

JNC CORPORATION

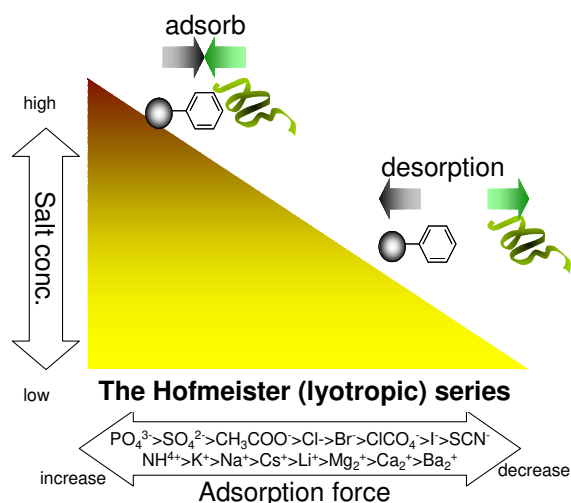
Operating Instructions

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



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

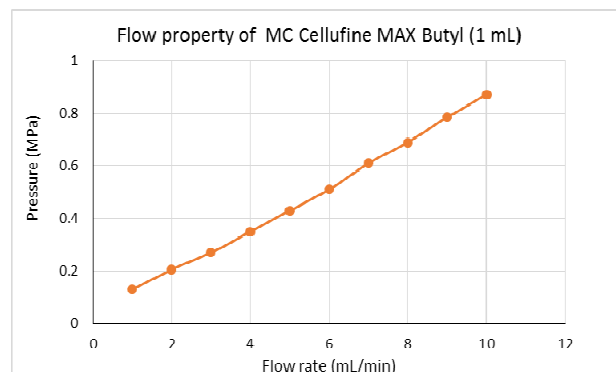
Column volumes	1 ml and 5 ml
Column dimensions (i.d. x L)	6.7 mm x 30 mm (1 ml) 14.6 mm x 30 mm (5 ml)
Ligand	Phenyl: Phenyl group Butyl: Butyl group
Binding capacity (BSA)	MAX Butyl: ≥ 9 mg/ml MAX Phenyl: ≥ 11 mg/ml MAX Phenyl LS (lowsub type): ≥ 4 mg/ml
Particle diameter	ca. 90 μm
Bead structure	Highly 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

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.

phospholipases A2 from the gills of the red sea bream, *Pagrus major*.
Iijima N, *et. al.*

J Biochem (Tokyo). (1998) 123(2) pp 219-25.

Purification and some characteristics of phosphatase of a
psychrophile. Tsuruta H, *et. al*

Protein Expr Purif. (1995) 6(5) pp 679-84.

Overexpression and purification of the trimetric aspartate
transcarbamoylase from *Bacillus subtilis*. Baker DP, *et. al.*

Biosci. Biotechnol., Biochem.(1993) 57(2) pp 177-80

Purification and characterization of *Actium lappa* L. (edible
burdock) polyphenol oxidase

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	5 x 5 ml	19711-55
Cellufine GH-25, 5 ml		

10. Contact information

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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

International Journal of Biochemistry & Cell Biology (2006) 38(4)
pp 521-532

"Primary structure and properties of Mn-superoxide dismutase from
scallop adductor muscle" Ikebuchi, Makoto; Takeuchi, *et. al.*

PDA Journal of GMP and Validation in Japan (2000) 2(1) pp 28-33

Purification of Recombinant Human Serum Albumin. Akinori SUMI,
et. al.

Lipids. (2000) 35(12) pp 1359-70.

Purification, characterization, and molecular cloning of group I

Cellufine™ is the trademark of JNC Corporation, Tokyo, Japan