JNC CORPORATION

Operating Instructions

Mini-Column Cellufine MAX AminoButyl and MAX IB

Cellufine™ MAX

1. Description

Mini-column Cellufine MAX AminoButyl and MAX IB are prepacked, easy to use columns for Cellufine Mixed mode chromatography (MIX). Cellufine MAX MIX is designed for concentration and purification of large molecules such as proteins, enzymes, polysaccharides, nucleic acids and virus or virus like particles. Especially Cellufine MAX IB is usually used as flow through mode. The Cellufine MAX MIX mini-columns are packed with Cellufine MAX mixed mode media. Cellufine media are spherical and rigid cellulose beads functionalized with both HIC and IEX 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 MIX 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	MAX AminoButyl: Butyl group (HIC) and primary amine group (anion IEX), N contents; 20 to 30 µmol/ml MAX IB: Polyallyl amine partially modified a butyl group, IEC; > 80 µequiv/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

Recommended Buffers

Adsorption buffer: Low ion strength (10 to 50 mM) buffer containing 10 mM to 50 mM NaCl is recommended. Phosphate, acetate or Tris, etc. can be used. Depending on the application, different buffer ions may be used. In general, adsorption strength varies inversely with pH and ionic strength. A slight Increase of ionic strength can aid in removing closely bound contaminants. In the case of using as HIC mode, high ionic strength buffer containing ammonium sulfate or NaCl is recommended. Phosphate, acetate, citrate and Tris, etc. can be available as buffer. 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: In general elution buffer containing more than 0.5 M NaCl or KCl. The exact concentration can be determined by gradient elution. Step gradients are typically employed for preparative applications. For VLPs purification with Cellufine MAX AminoButyl, detergent such as Triton is useful.

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 MIX 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. **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.

6. Further information

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

7. Ordering information

Product name	Quantity	Product number
Mini-column Cellufine MAX AminoButyl, 1 ml	5 x 1 ml	21500-51
Mini-column Cellufine MAX AminoButyl, 5 ml	1 x 5 ml	21500-15
Mini-column Cellufine MAX IB, 1 ml	5 x 1 ml	21600-51
Mini-column Cellufine MAX IB, 5 ml	1 x 5 ml	21600-15
Cellufine MAX AminoButyl	100 ml	21501
Cellufine MAX IB	100 ml	21602
Cellufine GH-25	100 ml	670 000 327
Mini-column Cellufine GH-25	5 x 5 ml	19711-55

8. Contact us

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This product can connect the tube and Cellufine Mini-column, which are generally used to chromatography systems, such as PEEK, Teflon, PP, etc.
Please read the instruction manual attached to this product before using it.