharose in 100 µL 2 x SDS-Sample Buffer for 10 minutes at 95°C to dissociate immune-complexes from Streptavidin sepharose. Streptavidin sepharose can be collected by centrifugation at 2500 x g for 2 minutes at 4°C and SDS-PAGE is performed with the supernatant.

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