Cellufine™ Phosphate HC

Cellufine Phosphate HC is an affinity chromatography media used to purify nucleic acid-related proteins such as protein kinases, restriction enzymes, nucleases, and polymerases. Its spherical cellulose particles are modified with phosphate ester groups as ligands. Compared to conventional Cellufine Phosphate, the pore size has been controlled to improve the adsorption capacity of large molecular weight proteins. This next-generation chromatography media is particularly effective for purifying high molecular weight enzymes such as T7 RNA polymerase.

Table 1 Characteristics of Cellufine Phosphate HC

	Cellufine Phosphate	Cellufine Phosphate HC
Ligand	Phosphate ester	Phosphate ester
Base resin	Spherical cellulose particle	Spherical cellulose particle
Particle size (μm)	40 - 130	40 - 130
MW exclusion limit (KDa)	30～40	150
Ion exchange capacity (meq/mL-gel)	0.3 - 0.8	0.2 - 0.8
Lysozyme adsorption (mg/mL-gel)	140	-
BSA adsorption (mg/mL-gel)	-	100
Operating pressure (MPa)	＜0.2	＜0.2
pH stability range	5 - 12	5 - 12
Storage	2-8 ºC in 20 % ethanol	2-8 ºC in 20 % ethanol

*The values in Table 1 do not indicate specifications.

Column Packing

Materials

- Cellufine resin
- Lab scale column, adapter, reservoir
- Pump
- Filtration equipment (Glass filter, Buchner-Roth, aspirator, etc.)
- Graduated cylinder
- Packing solution (water, NaCl solution※, buffer※)

・Mobile phase of packed column evaluation (water, NaCl solution※, buffer※)
・Sample of packed column evaluation (1-2 %(v/v)acetone or 1M NaCl)

※NaCl solution：low salt concentration solution such as 0.1M NaCl solution
※buffer：Adsorption buffer , etc.

Preparation of slurry

1) After the bottle is warmed to room temperature, shake it several times to equalize the slurry in the bottle.
2) Aspirate through a glass filter and wash 3 times with 5 times the volume of the packing solution. Remove 20% ethanol as a preservative. If necessary, the slurry may be washed by decantation.
3) Transfer the resin to a beaker and add packing solution to make a 50-60%(v/v) slurry. Suspend the resin and remove the air under decompression for 30-40 minutes. Slow stirring with a magnetic stirrer can effectively remove the air.
4) Pour the slurry into a graduated cylinder and leave it to stand for at least 4 hours. Measure bed height (volume) of a gravity settled bed and calculate the slurry concentration.
5) Adjust to a 50 % (v/v) slurry concentration of resin with packing solution.
6) Calculate the volume of slurry required to pack the column.

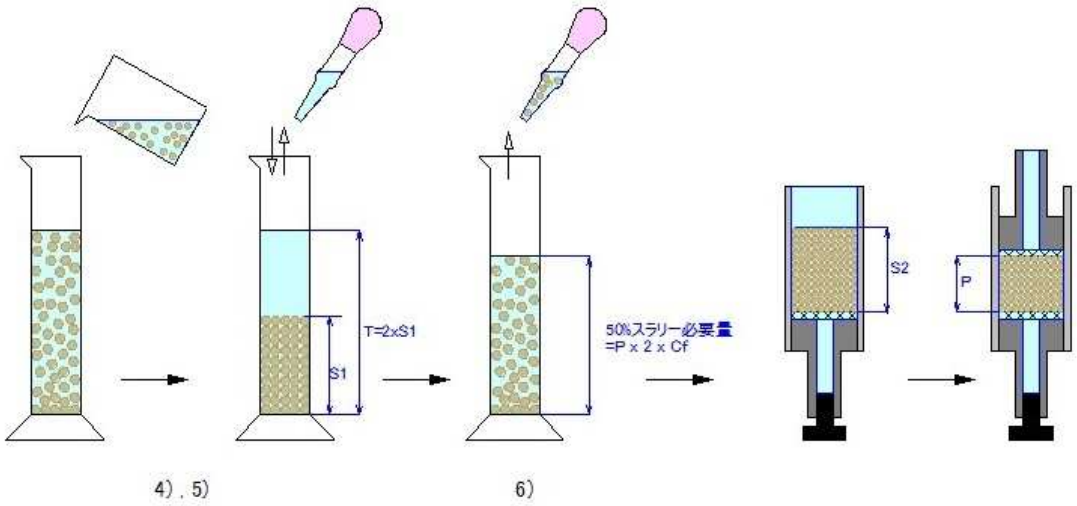

Figure1 Preparation of slurry

Slurry concentration (%)
= Gravity settled bed volume (S1) / Total slurry volume (T)×100

