JNC CORPORATION

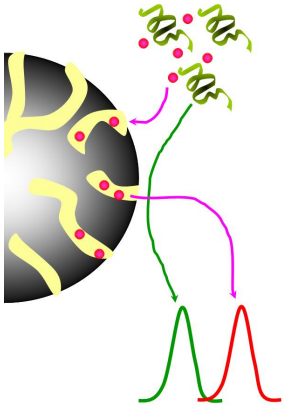
Operating Instructions

Mini-Column Cellufine GH-25

Cellufine™

1. Description

Mini-columns Cellufine GH-25 is prepacked, easy to use columns for Cellufine GH-25 gel filtration chromatography. Cellufine GH-25 is designed for remove under 3 kD molecular weight material such as alcohols, salts, detergents, fluorochromes, sugar, etc., from virtually any protein solution. The Cellufine GH-25 mini-columns are packed with Cellufine GH-25 media.



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 GH-25 characteristics

Column volumes	5 ml
Column dimensions (i.d. x L)	14.6 mm x 30 mm (5 ml)
Support matrix	Cellulose
Particle shape	Spherical
Particle diameter (μm)	ca 40 – 130
MW exclusion limit (kD)	3
pH stability range	1 – 14
Pressure limit	0.4 MPa (4 bar)
Recommend flow rate	0.1 – 5.0 ml/min
Storage	Cool and dark place in 20% EtOH

2. Operating Guidelines

General Operation

(1) Equilibrate column with 2 – 5 volumes of exchange buffer, or until the UV baseline has stabilized.

(2) Sample load (recommend sample volume is 1 ml)

(3) Elution with same as equilibrate buffer. (isocratic conditions)

(4) It measures in UV monitor or conductivity meter, and required fractions are collected.

Recommended Buffers

Common buffer solution can be used satisfactory.

Sample Preparation

Prepare samples at concentration of 1 – 20 mg/ml, in buffer. Remove insoluble material by centrifugation or microfiltration.

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” to avoid introducing air into the column.

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

(3) Wash out the preservative 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.

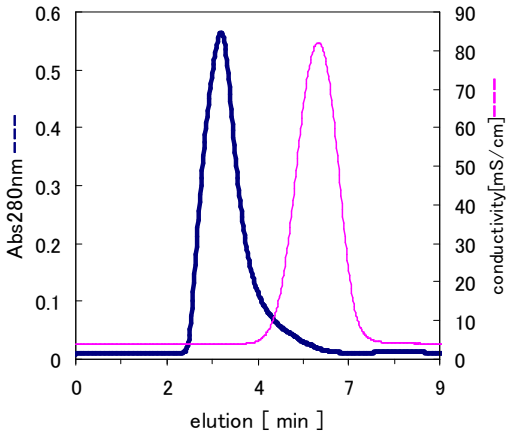
(6) Elute with 5 to 10 column volumes of elution buffer.

4. Regeneration and Depyrogenation

Cellufine GH-25 is typically regenerated and depyrogenated with high ionic strength (2.0 – 3.0 M) NaCl. If this is not sufficient, regenerate more aggressively with 3 – 10 column volumes of 0.1 M to 0.5 M NaOH at 2 – 10 °C, then wash with 2.0 – 3.0 M NaCl until pH drops to 7. Wash the column again with starting buffer until equilibrated.

5. Scaling up

Two or three of Cellufine GH-25 Mini-columns can be connected in series.



column: Mini-column Cellufine GH-25, 5mL
sample: Lysozyme 10mg/ml +0.5mol/L NaCl
injection : 1mL
buffer: 10mM phosphate buffer ,pH7.2
flow rate: 1mL/min

6. **Storage**

Wash the column with 5 to 10 column volumes of 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**

J Biochem Biophys Methods.2003, 56(1-3), pp69-78
Evaluation of Matrix Cellufine GH 25.
Vincent P, Compoint JP, Fitton V, Santarelli X.

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 GH-25,5 ml	5 x 5 ml	19711-55
Cellufine GH-25	100 ml	670 000 327

10. **Contact us**

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