Data sheet

Protein A SepFast™

Protein A SepFast™ HighRes

Protein A SepFast™ Large Bead

Protein A SepFast range is affinity chromatography media for the purification of immunoglobulins. Its purification power has been well documented in various antibody purification applications, such as isolation and purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media.

The range of Protein A SepFast media is developed and supported for production scale chromatography use. Regulatory Support File (RSF) is available to assist process validation and submissions to regulatory authorities.

Protein A SepFast: Suitable for most application from small scale to large bioprocessing scale

Protein A SepFast HighRes: Suitable for processing small quantity of antibodies at reduced time

Protein A SepFast Large Bead: Suitable for processing crude or very viscous feedstocks

1. Properties

Protein A is immobilised to highly porous and highly cross-linked agarose base matrix. Agarose has long been used for chromatographic separations due to its excellent hydrophilic and low non-specific-binding nature. The particles have an open pore structure with excellent mass transfer properties to large protein molecules. The medium shows high mechanical rigidity, so it can be operated at moderate to high flow velocities with moderate pressure drops.

BioToolomics offers both loose medium and pre-packed ready-to-use disposable columns.

Table 1: Characteristics of Protein A SepFast Media and Column:

Matrix	Highly cross-linked Agarose
Particle size	50 - 150 µm (Protein A SepFast) 20 - 50 µm (Protein A SepFast HighRes) 150 - 350 µm (Protein A SepFast Large Bead)
Binding capacity	>30 mg/ml human IgG/ml
Column material	Polypropylene (end-caps, stop plugs), acrylic or polypropylene (column body), polypropylene or polyethylene (frit or meshes), NBR O-rings
Operational pressure	Up to 3 bar (42 psi)
pH stability	2-10 (short term) and 3-9 (long term)
Working temperature	+4^{O}C to +30^{O}C
Chemical stability	Compatible with most commonly used reagents for antibody purifications
Sanitisation	Wash the packed column with 2% hibitane/20% ethanol
Storage	20% ethanol at +4^{O}C - +8^{O}C

2. General operations

Protein A SepFast can be used in batch stirred tank, gravity flow or packed bed operations.

The pre-packed resin is stored in 20% ethanol on delivery. It can be directly connected to a suitable chromatography system such as AKTA. Be sure that it is air bubble free. Normally, the end with the product label should be connected as the top inlet. If there is no label difference between those two ends, the column can be connected either way.

The resin or column should be equilibrated with at least 5 – 10 column volumes of the equilibration buffer until the pH and conductivity signals become stable, before a sample is loaded.

The running pressure shall not exceed 3 bars during the operation.

After each application, seal the column ends and store the column properly if re-use is expected.

3. Binding

Protein A SepFast binds IgG from most species at neutral pH (e.g. pH 7 to 7.4) and physiological ionic strength (e.g. phosphate saline buffer). The static binding capacity depends on the source of the particular immunoglobulin. For a column operation, the dynamic binding capacity is determined by a few factors such as flow rate (residence time), sample concentration and binding buffer.

4. Elution

The bound immunoglobulin is normally eluted by reduced pH, such as about pH 3.0. The general elution buffer includes 0.1M glycine pH 3.0 or 0.1M citric acid pH 3.0. For very strongly bound molecules, the pH may reduce to between 2 to 3.

For acid labile proteins, the eluted fractions can be quickly neutralized by adding (or with pre-added) 1M Tris/HCl, pH 9.0 (10% to 20% v/v).

5. Regeneration

After the elution, wash the medium with 2 – 3 volumes of the elution buffer following with 3 – 5 volumes of the equilibration buffer.

6. Cleaning-in-place (CIP)

In some applications, substances such as denatured proteins or lipids stay in the column after the regeneration step. The following cleaning procedure could be carried out.

To remove precipitated or denatured materials, wash the column with 2 column volumes of 6 M guanidine hydrochloride followed immediately with at least 5 column volumes of the binding buffer. To remove the bound hydrophobic components, wash the column with 1 column volume of a non-ionic detergent e.g. 0.1% Triton™ X-100 at 37°C followed immediately with at least 5 column volumes of the binding buffer.

Note: washing with concentrated alcohol is not recommended if the column body is made of acrylic material.

7. Sanitization

Equilibrate the column with a buffer containing 2% hibitane gluconate and 20% ethanol. Allow to stand for 6 to 8 hours. Re-equilibrate the column with at least 5 column volumes of sterile binding buffer.

8. Storage

Store the loose medium or the pre-packed column in the presence of 20% ethanol at 4-8°C. Never freeze the medium or the column.

9. Further information

Visit www.biotoolomics.com for further information or contact the technical team or sales representatives.

10. Ordering information

Product	Quantity	Code no.
Protein A SepFast HighRes	5 ml	230801-5ML
	25 ml	230801-25ML
	100 ml	230801-100ML
	250 ml	230801-250ML
	500 ml	230801-500ML
	1 litre	230801-1L
	1 ml HiSep column	230802-1ML
	5 ml HiSep column*	230802-5ML
Protein A SepFast	5 ml	230101-5ML
	25 ml	230101-25ML
	100 ml	230101-100ML
	250 ml	230101-250ML
	500ml	230101-500ML
	1 litre	230101-1L
	5 litre	230101-5L
	10 litre	230101-10L
	1 ml HiSep column	230102-1ML
	5 ml HiSep column*	230102-5ML
Protein A SepFast Large Bead	5 ml	230201-5ML
	25 ml	230201-25ML
	100 ml	230201-100ML
	250 ml	230201-250ML
	500ml	230201-500ML
	1 litre	230201-1L
	5 litre	230201-5L
	10 litre	230201-10L

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