50% slurry volume required to packing =
(Target packing volume (P) x 2） x Cf

※Cf = [gravity settled bed volume (S2) / Target packing volume (P)]

Note: *Compression factor (Cf) affects the column packing efficiency (see Appendix 1 for details) and may need to be optimized by adjusting the axial compression on the column.*

Column packing

1) Set up the column. After opening the column outlet, add packing solution to remove air remaining in the filter. Leave about 1 cm of packing solution from the bottom of the column to prevent air from entering.
2) Close the column outlet and pour the slurry into the column all at once to keep air out.
3) Open the column outlet to allow the resin to settle. As the resin settles, the liquid surface becomes clear. Close the outlet when the packing solution becomes clear to 2-3 cm from the liquid surface.
4) Carefully fill the column to the top with packing solution. Keep the settling resin from floating away.
5) Set the adapter on the column to prevent air from entering the liquid surface. Close the adapter O-ring, lower the adapter, and remove the air inside.
6) Connect the column to the pump and keep the packing solution flowing at a pressure lower than the operating pressure for 30 to 60 minutes. (＜0.2MPa)

Column size（ Diameter × Bed height）	Recommended Cf (approximately)
5.0 cm× 21.7 cm	1.30

Note: *The flow velocity ： Internal pressure at packing ＞ Operating pressure after packing*

7) After the resin height is stabilized, stop the flow. Close the column outlet. Disconnect the inlet piping at the top of the column. Slowly lower the adapter to the surface of the resin. At this time, the packing solution in the column flows out from the column inlet.
8) Connect to the adapter inlet with the piping filled with packing solution to exclude air.

Open the column outlet and pump the packing solution. (＜0.2MPa)

9) If the resin compresses and makes space between it and the adapter, lower the adapter to the surface of the resin.

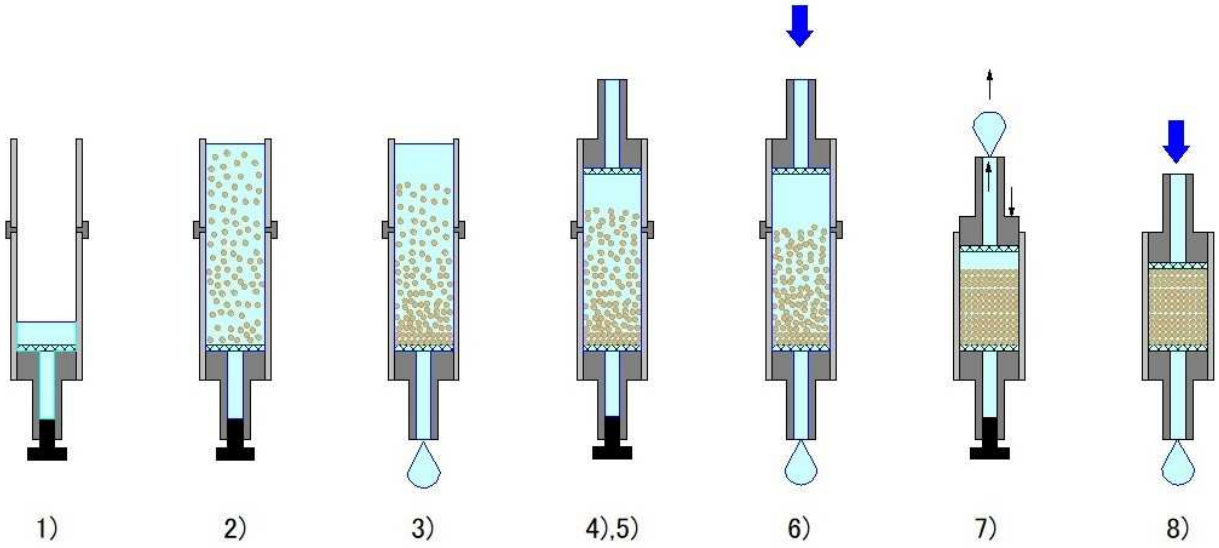

Figure 2 Process of column packing

10) Calculate column volume from column height. If the column volume is larger than the target column volume, lower the adapter to the target height. If the column volume is lower than the target column volume, the slurry concentration is low, or the resin may be excessively compressed. Remove the resin from the column and pack again.

