Rabbit IgG Beads 4FF

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1. Product Description

Rabbit IgG Beads 4FF is an affinity medium for rapid and convenient single step purification of protein A fusion protein. Rabbit IgG is coupled to highly cross-linked 4% agarose beads. The coupling technique is optimized to give a high binding capacity for IgG. This binding capacity , together with the excellent kinetic and flow properties of the highly cross-linked beads,makes the product suitable for large-scale purification.The characteristics of **Rabbit IgG Beads 4FF** are summarized in Table 1.

Table 1. Characteristics of **Rabbit IgG Beads 4FF**

Item	Description
Matrix	Highly cross-linked 4% agarose beads
Ligand	Rabbit IgG
Binding Capacity	>1mg Protein A/ml medium
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℃

2. Purification Procedure

2.1 Buffer Preparation

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

Binding/Wash Buffer: 50mM Tris, 0.15 M NaCl, 0.05% Tween-20, pH 7.6

Elution Buffer: 0.5M HAc, pH3.0 or 0.1 M glycine, pH 3.0

Neutralization Buffer: 1 M Tris-HCl, pH 8.5

2.2 Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascite fluid or cell culture supernatant at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight against Binding/Wash Buffer.

Note: Avoid reducing agents since the disulphide bonds in IgG will be affected.

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, **Rabbit IgG Beads 4FF** is packed at a constant pressure of approximately 3 bar (0.3 MPa). 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) 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 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. Regeneration

Re-equilibrate **Rabbit IgG Beads 4FF** with Binding Buffer until pH of the effluent is around 7.0. This is important since IgG might denature if the chromatography medium is left standing at a low pH.

If the **Rabbit IgG Beads 4FF** is not going to be used for a longer period of time, wash it with 5 column volumes 20% ethanol in Binding Buffer and store at 2°C - 8°C.

Rabbit IgG Beads 4FF must not be frozen.

4. Related Products

Product	Cat. No.	Size
Rabbit IgG Beads 4FF	SA082005	5 ml
	SA082025	25 ml
	SA082100	100 ml
	SA082500	500 ml
	SA08201L	1 L