Native conditions

Substance to analyze or purify

- **Proteins with specific Affinity**
  - Tagged Proteins → **Tag-Affinity Chromatography (AC)**
    - GST-tag
    - His-tag
  - Anti-bodies → **Antibody Affinity Chromatography (AC)**
    - Protein A
    - Protein G
- **Charged Biomolecules**
  - Anions → **Anion Ex-change (IEX)**
    - Weak anion ex-changer
    - Strong anion exchanger
  - Cations → **Cation Ex-change (IEX)**
    - Weak cation exchanger
    - Strong cation exchanger
- **Group separation of high and low molecular weight Biomolecules** → **Desalting/Buffer Exchange (SEC)**
  - Desalting

KNAUER Sepapure®

Find more information

Finding the best fitting column for your HPLC/UHPLC, GPC or FPLC application always starts with looking closely at the substances you want to analyse or purify.

This flow chart gives you a guideline how to select the right column for your application. Start at the top and follow the decision lines all the way down to find a column recommendation.

More details about KNAUER columns and phases can be found in the Column Product Selection Guide and online: www.knauer.net/columns

Sepapure®

In protein purification, a combination of different methods is needed for a successful separation. The purity of the wanted biomolecule is increased in three steps:

In the "capture step" the crude biomolecule is extracted from major side products. In the "intermediate step" further contaminations are removed, and the highly pure biomolecule is gained in the "polishing step". For each step a different method and therefore different columns are used.

FPLC Sepapure® columns are dedicated for purification of biomolecules. Sepapure® media for affinity chromatography (AC) and Ion-Exchange chromatography (IEX) is based on Agarose and depending on the specific purification mode functionalized with ligands e.g. Protein A, Ni-NTA or quaternary ammonium (Q). These FPLC media are available packed in 1 ml and 5 ml cartridges or as bulk media.

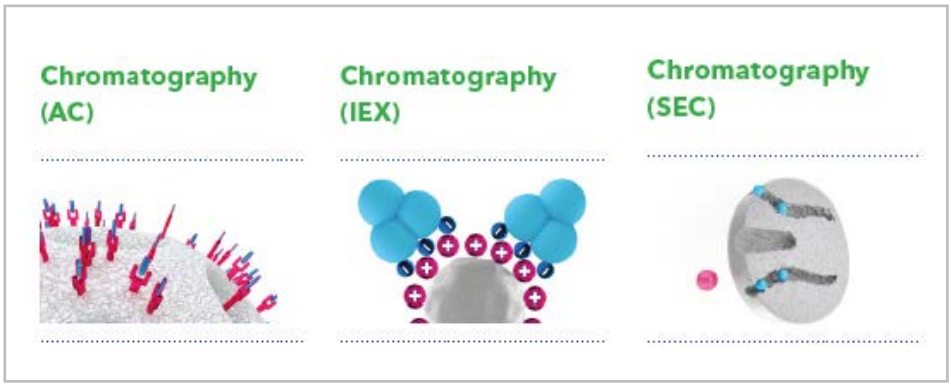
Physical properties FPLC Sepapure® columns

Resin	100 µm Agarose particles with 4 % or 6 % crosslinking
Flowrate	Recommended: 1 CV/ml
pH Stability	3 – 9 long term
Max. pressure	3 bar

Sepapure® Desalting columns are based on Dextran with an exclusion limit of 5 kDa and available in 1 ml and 5 ml cartridges.

Physical properties Sepapure® Desalting columns

Resin	20 – 50 µm Dextran particles
Flowrate	Recommended: 1 CV/ml
Exclusion limit	5 kDa
Max. pressure	3 bar



All available bulk media at one glance:

Resin Type / Volume	5 ml	10 ml	25 ml	50 ml	100 ml	150 ml	250 ml	500 ml	1000 ml
Ni-NTA			•		•		•	•	•
Protein A	•		•		•		•		•
Protein G		•	•						
IEX-Resins			•		•			•	•

Antibody Affinity Chromatography

FPLC media based on cross-linked agarose beads with a mean diameter of 100 µm.

The FPLC media is functionalized either with Protein A or Protein G ligands for the binding of antibodies or antibody fragments.

