Instructions Mini-column Cellufine® Phosphate (ver.2)

Mini-column Cellufine Phosphate , 1 ml and 5 ml.

1. Description

Mini-column Cellufine Phosphate is a prepacked, easy to use column for Cellfine Phosphate affinity chromatography. Cellufine Phosphate is an affinity medium designed for concentration, purification of proteins and, enzymes such as nucleic acid related proteins. Base of medium is spherical and rigid cellulose functionalized with Phosphate esters.

Column

Cellufine Mini-columns are made of polypropylene tube and polyethylene frits. The columns can be connected to syringe, peristaltic pump, or chromatography system with luer adaptors.

Table 1. Mini-column Cellufine Phosphate characteristics

Column volume	1ml and 5 ml
Column dimensions (i.d. x L)	9mm x 18 mm (1ml) 13mm x 44mm (5ml)
Ligand	Phosphate ester
ion exchange capacity	2 to 4 meq/ml
Binding capacity(Lyosozyme)	30 mg/ml
Particle diameter	20 to 120 μm
Bead matrix	Spherical Cellulose
Maximum back pressure	0.2 MPa
Maximum flow rate	10 ml/min
Recommend flow rate	5 ml/min
pH stability	3 to 12
Storage	+2 ºC to + 8ºC 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: 0.01 M sodium phosphate, 0.1 M NaCl, pH 7.5. Depending on the application, other buffer may be used. In general, adsorption strength varies inversely with pH and ionic strength. Increasing ionic strength slightly can aid in removing closely bound contaminants. Non-ionic detergents (Tween®20、Triton® X, etc.) may be also added to improve solubility.

Elution buffer: In general, use adsorption buffer containing 1 – 2 M NaCl or KCl. The exact concentration can be determined by gradient elution. Step gradients are typically employed for preparative applications.

Sample Preparation

Prepare samples at concentration of 1 – 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”, to avoid introducing air into the column.

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

(3) Wash out the preservative (20 % EtOH) 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 – 10 column volumes of adsorption buffer.

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

4. Regeneration and Depyrogenation

Cellufine Phosphate 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 0.05 – 0.15 N NaOH at 2 – 10°C, then wash with 2.0 – 3.0 M NaCl until pH drops below 9. Wash the column again with adsorption buffer until equilibrated.

5. Scaling up

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

6. Storage

Wash the column with 5 – 10 column volumes 20% ethanol. Store the column in 20% ethanol at +2ºC to +8 ºC.

Note: To prevent leakage it is essential to ensure that the end plugs are tight.

7. Reference

Nucleic Acids Research, 2006, Vol. 00, No. 00 1–8

Rachel Macmaster, Svetlana Sedelnikova, Patrick J. Baker, Edward L. Bolt1,Robert G. Lloyd1 and John B. Rafferty

RusA Holliday junction resolvase: DNA complexstructure—insights into selectivity and specificity

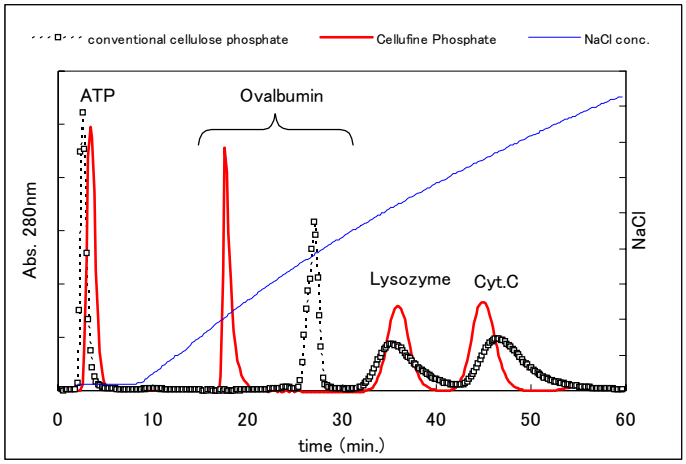

Fig.1 Separation of mixed sample

Column Size: ID 1.1 cm – Height 10 cm

Flow rate: 2 ml/min (126cm/h)

Buffer: 0.01M acetate buffer, pH4.8

Elution: 0 to 1 mol/L NaCl gradient

8. **Further information**

For further information, visit

http://www.chisso.co.jp/fine/en/cellufine/index.html

9. **Ordering information**

Product	Quantity	Product Number
Mini-column Cellufine Phosphate, 1 mL	5 x 1 ml	19551
Mini-column Cellufine Phosphate, 5 ml	1 x 5 ml	19515
Cellufine Phosphate	50 ml	19545
Cellufine GH-25	100 ml	670 000 327
Mini-Column Cellufine GH-25	5 x 5ml	19711-55

10. **Contact us**

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Appendix : Column connection

Cellufine Mini-column has luer adaptors.
You can connect up soft tube and rigid 1/16”(inch) tube with luer fittings.
The 1/16” tube is used by many chromatography systems.
It is possible to connect Cellufine Mini-column to a chromatography system using the Lure Tight™ Fittings.

1. For soft tube “Soft tube Fittings”

Picture 1. The example of connection of a flexible tube

(a) Connect tube with male luer

Fig.1 Male luer

(b) Feed buffer and purge air in the tube.
(c) Connect male l
(d) Take off plug
(e) Connect femal

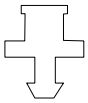

Fig.2 Fema

xample of connection of a rigid

(f) Connect tube v

2. For 1/16” tube “Lur

We have employed the I
UPCHURCH SCIENTIF
This product can connec
column, which are gene
systems, such as PEEK,
Please read the instructio
product before using it.

Picture 3. Syringe is directly connectable with Cellufine Mini-column.

Luer Tight™ Fittings is UPCHURCH SCIENTIFIC product.