Evaluation of column packing

Column packing efficiency is evaluated by checking HETP and asymmetry (As). (Appendix 1)

Operating Guidelines

How to use

1) Equilibrate the column with adsorption buffer. (3-5 column volume (CV))
2) Load the column with sample dissolved in adsorption buffer. (1-20mg/mL)
3) Wash with adsorption buffer to remove impurities. (2-3 CV)
4) Elute the adsorbed target substance with elution buffer.

Recommended Buffers

Adsorption buffer : 10mM to 50mM sodium phosphate, neutral pH. Depending on the application, other buffer ions such as tris, acetate, may be used. In general, adsorption

strength varies inversely with pH and ionic strength. Increasing ionic strength slightly can aid in removing loosely bound contaminants. Non-ionic detergents (Tween®20、Triton® X, etc.) may also added to improve solubility.

Elution buffer：In general use mobile phase consisting of 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 and Load

Prepare samples at a 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.

Flow Rate

The recommended linear velocity range for Cellufine Phosphate media is 50 – 250 cm/h.

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 0.2 N NaOH at 2 – 10°C, then wash with 2.0 – 3.0 M NaCl until pH drops below 9. Wash gel again with starting buffer until equilibrated.

Stability

pH range of 5 to 12 and operating temperature of 2 to 30°C are recommended.

Storage

Store unused resin in its container at a temperature of 2 to 8°C. Equilibrate opened resin and packed column in 20% ethanol and store at 2 to 8°C. Can be stored in buffer containing 1M NaCl in bulk and column conditions for up to 2 weeks. For long-term storage, add a preservative such as 20 % Ethanol. Do not freeze. Shelf Lifetime is 5 years from manufacture.

References

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

Product Ordering Information (Catalogue No.)

	Pack Size	Catalogue No.
Cellufine Phosphate	1 mL x 5 （Mini-Column）	19400-15
	5 mL x 1 （Mini-Column）	19400-51
	10 mL	19400
	50 mL	19401
	500 mL	19402
	5 L	19403
	10 L	19405

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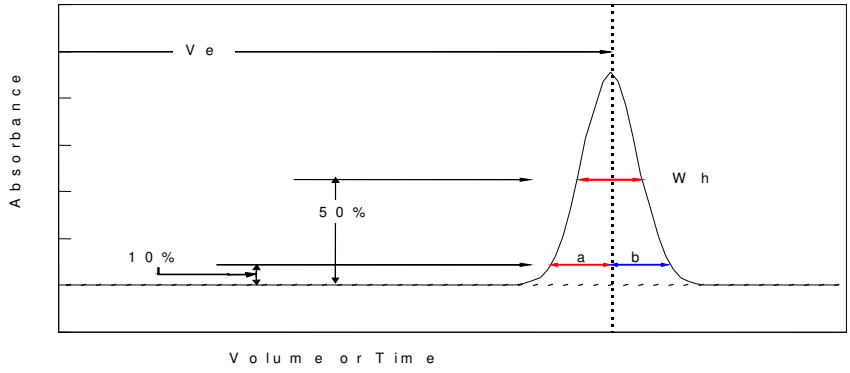
Appendix 1: Evaluation of column packing

The packing condition of the column is evaluated using indices such as theoretical number of stage plates (N), theoretical stage equivalent height (HETP), and asymmetry (As). These indices are affected by the measurement conditions. For example, these indices vary with column diameter/height differences, piping, solvent sample volume, flow rate, temperature, etc. Therefore, it is necessary to use the same measurement conditions each time. A flow velocity of 30 cm/h is recommended, but higher velocities are possible. However, the higher the flow velocity, the smaller the theoretical number of plates (N). The same conditions (flow rate, column size, mobile phase, and sample) must be used each time to evaluate the column.

