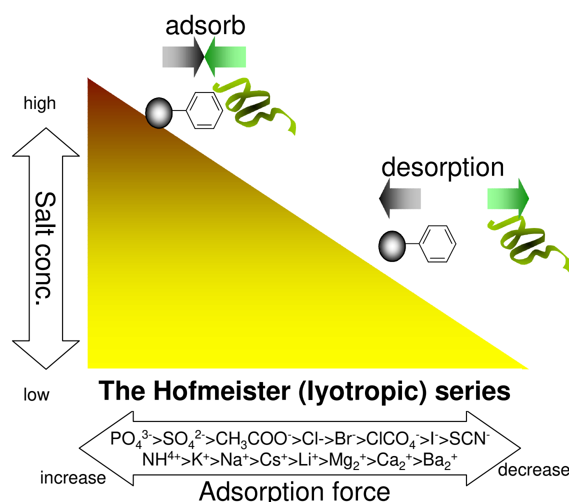
Operating Instructions

Mini-Column Cellufine MAX Butyl, MAX Phenyl, and MAX Phenyl LS

Cellufine™ MAX

1. Description

Mini-columns Cellufine MAX HIC (Butyl, Phenyl and Phenyl LS) are pre-packed, easy to use columns. Cellufine MAX HIC is designed for concentration and purification of large molecules such as proteins and enzymes. These mini-columns are packed with Cellufine MAX HIC media. Cellufine media are spherical and rigid cellulose beads functionalized with HIC groups. Especially, Cellufine MAX series are based on highly 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 HIC 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: Phenyl group Butyl: Butyl group
Binding capacity (BSA)	MAX Butyl: ≥9 mg/ml MAX Phenyl: ≥11 mg/ml MAX Phenyl LS (lowsub type): ≥4 mg/ml
Particle diameter	ca. 90 µm
Bead structure	Highly 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

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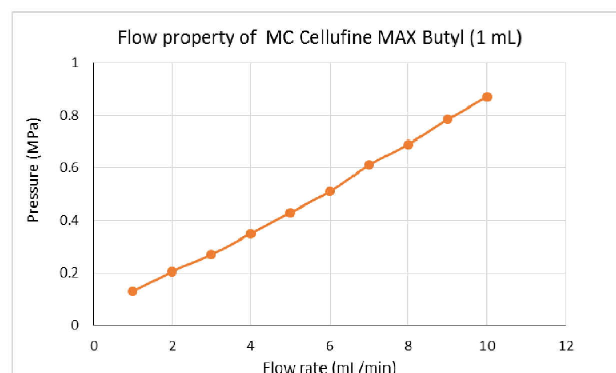
2. Operating Guidelines

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

Typical flow property of Cellufine MAX HIC (Cellufine MAX Butyl as example) measured by AKTA system (GE Healthcare).



Mobile phase: pure water (23 °C), Measured without using restrictor.

Recommended Buffers

Adsorption buffer: 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.

Elution buffer: 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.

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)

(Cleaning and sanitization)

If necessary, all resins are able to be used such as standard cleaning or sanitizing solution, NaOH (0.1 to 0.5 M), or 70% ethanol or non-ionic detergents or combinations, etc. After cleaning, the media should be re-equilibrated.

4. **Regeneration and Depyrogenation**

Cellufine HIC is typically regenerated and depyrogenated with water. If this is not sufficient, regenerate more aggressively with 3 to 10 column volumes of 0.1 N NaOH at 2 to 10 °C, then wash with water until pH drops to near neutral. Washing with 2 to 4 column volume of ethanol, acetone etc., can be also helpful. After wash the mini column with water and finally with starting buffer until equilibrated.

5. **Scaling up**

Two or three of Cellufine HIC 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/index.html

9. Ordering information

Product	Quantity	Product number
Mini-column Cellufine MAX Butyl, 1 ml	5 x 1 ml	21100-51
Mini-column Cellufine MAX Butyl, 5 ml	5 x 5 ml	21100-55
Mini-column Cellufine MAX Phenyl, 1 ml	5 x 1 ml	20700-51
Mini-column Cellufine MAX Phenyl, 5 ml	5 x 5 ml	20700-55
Mini-column Cellufine MAX Phenyl LS, 1 ml	5 x 1 ml	20800-51
Mini-column Cellufine MAX Phenyl LS, 5 ml	5 x 5 ml	20800-55
Cellufine MAX Butyl	100 ml	21100
Cellufine MAX Phenyl	100 ml	20700
Cellufine MAX Phenyl LS	100 ml	20800
Cellufine GH-25	100 ml	670 000 327
Mini-column Cellufine GH-25, 5 ml	5 x 5 ml	19711-55

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