Operating Instructions

Hydrophobic Interaction Chromatography Media

Cellufine Phenyl EX

Description

Cellufine Phenyl EX is useful for the chromatography of hydrophobic proteins. Many proteins have hydrophobic amino acid residues which will interact with the phenyl functional groups. Factors that influence this hydrophobic interaction include salt concentration, temperature, pH, organic solvents, and surfactants. Protein adsorption usually occurs at high ionic strength, while elution occurs at lower salt concentrations. This is the opposite and complementary to ion exchange chromatography. This is the opposite of ion exchange chromatography and offers complementary separation benefits.

In addition, Cellufine Phenyl EX can be suitably used for removing aggregates of monoclonal antibodies. With a low electrical conductivity of a 6 mS / cm, Cellufine Phenyl EX exhibits excellent performance in removing aggregates generated in the production of monoclonal antibodies.

Physical-Chemical Characteristics

	Cellufine Phenyl EX	(Reference) Cellufine MAX Phenyl	(Reference) Cellufine MAX Phenyl LS
Support matrix	Cross-linked cellulose	Highly cross-linked cellulose	Highly cross-linked cellulose
Particle shape	Spherical	Spherical	Spherical
Particle diameter [μm]	ca. 40 – 130	ca. 40 – 130	ca. 40 – 130
Ligand type	Phenyl	Phenyl	Phenyl
BSA capacity [mg/ml]	13	11	4
BSA elution efficiency [%]	30	40	90
pH stability range	2 – 13	2 – 13	2 – 13
Operating pressure	< 0.2 MPa	< 0.3 MPa	< 0.3 MPa
Supplied	Suspension in 20 % EtOH	Suspension in 20 % EtOH	Suspension in 20 % EtOH

Flow pressure property

Cellufine Phenyl EX have superior flow-pressure properties because of its cross linking in the resin.

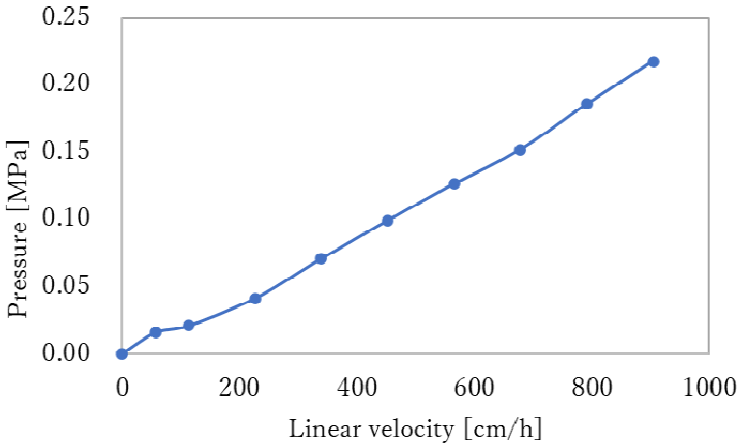

Fig.1 Flow property of Cellufine Phenyl EX
Column: 2.6cm Diam. x 19.3 cm L
Mobile phase: water, 23 – 25 °C
Compression factor: 1.35
The pressure applied to the resin excluding the system pressure is described.

Retention of model proteins

The figure bellow shows separation of proteins with Cellufine Phenyl EX. Cellufine Phenyl EX is a resin designed with a high ligand concentration. Therefore, these model proteins are eluted at a low salt concentration.

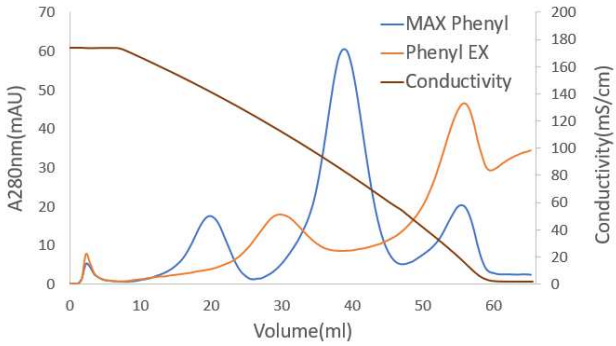

Fig.2 Separation property of model proteins
Column: 6.6 mm Diam. × 50 mm L
Buffer A: 10 mM Phosphate buffer, pH 7
Buffer B: 10 mM Phosphate buffer, pH 7 +1.5 M Ammonium sulphate
Proteins: Ribonuclease A, Lysozyme, α-Chymotripsinogen A

