ChangZhou Smart-Lifesciences Biotechnology Co., Ltd.
Hotline Service: 0086-519-83820182 **E-mail:**sales@smart-lifesciences.com
West Taihu science and Technology Industrial Park,Wujin District,Changzhou City,Jiangsu Province,China

Streptavidin ST Beads 4FF

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1. Product Description

Streptavidin ST Beads 4FF is a chromatography medium for one-step purifying Strep-tag II fusion proteins from various of expression system. The Strep-Tag II peptide is an eight amino acid fusion tag(Trp-Ser-His-Pro-Gln- Phe-Glu-Lys), which has negligible effects on recombinant proteins. The ligand immobilized on high-crossed linked 4% agarose is a specially recombinant protein. Purification under physiological conditions and mild elution preserves the activity of the target protein.

Table 1. Characteristics of **Streptavidin ST Beads 4FF**

Item	Description
Matrix	Highly cross-linked 4% agarose beads
Ligand	Streptavidin ST
Capacity （/ml medium)	6 mg Strep-tag II fusion proteins
Particle Size (μm)	45-165
Maxi Pressure	0.3 MPa, 3 bar
pH	3-10
Storage Buffer	1XPBS containing 20% ethanol
Storage Temperature	2℃ - 8℃

Table 2. Chemical compatibilities for **Streptavidin ST Beads 4FF**

Reagent	Concentration
Reduction Agents	
DTT	50 mM
β-mercaptoethanol	50 mM
Non-Ionic Detergents	
C8E4 Octyltetraoxyethylene	Max.0.88 %
C10E5; Decylpentaoxyethylene	0.12 %
C10 E6	0.03 %
C10E8	0.005 %
C12E9; Dodecyl nonaoxyethylene (Thesit)	0.023 %
DM; Decyl-β-D-maltoside	0.35 %
LM; N-dodecyl β-D-maltoside	0.007 %
NG; N-nonyl-β-D-glucopyranoside	0.2 %
OG; N-octyl-β-D-glucopyranoside	2.34 %
TX; Triton X-100	2 %
Tween-20	2 %
Ionic Detergents	
N-lauryl-sarcosine	2 %
8-HESO;N-octyl-2-hydroxy-ethylsulfoxide	1.32 %
SDS; Sodium-N-dodecyl sulfate	0.1 %
Zwitter-Ionic Detergents	
CHAPS	0.1 %
DDAO; N-decyl-N,N-dimethylamine-N-oxide	0.034 %
LDAO; N-dodecyl-N,N-dimethylamine-N-oxide	0.13 %

Other reagent	
Ammonium sulfate $(NH4)_2SO_4$	2 M
$CaCl_2$	Max.1 M
Ethanol	10%
EDTA	50 mM
Guanidine	Max.1 M
Glycerol	Max.25 %
Imidazole	Max.250 mM
$MgCl_2$	1 M
NaCl	5 M
Urea	Max.1 M

2. Purification Procedure

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be high purity. It is recommended to filter the buffers by passing them through a 0.22 μm or 0.45 μm filter before use.

Binding/Wash buffer: 100 mM Tris-HCl,150 mM NaCl,1mM EDTA,pH 8.0 or PBS

Elution Buffer: 2.5mM desthiobiotin in binding buffer

Regeneration Buffer: 1mM HABA in binding buffer

2.2 Sample preparation

The sample should be adjusted to the composition of the binding buffer. This can be done either by diluting the sample with binding buffer or by buffer exchange. It is recommended to filter the sample solution by passing them through a 0.22 μm or 0.45 μm filter before use.

2.3 Packing Columns

Streptavidin ST Beads 4FF is easy to pack and use,and its high flow properties make it excellent for industrial scaling-up.The method of packing the column is described below.

1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.

2) Close the column outlet leaving the net covered with packing buffer.

3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **Streptavidin ST Beads 4FF** is packed at a constant pressure of approximately 3bar(0.3MPa).If the packing equipment does not include a pressure gauge,use a packing flow velocity of approximately 400cm/h(10 cm bed height,25℃, low viscosity buffer). If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Don't exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5) When the bed has stabilized, close the bottom outlet and stop the pump. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

6) With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

7) Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

8) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

2.4 Sample Purification

1) Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, "drop to drop" to avoid introducing air into the column. Remove the snap-off end at the column outlet.

2) Wash the column with 10 column volumes of binding buffer.
3) Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.
4) Wash with 5 to 10 column volumes of wash buffer or until no material appears in the effluent.
5) Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

2.5 Analysis

Identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or western blot.

3. Regeneration

Regeneration: Wash the column with 3CV distilled water followed by 15CV regeneration buffer and 30CV binding buffer. The displacement is detected by the change in color of the edium in the column from yellow to red. This color change is due to the accumulation of HABA/Streptavidin ST complexes. The HABA is washed away with the binding buffer.
Equilibration:Before next use, balance with 5 times column volume of equalizer.

4. Related Products

Product	Cat. No	Size
Streptavidin ST Beads 4FF	SA053005	5 ml
	SA053025	25 ml
	SA053100	100 ml
	SA053500	500 ml
	SA05301L	1 L
	SA05310L	10 L
PreCap Streptavidin ST	SA053C11	1X1 ml
	SA053C51	5x1 ml
	SA053C15	1X5 ml
	SA053C55	5X5 ml
	SA053CS	3X1 ml+1X5 ml