Product Information

Product Name:	rProtein G Beads 4FF
Product Code:	SA015005/SA015025
Size(s):	5ml & 25ml

Product Description:

rProtein G Beads 4FF is an affinity chromatography medium designed for the easy one-step purification of immunoglobulins from biological fluids and cell culture media. Coupling of recombinant protein G ligand to highly cross-linked 4% agarose beads optimized the binding capacity of rProtein G Beads 4FF to immunoglobulins. With the chemical stability and high batch-to-batch reproducibility of the medium, rProtein G Beads 4FF is excellent for isolating immune complexes.

Protein G is a bacterial cell wall protein in *G. streptococci*. Likewise to Protein A, Protein G binds to the Fc regions of mammalian IgG. The non-specific albumin and cell surface binding sites in recombinant protein G have been eliminated, while maintaining the IgG binding sites. The binding characteristics of protein G and protein A are attributed to their significantly different amino acid compositions. In comparison with protein A, Protein G can be used for the purification of a wider spectrum of mammalian (i.e. human, mouse and rat) monoclonal and polyclonal IgG-class antibodies at high binding affinity, but not with other classes of immunoglobulins (i.e. IgM, IgD and IgA).

Product Specification:

Chromatography Technique:	Antibody affinity purification
Matrix:	Highly cross-linked 4% agarose beads (supplied as 50% slurry)
Ligand:	Recombinant protein G
Binding Capacity:	>30mg human IgG/ml medium
Particle Size:	45 - 165μm
Maximum Pressure:	1bar (0.1MPa)
pH Stability*:	Short Term: pH 2 - 10 Long Term: pH 3 - 9
Chemical Stability:	IgG binding capacity is maintained after storage for: **1) 7 days @37°C in:** 1M acetic acid, pH 2.0; 20mM sodium phosphate, 1% SDS, pH7.0; 6M Guandine-HCl, pH7.0; 70% ethanol **2) 2 hours at room temperature in:** 0.1M HCl, pH1.0; 8M urea, pH10.5; 0.1M Glycine-NaOH, pH11
Storage Conditions	2 – 8°C, 1XPBS containing 20% ethanol (Do not freeze)

* The stability of protein G in the given pH range are estimated values based on our product knowledge and experience

Note: The long-term pH stability refers to the pH range for when the medium is stabilized over a period of time, without adverse effects on subsequent chromatographic performance. The short term pH stability refers to the pH range for regeneration and cleaning-in-place. Recombinant protein G may hydrolyse at very low pH

Test Items	Specifications
Appearance	White or white-like beads
Volume	50% suspension
Binding Capacity	>30mg 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: 1M 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. It is recommended to filter the sample solutions through a 0.22μm or 0.45μm filter before usage

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 G is packed at a constant pressure of approximately 1bar (0.1MPa) to provide a reasonably well-packed bed.
i. **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. Cleaning-in-Place

rProtein G Beads 4FF can be used several times without regeneration. If there is a loss of flow rate and combined loads, it could be resulted from precipitation and protein aggregation. The medium would then have to be cleaned.

i. Removal of precipitated or denatured substances:

a. Wash the column with 2 column volumes of 6M guanidine hydrochloride, followed by 5 column volumes of PBS (pH7.4) immediately.

ii. Removal of bounded hydrophobic substances:

a. Wash the column with either 3 - 4 column volumes of 70% ethanol or 1% Triton™ X-100, followed by 5 column volumes of PBS (pH7.4) immediately.

Storage

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

Troubleshooting Guide

Problems	Possible Causes	Solutions
The back pressure exceeds 1bar	The column is clogged	Refer to "Cleaning-in-Place" **(section 6)**
	The sample solution contains precipitate	Filter the sample solution through a 0.22μm or 0.45μm filter before usage
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	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 G	Experiment with other affinity chromatography medias to purify the antibodies, such as media conjugated with rProtein A or rProtein A/G
The antibody is degraded	The antibody is sensitive to low-pH elution buffer	Neutralize the eluted fractions with neutralization buffer immediately after elution