Parameter	Condition
Sample volume	1 -2.5% of column volume (CV)
Sample concentration	1-2 %(v/v) acetone (mobile phase：water or adsorption buffer)
	1M NaCl （mobile phase： 0.1～0.4M NaCl aq）
Flow rate (cm/h)	30 cm/h
Detector	UV, Conductivity

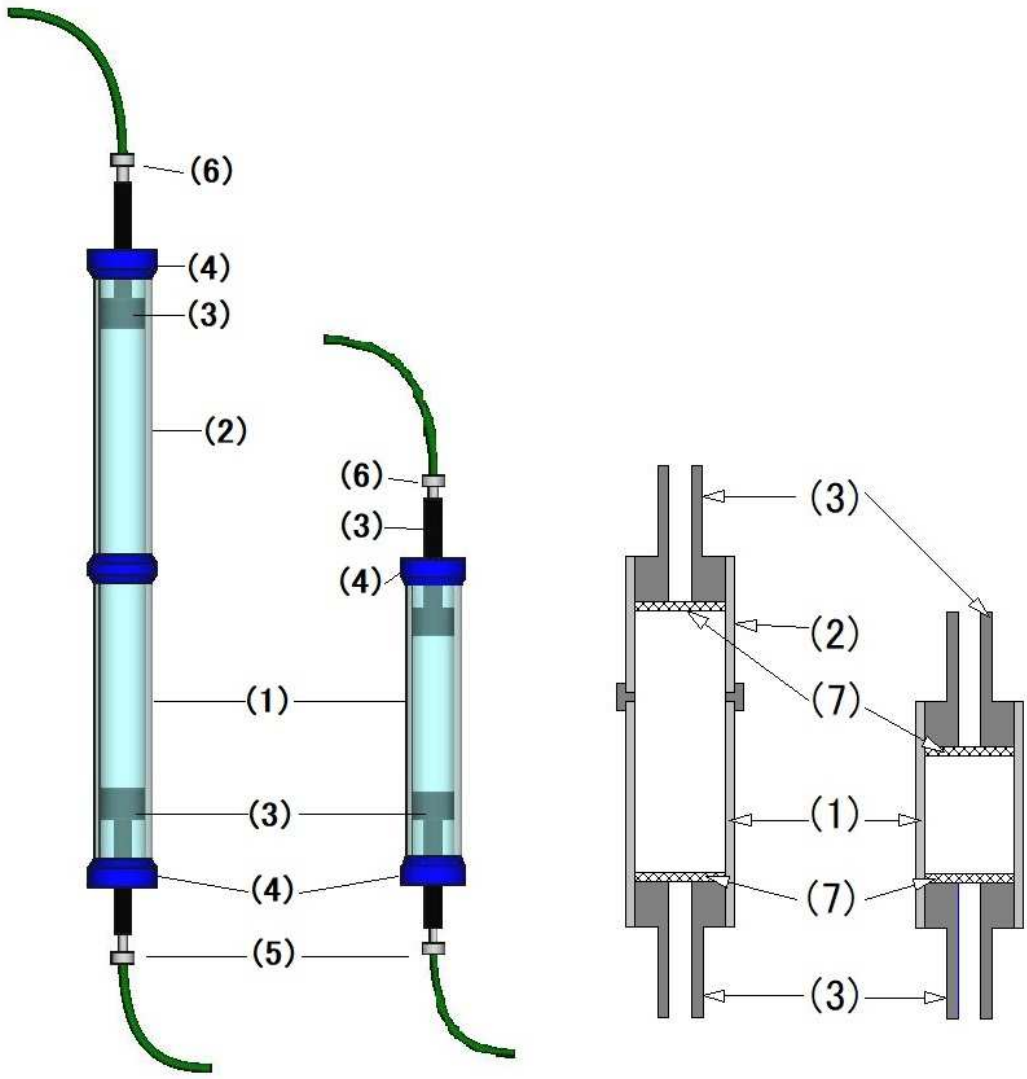


L	Column length [cm or m]
V_e	Elution time or volume
W_h	Half of width of peak
a, b	Peak width of 10% peak hight (a) front (b) rear
Note	Ve,Wh and a, b should have same dimensional units

HETP = L/N N = 5.54 x $(Ve/Wh)^2$ As = b/a

Generally, number of (theoretical) plates (N) is good if it is over 3000. Acceptable asymmetry factor (As) values range from 0.7-1.5.

Appendix 2 : Figure of columns



The Operating Instructions use a simple cross-sectional figure of the column, as shown on the right.

(1)	Column tube	(4)	Column end
(2)	Reservoir	(5)	Column outlet
(3)	Adapter	(6)	Column inlet
(7)	Filter		

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