Streptavidin Beads 6FF

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1. Product Description

Recombinant streptavidin isolated from Streptomyces avidinii is immobilized on high-crossed linked 6% agarose beads. **Streptavidin Beads 6FF** can be used to bind biotin and biotinylated substances. The interaction between streptavidin and biotin is very strong and requires denaturating conditions for elution, which may destroy both the ligand and the sample. The interaction between 2-iminobiotin and streptavidin is weak, it can be eluted at pH4.0, which preserve the biomolecules activity.

The crosslinking of the base matrix has been optimized to give the matrix good flow properties and high physical and chemical stability, both of which are key factors for cost-effective, large-scale use.

Table 1. Characteristics of **Streptavidin Beads 6FF**

Item	Description
Matrix	Highly cross-linked 6% agarose
Ligand	Streptavidin
Capacity (/ml medium)	>200nmol Biotin/ml medium
Particle size (μm)	45-165
Maxi pressure	0.3 MPa, 3 bar
pH stability	2-10
Storage buffer	1×PBS containing 20% ethanol
Storage	2°C - 8°C

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 0.22μm or 0.45 μm filter before use.

Binding of biotin or biotinylated substances

Binding Buffer: 20mM NaH_2PO_4, 0.15M NaCl, pH7.4

Elution Buffer: 8M Guanidine-HCl, pH1.5

Note: The harsh elution condition may affect the activities of the sample and the ligand. Streptavidin Beads 6FF cannot be re-used after elution under these conditions.

Purification of iminobiotinylated subatances

Binding Buffer: 50mM ammonium carbonate, 0.5M NaCl, pH10.0

Elution Buffer: 50mM ammonium acetate, 0.5M NaCl, pH4.0

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. The sample should be filtered through a 0.22um or 0.45 μm filter or centrifuged before use.

2.3 Packing Columns

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 Beads 6FF** 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 400 cm/h (10 cm bed height, 25°C, 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. Do not 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) Load the sample by using a syringe fitted to the connector or by pumping it onto the column.

4) Wash the column with 5 to 10 column volumes of binding 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. Related Products

Product	Cat. No.	Size
Streptavidin Beads 6FF	SA021005	5 ml
	SA021025	25 ml
	SA021100	100 ml
	SA021500	500 ml
	SA02101L	1 L
	SA02110L	10 L
PreCap Streptavidin	SA021C11	1×1 ml
	SA021C51	5×1 ml
	SA021C15	1×5 ml
	SA021C55	5×5 ml
	SA021CS	3×1 ml+1×5 ml