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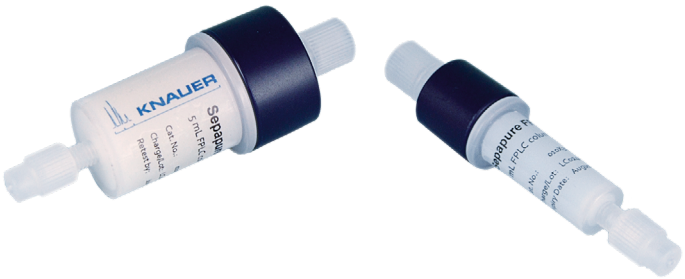
Sepapure protein A FPLC column
Short guide



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Note: For your own safety, read the instructions and observe the warnings and safety information on the device and in the instructions. Keep the instructions for future reference.

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Legal information

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1. Specifications

Definition Sepapure Protein A columns are designed for the purification of antibodies and antibody fragments via the Protein A – Fc region interaction.

The columns are designed to be used with low-pressure FPLC-type automated purification systems and operated below 3 bar (0.3 MPa, 2.96 atm).

1.1 Hardware specifications

Column housing	Polypropylene
Frits	Polyethylene (nominal 20 µm porosity)
Fittings	10/32
Bed volume	1 and 5 mL

1.2 Resin specifications

Resin name	Sepapure Protein A FF
Base matrix material	4% cross-linked, beaded agarose
Mean bead diameter	100 µm
Ligand	Recombinant protein A
Ligand density	6 mg/mL
Static antibody binding capacity	> 30 mg/mL human IgG
Flow rate	> 300 cm/hr
pH stability (with metal ion loaded)	2 – 10 (short term) 3 – 9 (long term)

Note: Before use, inspect the column for damage. If any damage is observed, do not use the column.

Note: Flow rates shown in this manual are for guidance only. Always ensure that system pressure is below the maximum for the column and resin.

1.3 Buffers

Sepapure columns are designed to be used with most aqueous phase chromatography buffers. A suggested buffer system is shown below, although other buffers may be used. Please check the resin specifications for further details.

Equilibration buffer	PBS, pH 7.4
Wash buffer	PBS, pH 7.4
Elution buffer	0.1 M glycine, pH 3.0 or 0.1 M citric acid, pH 3.0

2. Preparing the column

Sepapure Protein A columns are supplied with 20% ethanol as the storage buffer. This must be removed prior to purification.

Process Remove the end-plugs and connect the column to the control system, taking care to avoid introduction of air into the system. Do not over-tighten fittings as this can strip the screw connections and lead to column leakage. Flush the column with 3 to 5 Column Volumes (CVs) of binding buffer at a flow rate of 1 to 2 CV/min to remove the storage buffer.

Equilibrate the column with 3 to 10 CVs of binding buffer at 1 CV/min to ensure that pH, conductivity and UV_{280} signals are stable.

Result The column is now ready for use.

3. Sample preparation

Sepapure Protein A columns should only be used with clarified (particle-free) samples. Most antibodies will bind to Protein A in the pH range 7.0 – 7.4 and under physiological conditions (such as phosphate buffered saline), although some may exhibit better binding at slightly higher pH.

Note: Avoid excessively high concentrations of NaCl as whilst this may give higher binding capacity, it also increases binding on other proteins to the solid phase.

4. Antibody purification

Load the sample at a flow rate of approximately 1 CV/min. Some protein A - antibody binding kinetics and sample viscosities will require the flow rate to be adjusted. To avoid overloading the column, the amount of sample that is applied to the column should not exceed the binding capacity of the solid phase to the target molecule.

Once the sample has been loaded, wash the column with binding buffer until the UV_{280} trace reaches baseline.

If a wash buffer is used, then also wash the column with 3 to 5 CVs of this reagent.

Elute the protein using the selected elution buffer. A stepwise or linear gradient may be used to determine the precise elution point of the target antibody. Antibodies can be eluted in a pH range starting from 5.5 and decreasing to as low as 3.0. Strongly bound antibodies may require an elution pH of between 2.0 and 3.0. Initially, all elution fractions should be collected for further analysis. Buffer exchange and/or desalting might be required following elution and we recommend Sepapure Desalting Columns (010X460SPZ and 020X460SPZ) for this purpose.

Acid-labile antibodies should be neutralised immediately on elution with 1 M Tris-HCl, pH 9.0 (10 - 20% v/v).

5. Column regeneration

To remove contaminants (lipids, non-specifically bound proteins, etc.) from the column following elution, wash with a further 3 to 5 CVs of elution buffer, followed by 3 to 5 CVs of equilibration buffer.

Some contaminants may not be fully removed by the above procedure, in which case a more stringent column regeneration/sanitization procedure should be used. Wash the column with 2 CVs 6 M Guanidine HCl followed by 5 to 10 CVs equilibration buffer. To remove hydrophobic contaminants, use 1 CV of a non-ionic detergent (eg.: 0.1% Triton-X100) at 37°C immediately followed by 5 to 10 CVs of equilibration buffer.

0.1 M NaOH may also be used to clean the column although the solid phase should only be exposed to this reagent for short periods (<10 minutes). Equilibrate the column using at least 5 to 10 CVs of equilibration buffer following NaOH treatment.

6. Column storage

Columns should be stored in PBS supplemented with 20% ethanol.

Note: Columns should be stored at +4°C.
Do not freeze!

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