Column Packing

1. Calculate volume required for the desired bed dimension.
   (a) Packed bed volume= column cross sectional area (cm^2) x bed height (cm)
   (b) Required sedimented gel volume=Packed bed volume x (from 1.30 to 1.35)
2. Wash the gel with water or the appropriate buffer.
3. Prepare 40– 60 % (v/v) slurry with water or 0.1M NaCl or appropriate buffer.
4. Gently stir. If required place under vacuum to degas in an hour.
5. Column
   (a) A column is prepared according to the instructions from the column supplier.

(b) The bed support wetted in a packing solution or 20 % ethanol before use to remove air.

(c) Pour the packing solution into the column tube and it check that solution flows out from the column exit. Shut the exit valve when approximately 0.5 to 1cm high of the solution remains.

6. Carefully pour the slurry into the column and packing connector without creating air bubbles. Depending on the volume, a filler tube may be necessary.
7. Mount the top adapter on the top of packing connector.
8. Open the column outlet and begin pumping elution buffer for 10 min. Caution: do not excess the operation pressure limit for the selected column.
9. Mark the column height when the bed is compressing with mobile phase. Stop the pump and shut the column outlet valve.
10. Disconnect the top adapter line from the pump. After opening the inlet of the top adapter, lower the top adapter to the marked position to compress the resin. If the column height is set in advance, fill the column with 1.30-1.35 times the sedimentation volume and compress it to a predetermined height.
11. Mount the top adapter prevent air bubbles from entering, lock the adapter and reconnect the line from the pump.
12. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

Packing to the fixed length column

1. Calculate volume required of the desired bed dimension.
   (a) Packed bed volume= column cross sectional area (cm^2) x bed height (cm)
   (b) Required sedimented gel volume=Packed bed volume x (from 1.3 to 1.35)
   Note: When using a packing connector, the amount of excess gel corresponds to the required sedimentary gel volume.
2. Wash the gel with water or the appropriate buffer.
3. Prepare 40– 60 % (v/v) slurry with water or the appropriate buffer (high salt).
4. Gently stir. If required place under vacuum to degas.
5. Column
   (a) A column is prepared according to the instructions from the column supplier.
   (b) The bed support wetted in a packing solution or 20 % ethanol before use to remove air.
   (c) Pour the packing solution into the column tube and it check that solution flows out from the column exit.
   (d) Shut the exit valve when approximately 0.5 to 1cm high of the solution remains.
5. Carefully pour the slurry into the column and packing connector without creating air bubbles. Depending on the volume, a filler tube may be necessary.
6. Mount the top adapter on the top of packing connector.

7. Open the column outlet and begin pumping packing buffer for 10 min at 20 – 30 % high flow rate than operating flow rate. Caution: do not excess the operation pressure limit for the selected column.
8. Stop the pump and shut the column outlet valve.
9. Disconnect the top adapter line from the pump. Remove the packing connector. Before remove excess medium from the packing connector, if necessary.
10. Mount the top adapter prevent air bubbles from entering, lock the adapter and reconnect the line from the pump.
11. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

Operating Guidelines

1. How to use as a general hydrophobic interaction chromatography

Operating flow rate

The recommended flow pressure of Cellufine Phenyl EX is less than 0.2 MPa. Generally, low flow rates are used for binding and elution, while higher flow rates are acceptable for washing and regeneration steps.

Column preparation

Equilibrate column with 2 – 5 column volumes of elution buffer, then wash with the same amount of loading buffer. The typical loading buffer is 50 mM of sodium phosphate, pH 7.0 containing 0.5 – 2.5 M Na_2SO_4 , $(NH_4)_2SO_4$ or NaCl. Adsorption strength is a function of salt concentration, pH and temperature. In general, high concentration promotes adsorption. Elution is then accomplished by lowering the salt concentration. For more information, see References.

