

**Affinity Chromatography Media**  
**Cellufine<sup>®</sup> 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 rate 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/g-dry)	2 to 4
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 a 40 – 60 % (v/v) slurry with the appropriate elution buffer (high salt). 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 be 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 3 – 12, when operated at temperatures between 2 – 30°C.

Autoclavable in suspension at neutral pH for 30 minutes at 121°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 % chloroxon 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. Bolt<sup>1</sup>, Robert G. Lloyd<sup>1</sup> and John B. Rafferty

RusA Holliday junction resolvase: DNA complex structure—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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11. **Modifications, Waiver, Termination** - This contract may be modified and any breach hereunder may be waived only by a writing signed by the party against whom enforcement thereof is sought.

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.

13. **Arbitration** - Any and all disputes or controversies arising under, out of or in connection with this contract or the sale or performance of the Products shall be resolved by final and binding arbitration in Tokyo under the rules of the Japan Arbitration Association then obtaining. The arbitrators shall have no power to add to, subtract from or modify any of the terms or conditions of this contract. Any award rendered in such arbitration may be enforced by either party in either the courts of the Commonwealth of Japan District Court for the District of Japan, to whose jurisdiction for such purposes JNC Corporation and Buyer each hereby irrevocably consents and submits.