

Data sheet

Protein A SepFast™ Medium and Pre-packed Column

Protein A SepFast is an affinity chromatography medium 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.

Protein A binds to the Fc region of immunoglobulins. The binding is highly specific so high purity can be achieved in a single step.

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 particle has open pore structure with excellent mass transfer property to large protein molecules. The medium shows high mechanical rigidity. So it can be operated at moderate to high flow velocities with moderate pressure drop.

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

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

Matrix	Highly cross-linked Agarose
Particle size	50 - 150 µm
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°C to +30°C
Chemical stability	The commonly used reagents for antibody purifications
Sanitisation	Wash the packed column with 2% hibitane/20% ethanol
Storage	20% ethanol at +4°C - +8°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 air bubble is free in the liquid flow path. Normally, one 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 volume of the equilibration buffer and 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 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.biotooolomics.com for further information or contact the technical team or sale representatives.

10. Ordering information

Product	Quantity	Code no.
Protein A SepFast	1 ml	230103
	5 ml	230104
	25 ml	230105
	100 ml	230106
Protein A SepFast pre-packed column	1 x 1 ml	230107
	1 x 5 ml	230108
Protein A SuperSpin	50 / pack	230102



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