

Operating Instructions

Affinity Chromatography Media Cellufine[®] Sulfate

Description

Cellufine Sulfate is an affinity chromatography media designed for the concentration, purification and depyrogenation of virus, viral coat and microbial antigens and specific proteins. This media is based on spherical cellulose beads functionalized with a low concentration of sulfate esters. The low density of sulfate groups give the gel unique chromatographic selectivity that, in some cases, is similar to immobilized heparin or sulfated dextran.

Due to Cellufine Sulfate's low exclusion limit of 3 kD, large molecules adsorb primarily on the packing's exterior, resulting in rapid adsorption and desorption times. Its superior rigidity allows high flow rates, and thus, rapid processing times. Because pyrogens have no affinity for Cellufine Sulfate, the gel can typically be depyrogenated with several column volumes of purified and depyrogenated, simultaneously.

Physical-Chemical Characteristics

| | |
|-------------------------------|-------------------------|
| Support matrix | cellulose |
| Particle shape | spherical |
| Particle diameter (µm) | ca 40 – 130 |
| Sulfur content (µg/g dry gel) | ≥ 700 |
| Lysozyme capacity (mg/ml) | ≥ 3 |
| MW exclusion limit (kD) | 3 |
| 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 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 at pH 7.2 \pm 0.2.
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 ions 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 Sulfate is 50 – 250 cm/h (Buffer) and 30 – 60 cm/h (Alkaline for cleaning)

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 Sulfate 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 gel again with starting buffer until equilibrated.

Storage

Store unopened container at ambient temperature. Do not freeze.

Short term (2 weeks or less), bulk and column can be stored in 1 M NaCl in neutral buffer at 2 – 8°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.

Shelf Lifetime:

5 years from date of manufacture

References

1. Janson, J. C. and Ryden, L., *Protein Purification: Principles, High Resolution Methods and Applications*. 2nd ed. New York; John Wiley & Sons, Inc., 1998.
2. O'Neil, P.F. and Balkovic, E.S., *Bio/technology* 11 (1993): 73.

Product Ordering Information (Catalogue No.)

| Media type | Pack Size | | | | | |
|-------------------|-----------|-------------|-------|-------|-------|-------|
| | 5 x 1ml | 10ml | 50ml | 500ml | 5 lt | 10 lt |
| Cellufine Sulfate | 19845-51 | 676 943 324 | 19845 | 19846 | 19847 | 19849 |

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disclaimers set forth in paragraph 5 above.

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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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