Properties:

Sepapure® Affinity media for antibodies or antibody fragments is designed to be used with most aqueous buffer systems. It is long lasting when correctly handled and are compatible with common CIP strategies. All Sepapure® media is stored in 20 % ethanol upon delivery. Available as prepacked 1 ml or 5 ml cartridge or as bulk material.

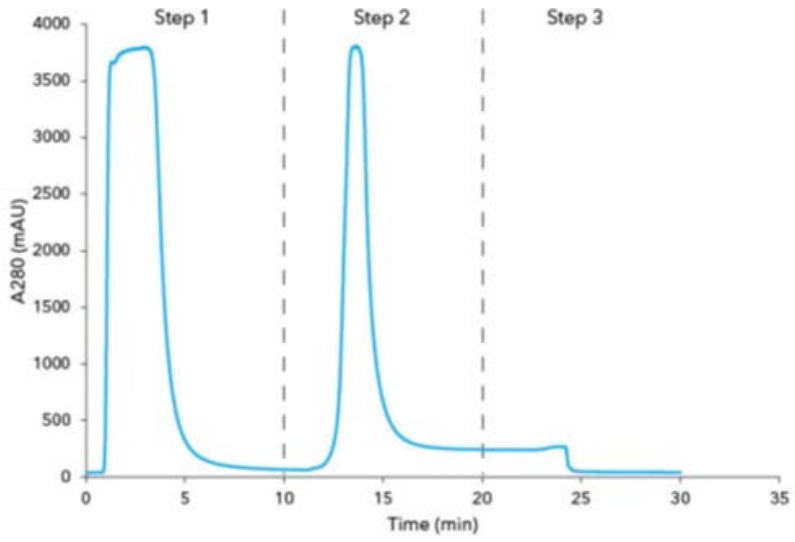
Technical data:

Agarose beads with typical loading ranges of 1 - 30 mg/l (Protein A column) or 1 - 15 mg/ml (Protein G column). The maximum operating pressure the Sepapure® columns should be used at is 3 bar, while the recommended flowrate is 1 CV/ml.

Recommended application areas:

Typically used in the first step („capture“) of an FPLC purification procedure.

Sepapure Protein A FF 1 ml
Article number: 010X40USPZ



Step 1: injection peak of human plasma and column washing

Step 2: elution peak of IgG

Step 3: column equilibration

Column type	Cartridge	
	1 ml	5 ml
Sepapure® Protein A FF	010X0USRZ	020X40USRZ
Sepapure® Protein G FF	010X40VSPZ	020X40VSPZ

Resin type	Media						
	5 ml	10 ml	25 ml	50 ml	100 ml	250 ml	1000 ml
Sepapure® Protein A FF	00GX40USRZ	-	00IX40USRZ	00JX40USRZ	00KX40USRZ	00NX40USRZ	00QX40USRZ
Sepapure® Protein G FF	-	00HX40VSPZ	00IX40VSPZ	-	-	-	-

Tag-Affinity Chromatography

FPLC media based on cross-linked agarose beads with a mean diameter of 100 µm.

The FPLC media is functionalized with NTA ligands for His-Tag.

Properties:

Sepapure® Affinity media for recombinantly tagged proteins is designed to be used with most aqueous buffer systems. It is long lasting when correctly handled and compatible with common CIP strategies. All Sepapure® media is stored in 20 % ethanol upon delivery. Available as prepacked 1 ml or 5 ml cartridge or as bulk material.

Technical data:

Agarose beads with typical loading ranges of 1–40 mg/l (Ni-NTA). The maximum operating pressure the Sepapure® columns should be used at is 3 bar, while the recommended flowrate is 1 CV/ml.

