Affinity Chromatography Media

Cellufine® Phosphate

Description

Cellufine Phosphate an affinity chromatography media designed for the concentration, purification protein kinase, nucleic acid related proteins such as restriction enzymes, nuclease, polymerase. This media is based on spherical cellulose beads functionalized with a phosphate ester.

Compared with the conventional product, Cellufine Phosphate has resistance to pressure and use in a large-sized column with high flow late is also possible. This is because Cellufine Phosphate is a spherical particle, but conventional product is a fiber.

Physical-Chemical Characteristics

Support matrix	cellulose
Particle shape	Spherical & grain
Ion exchange capacity (meq /ml)	0.3 - 0.8
Lysozyme capacity (mg/ml)	≥ 20
MW exclusion limit (kD)	100 (PEG)
pH operating range	5 - 12
pH stability range	5 - 12
Operating pressure	< 2 bar (29 psi)
Supplied	suspension in 20 % EtOH

Column Packing

1. Calculate volume required for the desired bed dimension.
2. Prepare 40 – 60 % (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer. Allow the gel to equilibrate at ambient temperature for one hour.
3. Gently stir or place under vacuum to degas.
4. With column outlet closed, carefully pour the slurry into column. Depending on the volume, a filler tube may be necessary.

5. With the inlet open to release air, insert and affix the top adjuster assembly at the slurry interface.
6. Open the column outlet and begin pumping elution buffer at rate 10 % – 20 % greater than the operational flow rate.
7. After the bed stabilizes, close the column outlet. Then with the inlet open, reposition the end cell on top of the bed. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

Operating Guidelines

General Operation

1. Equilibrate column with adsorption buffer.
2. Load sample.
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: 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 is 50 – 250 cm/h.

Chemical and Physical Stability

pH 5 – 12, when operated at temperatures between 2 – 30 °C.

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.

Storage

Short term (2 weeks or less), bulk and column can be stored in 1 M NaCl in neutral buffer at 2 – 4°C. Longer term storage can be conducted under identical conditions; however, a preservative (e.g. 0.1 % formalin, 0.05 % chloreton or 0.02 % sodium azide) should be added to the buffer. Store at 2 – 8 °C. Do not freeze.

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.)

Media type	Pack Size						
	Mini-Column		10ml	50ml	500ml	5L	10L
	1ml x 5	5ml x 1					
Cellufine Phosphate	19551	19515	19524	19545	19546	684 987 330	684 987 335

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provide such information which is provided without charge at the Buyer's risk and which **is provided subject to the**

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12. **Governing Law** - This contract shall be governed by and construed in accordance with the laws (other than those relating to conflict of laws questions) of the Commonwealth of Japan.

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