Cellufine™ Mini-Columns

Pre-packed Mini-columns of Cellufine™ SPA-HC resin are easy to use for rapid screening of rProtein A antibody affinity applications.

Cellufine SPA-HC resins are designed for capture of antibodies from cell culture material. Cellufine media are based on spherical and rigid cellulose beads functionalized with a novel alkali stable rProtein A affinity ligand. Mini-column characteristics are summarized in Table 1.

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, Characteristics of Cellufine Mini-columns

Column volumes	1 mL and 5 mL
Dimensions	6.7 mmID x 3.0 cmL (1.0 ml) 14.6 mmID x 3.0 cmL (5.0 ml)
Particle diameter	Average 70 µm
Matrix structure	Highly cross-linked cellulose
Pressure limit	0.4 MPa (4 bar)
Recommend flow rate	0.1-1.0 mL/min (1 mL) 0.1-5.0 mL/min (5 mL)
pH stability	3 to 12
Storage (Long term)	20%(V/V) ethanol at 2-8oC

Operating Guidelines

General Operation

1. Equilibrate column with adsorption buffer
2. Sample load (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, usually low pH

Recommended Buffers

Adsorption buffer: Low ion strength (10 mM to 50 mM) buffer containing 150mM NaCl is recommended. Phosphate, acetate or Tris, etc. can be used. Depending on the application, different buffer ions may be used. In general, adsorption strength varies inversely with pH and ionic strength. A slight Increase of ionic strength can aid in removing closely bound contaminants.

Elution buffer: Recommended elution buffers is 60 mM Acetic Acid pH 3.0. If the antibody is acid labile the buffer pH can be raised to pH 3.5. Other acidic buffers such as, 0.1M Glycine pH 3.0 or 0.1 M Citric acid pH 3.5 can also be used.

Sample Preparation

Prepare cell culture samples by adjusting the pH to 7.5 and adding NaCl if necessary to raise the ionic strength to 12 mS/cm. Remove insoluble material by centrifugation or microfiltration. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography such as Cellufine™ GH-25.

Purification Procedure

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 avoiding introduction of 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.

Recommended Polishing Steps after rProtein A Capture

After Protein A capture the following contaminants need to be removed in the subsequent “polishing” steps; a) host cell protein (HCP), b) leached Protein A ligand, c) residual dsDNA and d) mAb aggregates > 300 kDa molecular weight. In some cases, removal of mAb dimers is also required. Cellufine resins are available pre-packed in the 1 and 5 mL mini-column format to remove these contaminants and are listed below. More details on these resins can be found on the web site at http://www.jnc-corp.co.jp/fine/en/cellufine/

Cellufin MAX IB, a new mixed mode primary amine based hydrophobic modified resin for flow through removal of HCP, leached Protein A and mAb aggregates.

- Adjust to pH 7.0 with Tris base,
- Tolerant of a wide conductivity range up to 0.2 M NaCl.

Cellufine MAX GS, a dextran coated cation exchange resin with high binding capacity for bind and elute removal of dimers and aggregated mAb's.

- Adjust to pH 4.5 with Tris base and lower conductivity < 5 mS/cm.

Cellufine MAX Q-h, a high capacity anion exchange resin that can be used to remove HCP, leached Protein A and residual dsDNA in a flow through format.

- Adjust to pH 8.5 with Tris base and conductivity ~12 mS/cm.

Regeneration and Depyrogenation

Cellufine SPA-HC is typically regenerated with 0.1M Acetic acid after initial elution. If necessary, the column can be "cleaned in place" (CIP) with 0.1M NaOH (static soak for 30 min followed by up flow to displace any material on the head of the column. After CIP of the column with 10 CV follow with 5 CV of the equilibration buffer or 5 CV of 20 % Ethanol for storage.

Scaling up

Two or three Cellufine™ SPA-HC Mini-columns can be connected in series.

Storage

Wash the column with 5 – 10 column volumes of 20 % (v/v) ethanol. Store the column in 20% ethanol at 2-8 ℃. **Note**: To prevent leakage it is essential to ensure that the end plugs are tight.

Further information

For further information, visit
http://www.jnc-corp.co.jp/fine/en/cellufine/index.html

Description	Quantity	Catalogue No.
Cellufine SPA-HC	5 x 1 mL mini column	21900-51
	1 x 5 mL mini column	21900-15

Purchase/Technical Support

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