JNC CORPORATION

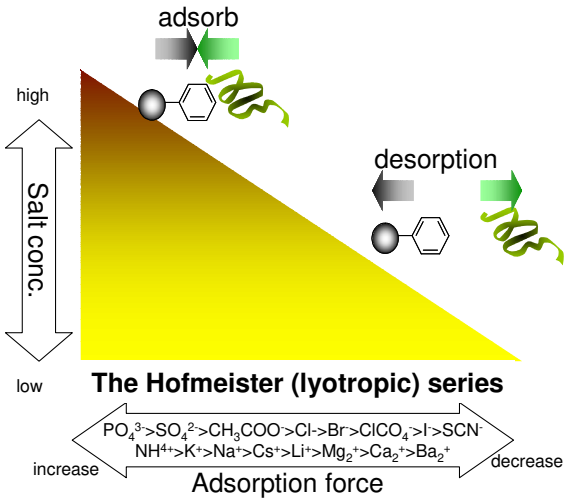
Operating Instructions

Mini-Column Cellufine Phenyl EX

Cellufine™

1. Description

Mini-column Cellufine Phenyl EX is pre-packed, easy to use columns. Cellufine Phenyl EX is designed for concentration and purification of macromolecules such as antibody aggregates, proteins, enzymes and polysaccharides. Cellufine Phenyl EX is spherical and rigid cellulose beads functionalized with phenyl group. Cellufine Phenyl EX is based on cross-linked cellulose particles.



Column

Cellufine Mini-columns are made of polypropylene tube and UHMW-PE frits. The columns can be connected to chromatography system with 10-32UNF thread for connection of 1/16 inch OD tubing.

Table 1. Mini-column Cellufine Phenyl EX characteristics

Column volumes	1 ml and 5 ml
Column dimensions (i.d. x L)	6.7 mm x 30 mm (1 ml) 14.6 mm x 30 mm (5 ml)
Ligand	Phenyl group
Binding capacity (BSA)	≥ 13 mg/ml
Particle diameter	ca. 90 µm
Bead structure	Cross-linked Cellulose
Pressure limit	0.4 MPa (4 bar)
Recommend flow rate	0.1 – 1.0 ml/min (1 ml) 0.1 – 5.0 ml/min (5 ml)
pH stability	3 – 12
Storage	Cool and dark place in 20% ethanol

2. Operating Guidelines

General Operation

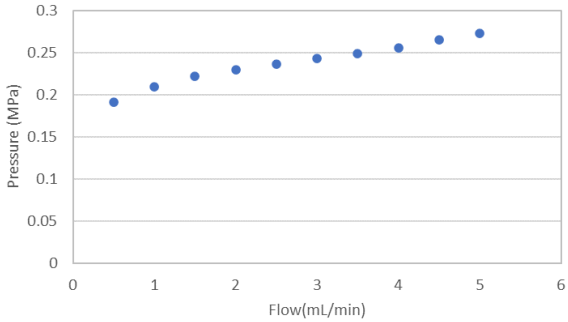
(1) Equilibrate column with adsorption buffer

(2) Load sample (preferably in adsorption buffer.)

(3) Wash with several bed volumes of adsorption buffer to remove non-binding contaminants.

(4) Elute bound solute(s) with desorption buffer

The flow property of Cellufine Phenyl EX was measured by AKTA system (Cytiva).



System: Akta avant 25

Flow Ristrictor FR-902:in line

Mobile phase: Water

Temperature:20-25 ℃

connection piping: ID 5mm x 20 cm

Recommended Buffers

Adsorption buffer:

1) When used as a general-purpose hydrophobic chromatography

Buffer containing ammonium sulfate of concentration with which a sample does not precipitate by salting-out is recommended. Phosphate, acetate and Tris, etc. buffer can be used. In general, adsorption strength varies proportionately with lyotropic ion concentration. Depending on the application, different lyotropic salts may be used. A slight decrease of salt concentration can aid to remove closely bound contaminants. Non-ionic detergents (Tween®20, Triton® X, etc.) may be also added to improve solubility.

2) When used for purification of antibody aggregates in flow-through mode

Phosphoric acid, acetic acid, tris, etc. can be used. Adjust the pH and conductivity according to the antibody solution to be loaded. Antibody aggregates can be adsorbed with an electrical conductivity of about 5-15 mS / cm by Cellufine Phenyl EX.

Elution buffer:

1) When used as a general-purpose hydrophobic chromatography

Elution occurs by decreasing of ammonium sulfate contained in adsorption buffer solution. The exact concentration can be determined by gradient elution. Step gradients are typically employed for preparative applications. If this is not sufficient, elution can be done by adding of chaotropic agents or ethylene glycol.

2) When used for purification of antibody aggregates in flow-through mode

For elution of impurities such as antibody aggregates adsorbed

on the column, use a buffer solution with conductivity of 5 mS / cm or less or pure water. For cleaning-in-place, pass 0.5 M NaOH, 30% isopropanol at 10 CV. A mixture of 0.5 M NaOH and 30% isopropanol may be used.

Sample Preparation:

Prepare samples at concentration of 1 to 20 mg/ml in adsorption buffer. Remove insoluble material by centrifugation or microfiltration. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography such as Cellufine GH-25.

3. **Purification procedure**

(1) Fill the pump tubing or syringe outlet with adsorption buffer. Remove the inlet plug (top of the column) and connect the column to the pump tubing, or syringe, “dripping the buffer”, avoiding of air introduction into the column.

(2) Remove the outlet plug (end of the column).

(3) Wash out the preservative (20% ethanol) and equilibrate the column with 10 column volumes of adsorption buffer.

(4) Apply the sample, using a syringe or by pumping it on the column.

(5) Wash with 5 to 10 column volumes of adsorption buffer.

(6) Elute with 5 to 10 column volumes of elution buffer (gradient elution or step-wise)

(7) Cleaning-in-place with a cleaning solution containing 30% isopropanol in 0.5 M NaOH. After washing, re-equilibrate with an adsorption buffer.

When the antibody is purified as flow-through mode, passage samples of (4) and (5) are collected.

4. **Regeneration and Depyrogenation**

For cleaning-in-place, pass 0.5 M NaOH, 30% isopropanol at 10 CV. A mixture of 0.5 M NaOH and 30% isopropanol may be used. After washing, wash with an adsorption buffer to prepare for the next operation.

5. **Scaling up**

Two or three of Mini-columns can be connected in series.

6. **Storage**

Wash the column with 5 to 10 column volumes 20% ethanol. Store the column in 20% ethanol at cool and dark place.
Note: To prevent leakage it is essential to ensure that the end plugs are tight.

7. Reference

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8. Further information

For further information, visit
http://www.jnc-corp.co.jp/fine/en/cellufine/

9. Ordering information

Product	Quantity	Product number
Mini-column Cellufine Phenyl EX, 1 ml	5 x 1 ml	22000-51
Mini-column Cellufine Phenyl EX, 5 ml	5 x 5 ml	22000-55

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