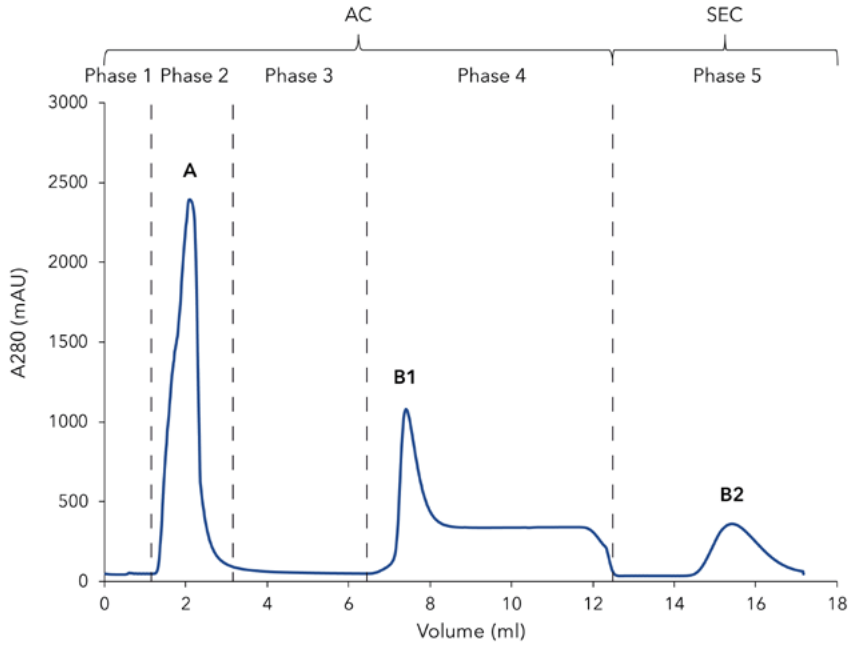
Available as prepacked 1 ml or 5 ml cartridge or as bulk material.

Recommended application areas:

Typically used in the first step („capture“) of an FPLC purification procedure.

AC - Sepapure Ni-NTA FF 1 ml
Article number: 010X39FPSZ

SEC - Sepapure Desalting 5 ml
Article number: 020X460SPZ



A: flow through of unbound protein

B1: elution peak of 6xHis-GFP from Ni-NTA column

B2: elution peak of 6xHis-GFP from Desalting column

Column / Resin type	Cartridge		Media					
	1 ml	5 ml	5 ml	25 ml	100 ml	250 ml	500 ml	1000 ml
Sepapure® Ni-NTA FF	010X39FPSZ	020X39FSPZ	-	00IX39FPSZ	00KX39FPSZ	00NX39FPSZ	00PX39FPSZ	00QX39FPSZ

Ion-Exchange Chromatography

FPLC media based on cross-linked agarose beads with a mean diameter of 100 µm.

The FPLC media is functionalized with different linkers ranging from strong anion exchange ligands to weak cation exchange ligands.

Properties:

Sepapure® Ion-Exchange media is designed to be used with most aqueous buffer systems. It is long lasting when correctly handled and is compatible with common CIP strategies. All Sepapure® media is stored in 20 % ethanol upon delivery.

Technical data:

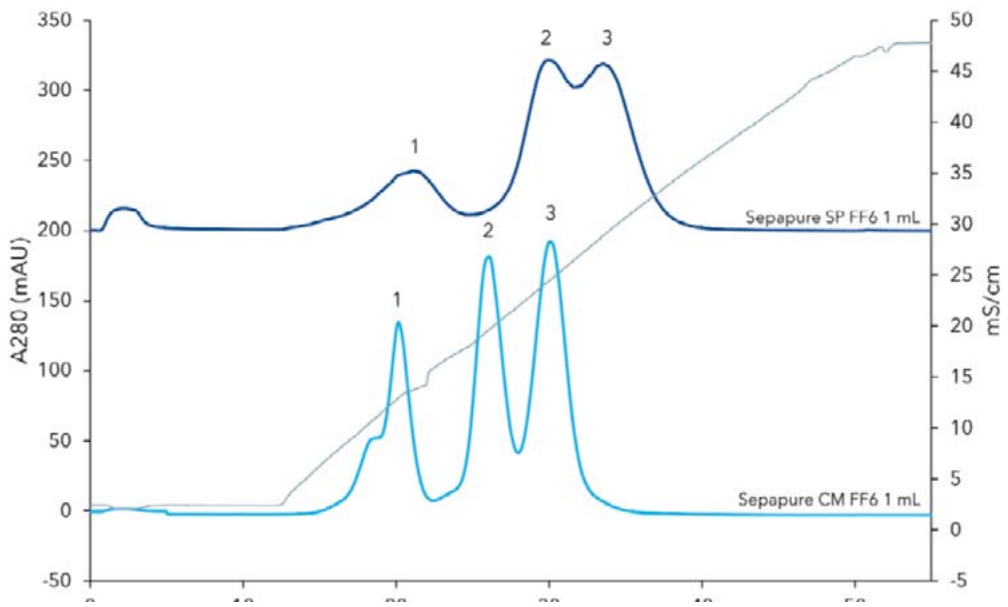
The agarose beads are functionalized with a quaternary ammonium for the strong anion exchanger (Q) and with diethylaminoethyl for the weak anion exchanger (DEAE). The strong cation exchanger is functionalized with sulphopropyl (SP) and the weak cation exchanger with carboxymethyl (CM).

