Product Information

Product Name:	rProtein A Beads 4FF
Product Code:	SA013005/SA013025
Size(s):	5ml & 25ml

Product Description:

rProtein A Beads 4FF is an affinity chromatography medium designed for the one-step purification of immunoglobulins from biological fluids and cell culture media. Coupling of recombinant protein A ligand to highly cross-linked 4% agarose beads optimized the binding capacity of rProtein A Beads 4FF to immunoglobulins, especially IgG-class antibodies. The dynamic binding capacity of rProtein A Beads 4FF is dependent on various factors, such as target antibody and flow rate.

Protein A is a bacterial cell wall protein in *Staphylococcus aureus*. IgG-class antibodies bind to the IgG binding domains of the immobilized recombinant protein A ligand through interactions with the Fc regions of mammalian IgG, with high specificity and affinity. The Fab region in recombinant protein A is involved in the binding of antigens, making rProtein A Beads 4FF excellent for isolation of immune complexes.

Product Specification:

Chromatography Technique:	Antibody affinity purification
Matrix:	Highly cross-linked 4% agarose beads
Ligand:	Recombinant protein A
Binding Capacity:	>40mg human IgG/ml medium
Particle Size:	45 - 165μm
Maximum Pressure:	3bar (0.3MPa)
Temperature Stability:	2 – 40°C
Flow Rate:	30 – 300cm/h
pH Stability:	Working range: pH 2 - 9 Cleaning-in-Place: pH 2 - 11
Chemical Stability:	Stable in commonly used aqueous buffers (i.e. 6M guanidine-hydrochloride and 20% ethanol)
Storage Conditions	2 – 8°C, 1XPBS containing 20% ethanol (Do not freeze)

Test Items	**Specifications**
Appearance	White or white-like beads
Volume	50% suspension
Binding Capacity	>40mg hIgG/ml medium

Operation Protocol

1. Buffer Preparation

Water and chemicals of high purity should be used. It is recommended to filter all buffers through a 0.22μm or 0.45μm filter before usage.

Binding/Wash Buffer: 0.15M NaCl, 20mM Na_2HPO_4, pH7.0
Elution Buffer: 0.1M Glycine, pH3.0
Neutralization Buffer: 1 M Tris-HCl, pH8.5

2. Sample Preparation

To maintain the ionic strength and pH for optimal binding, samples such as serum, ascites fluid and cell culture supernatant have to be diluted at 1:1 with Binding/Wash buffer. Alternatively, the samples could also be dialyzed overnight against Binding/Wash buffer

3. Column Packing

3.1. Flush the end-piece and adaptor with Binding Buffer to remove the air from the column dead spaces. Ensure that there is no air trapped under the column net
3.2. Close the column outlet, while leaving the column net covered with Binding Buffer
3.3. Shake the beads container to resuspend the stored beads (Avoid stirring the sedimented medium). To minimise the introduction of air bubbles, pour the slurry down a glass rod while holding it against the column wall.
Note:
If a packing reservoir is in use, fill the remaining column and reservoir with Binding Buffer immediately. Mount the adaptor/lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adaptor or in the inlet tubing.
3.4. Open the bottom outlet of the column and set the pump to run at the desired flow rate. The rProtein A is packed at a constant pressure of approximately 3bar (0.3MPa) to provide a reasonably well-packed bed.
   i. If the packing equipment does not include a pressure gauge, a packing flow velocity of approximately 400cm/h (10cm bed height, 25°C, low viscosity buffer) should be used
   ii. If the recommended pressure or flow velocity cannot be obtained, use the maximum flow velocity the pump can deliver to provide a reasonably well-packed bed. **DO NOT** exceed 75% of the packing flow rate in subsequent chromatographic procedures
3.5. When the bed is stabilized, close the bottom outlet and stop the pump.
Note:
   i. If a packing reservoir is in use, disconnect the reservoir and fit the adaptor to the column.
   ii. If the column is in use, carefully place the top filter on top of the bed before fitting the adaptor to the column
3.6. With the adaptor inlet disconnected, push the adaptor (approximately 2mm) down into the bed. This allows the packing solution to flush the adaptor inlet.
3.7. Connect the pump and open the bottom outlet, before resuming packing. The bed should be further compressed and a space will be formed between the bed surface and the

adaptor.

3.8. Close the bottom outlet. Disconnect the column inlet and lower the adaptor (approximately 2mm) into the bed. Connect the pump. The column is now ready for use

4. Column Purification Protocol

4.1. Fill the syringe/pump tubing with Binding Buffer. Remove the stopper and connect the column to the syringe/pump tubing (using the luer connector provided) in a “drop to drop” motion, to avoid introduction of air into the column. Remove the snap-off end at the column outlet

4.2. Wash the column with 10 column volumes of Binding Buffer

4.3. Apply the sample, either through the syringe fitted to the luer connecter or pumping it into the column

4.4. Wash the column with 5 - 10 column volumes of Binding Buffer, until there is an absence of materials in the effluent

4.5. Elute with 5 column volumes of Elution Buffer. (Volumes of Elution Buffer varies with the ligand-antibody interaction)

5. Results Analysis

The eluted fractions can be analysed using UV absorbance, SDS-PAGE and western blot

6. Regeneration

Wash the rProtein A 4FF beads with 10 column volumes of Elution Buffer, before equilibrating with 5 column volumes of Binding/Wash buffer. The columns can be regenerated up to 10 times, without significant losses to the binding capacity

Storage

Store the rProtein Beads 4FF medium in Binding/Wash Buffer containing 20% ethanol at 2 - 8°C. Do not freeze.

Troubleshooting Guide

Problems	Possible Causes	Solutions
The flow rate is significantly low	Tiny air bubbles from the buffer or particles from the sample could have blocked the gel pores	De-gas the buffers and samples. Do not allow the column to dry
The specific antibody of interest is not detected in the elution fraction	Concentration of the target antibody is very low	Purify the antibodies using a specific antigen coupled to a support matrix (eg. PabPur SulfoLink Beads, Cat. No. SA018001, or NHS-Activated Beads 4FF, Cat. No. SA039001)
	The IgG subclass does not bind to protein A	Experiment with other affinity chromatography medias to purify the antibodies, such as media conjugated with rProtein G or rProtein A/G or

		Protein L
The antibody is degraded	The antibody is sensitive to low-pH elution buffer	Neutralize the eluted fractions with neutralization buffer immediately after elution