Sample Preparation and Load

Samples are ideally prepared in loading buffer. Filtration may be required to remove insoluble matter. If necessary, buffer exchange may be accomplished using diafiltration or desalting chromatography. Protein adsorption and recovery will vary with loading samples. The sample (prepared in the loading buffer) is applied after washing of column with the loading buffer. After loading of sample, flush with 5 column volumes of loading buffer to remove unbound material. Subsequently, bound product can be eluted.

Elution

Elution of bound material is accomplished by step or gradient elution with low concentration of salt (e.g., less than 0.5 M). The use of chaotropic agents (e.g., KSCN), surfactants (e.g., Octyl Glucoside, CHAPS, Triton X, CHAPS or Tween), denaturating agent (e.g., guanidine hydrochloride, urea, ethanol) will improve the recovery of tightly adsorbed proteins.

2. How to use as removing antibody aggregates

Operating flow rate

The recommended flow pressure of Cellufine Phenyl EX is less than 0.2 MPa. Generally, low flow rates are used for binding and elution, while higher flow rates are acceptable for washing and regeneration steps.

Column preparation

Equilibrate with an adsorption buffer of 2-5 CV. Generally adsorption buffers can be used such as phosphate and Tris, but it is recommended to examine optimum conditions for salt concentration and pH.

Sample preparation and sample loading

After the protein A column, the elution sample is adjusted to the desired pH and conductivity by buffer exchange, dilution, acid / base addition, and filtered through a 0.2 um filter. After equilibrating the column with an adsorption buffer, load the sample. Since the flow-through is the fraction in which the monoclonal antibody is present, it is collected. After loading the sample, wash with 5-10 CV adsorption buffer. Since the monoclonal antibody is also present in the flow-through fraction of the washing liquid, it is collected too.

Elution of impurities

Use 10 CV pure water to elute impurities such as antibody aggregates and impurities adsorbed on the column. For clean-in-place, pass 0.5 M NaOH, 30% isopropanol at 10 CV. A mixture of 0.5 M NaOH and 30% isopropanol may be used.

Chemicals and Physical Stability

pH 2 – 13, when operated at room temperature. Stable in most salts (NaCl, $(NH_4)_2SO_4$, etc.) and most detergents (SDS, Tween etc.) and other chemicals (70% ethanol, 30% isopropyl alcohol, 6M Guanidine hydrochloride and Urea). The products can be cleaned using 0.5 N NaOH. Autoclavable in suspension at neutral pH for 20 minutes at 121 ºC.

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Wash the column with 5 – 10 column volumes of 0.5N NaOH or 30% isopropyl alcohol. In some cases, an additional wash with 2 - 5 bed volumes of 70 % EtOH/30 % DIW /0.1 M AcOH followed by distilled water may be required to remove adsorbed lipids. Strongly adsorbed impurities may be washed with chaotropic reagents (KSCN), detergents (Octyl glycoside, CHAPS, Triton X, Tween) and denaturants (guanidine hydrochloride, urea, ethanol).

Storage

Store in a neutral buffer containing 20% ethanol at 2-25 ℃. Do not freeze. For long-term

storage, store at 4 ℃ is desirable.

Shelf Lifetime

5 years from date of manufacture

References

1. Harris, E.L.V. and Angal, S., *Protein Purification Methods: A practical Approach*. New York: Oxford University Press, 1989.
2. Janson, J.-C. and Ryden, L., *Protein Purification: Principles, High Resolution Methods, and Applications.* 2nd ed. New York: John Wiley & Sons, Inc., 1998
3. Sanchayita G., Yinying T., Lynn C. and Douglas C., *mAbs 5:5, 795–800; September/October 2013*

Product Ordering Information (Catalogue No.)

Product	Pack size					
	MC* 1 ml x 5	MC 5 ml x 5	100ml	500ml	5 lt	10 lt
Cellufine Phenyl EX	22000-51	22000-55	22000	22001	22002	22003

MC = Mini-Column

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