The maximum operating pressure the Sepapure® columns should be used at is 3 bar, while the recommended flowrate is 1 CV/ml.

Available as prepacked 1 ml or 5 ml cartridge or as bulk material.

Recommended application areas:

Typically used in the intermediate step of an FPLC purification procedure.



Sepapure SP FF6 1ml
Article number:
010X15RSPZ

Sepapure CM FF6 1ml
Article number:
010X15QSPZ

1. Ribonuclease A
2. Cytochrome C
3. Lysozyme

Column / Resin type	Cartridge		Media			
	1 ml	5 ml	25 ml	100 ml	500 ml	1000 ml
Sepapure® DEAE FF	010X15ISPZ	020X15ISPZ	00IX15ISPZ	00KX15ISPZ	00PX15ISPZ	00QX15ISPZ
Sepapure® Q FF	010X15HSPZ	020X15HSPZ	00IX15HSPZ	00KX15HSPZ	00PX15HSPZ	00QX15HSPZ
Sepapure® CM FF	010X15QSPZ	020X15QSPZ	00IX15QSPZ	00KX15QSPZ	00PX15QSPZ	00QX15QSPZ
Sepapure® SP FF	010X15RSPZ	020X15RSPZ	00IX15RSPZ	00KX15RSPZ	00PX15RSPZ	00QX15RSPZ

Desalting Columns

FPLC media based on dextran beads with a mean diameter of 20–50 µm.

Properties:

Sepapure® Desalting media is designed to be used for removal of small molecules below the exclusion limit and for covenient rebuffering. It is long lasting when correctly handled and compatible with common CIP strategies.

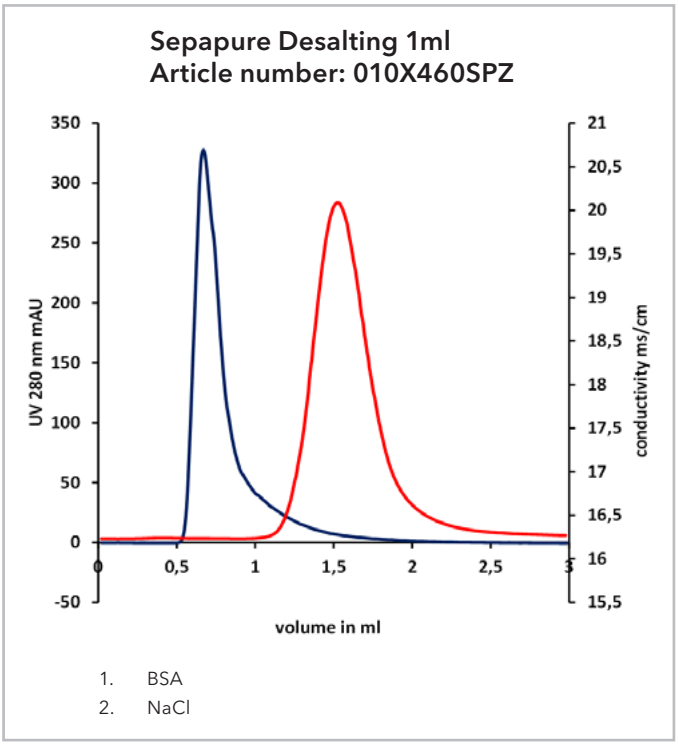
All Sepapure® media is stored in 20 % ethanol upon delivery. Available as prepacked 5 ml cartridge or as bulk material.

Technical data:

The Sepapure® dextran beads have an exclusion limit of 5 kDa. The maximum operating pressure of the Sepapure® columns is 3 bar, while the recommended flowrate is 1 CV/ml.

Recommended application areas:

Typically used in the final step of an FPLC purification procedure or inbetween steps for fast buffer exchange.



1. BSA
2. NaCl

Column type	Cartridge
	5 x 5 ml
Sepapure® Desalting	040X460SPZ