# JNC CORPORATION

Operating Instructions

Mini-column Cellufine Phosphate

Cellufine™

## 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 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 Phosphate characteristics

| | |
|---|---|
| Column volume | 1 ml and 5 ml |
| Column dimensions (i.d. x L) | 6.7 mm x 30 mm (1 ml)<br>14.6 mm x 30 mm (5 ml) |
| Ligand | Phosphate ester |
| Ion exchange capacity | 0.3 – 0.8 meq/ ml |
| Binding capacity (Lysozyme) | 20 mg/ml |
| Particle diameter | 40 to 120 µm |
| Bead matrix | Spherical Cellulose |
| Pressure limit | 0.4 MPa (4 bar) |
| Recommend flow rate | 0.1 – 1.0 ml/min (1 ml)<br>0.1 – 5.0 ml/min (5 ml) |
| pH stability | 5 – 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:** 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 to 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 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

(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 Phosphate is typically regenerated and depyrogenated with high ionic strength (2.0 to 3.0 M) NaCl. If this is not sufficient, regenerate more aggressively with 3 to 10 column volumes of 0.05 to 0.15 N NaOH at 2 to 10 °C, then wash with 2.0 to 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 Phosphate 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

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 complex structure—insights into selectivity and specificity

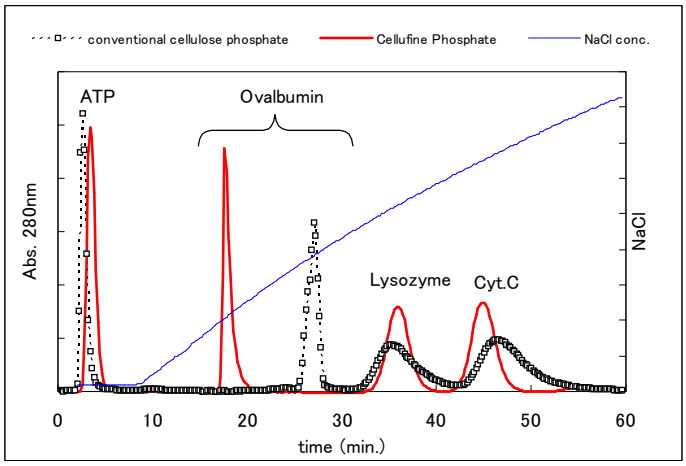


Fig. 1 Separation of mixed sample

Column Size: ID 1.1 cm – Height 10 cm

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

Buffer: 0.01 M acetate buffer, pH4.8

Elution: 0 to 1 mol/l NaCl gradient

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 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 5 ml | 19711-55 |

10. **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.