EconoFit UNOsphere SUPrA Columns, 1 and 5 ml

Instruction Manual

Catalog number

12009322
12009323

Please read the instructions in this manual prior to using EconoFit UNOsphere SUPrA Columns. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories representative.

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Section 1
Introduction

EconoFit UNOsphere SUPrA Columns are convenient, disposable, prepacked low-pressure chromatography columns. EconoFit Columns offer both increased run-to-run uniformity and high purity of protein through the column design and novel resin technology. Compatible with aqueous buffers most commonly used for protein purification, EconoFit Columns offer improved performance for your protein separation needs.

UNOsphere SUPrA Resin is a chromatographic support based on recombinant Protein A. The resin is designed for process-scale purification of monoclonal antibodies. The Protein A ligand is produced in *E. coli* without the use of material from animal origin. The UNOsphere base bead is a macroporous polymeric bead that is designed for robust and scalable applications. See Tables 1 and 2 for a technical description of the product.

UNOsphere SUPrA Resin is built on the proven UNOsphere base bead, which ensures an easy scale-up path for process applications. The outstanding flow pressure performance of UNOsphere Chromatography Resin allows it to be used in process applications without concern for exceeding the pressure limits of either the resin or the chromatography system. The flow characteristics of UNOsphere SUPrA packed in a large column format are shown in Figure 1.

UNOsphere SUPrA Affinity Chromatography Resin comes with full regulatory support and is backed by the support of the Bio-Rad global application and development team.

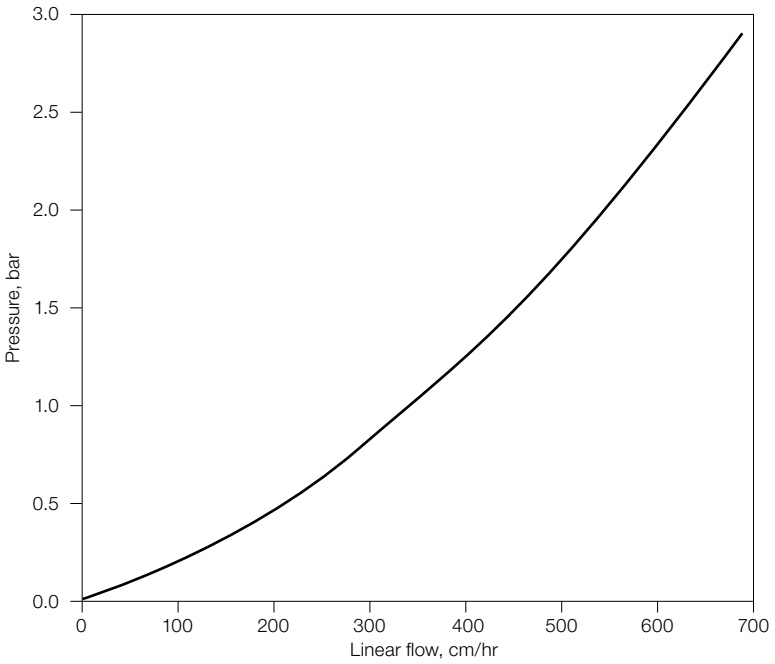

Fig. 1. Flow performance of UNOsphere SUPrA Resin in Bio-Rad EasyPack Column (20 x 20 cm) packed to 13.1% axial compression.

Section 2
Product Information

EconoFit Columns are disposable, easy-to-use, prepacked chromatographic columns supplied ready for use in convenient 1 and 5 ml sizes. They are quickly connected to liquid chromatography systems using 10-32 fittings. Columns are available for a variety of chromatographic techniques, including desalting (size exclusion [SEC]), ion exchange (IEX), affinity (AC), mixed-mode, and hydrophobic interaction chromatography (HIC). See Table 1 for specifications. Refer to **bio-rad.com/ResinsandColumns** for a complete listing of items in the EconoFit Column product line.

Table 1. EconoFit UNOsphere SUPrA Column specifications.

Property	Description
Size	1 and 5 ml bed volumes
Bed dimensions	1 ml: 25 mm length x 7 mm inner diameter 5 ml: 25 mm length x 16 mm inner diameter
Maximum pressure tolerance	72 psi
Recommended flow rate	1 ml: 1–2 ml/min (240–480 cm/hr) 5 ml: 5–10 ml/min (140–480 cm/hr)
Maximum flow rate	1 ml: 3 ml/min (730 cm/hr) 5 ml: 15 ml/min (722 cm/hr)
Fittings	10-32 (1/16"), female inlet and male outlet
Column material	Polypropylene
Frit material	High-density polyethylene
Shipping solution	20% ethanol
Storage conditions	20% ethanol
Autoclavability	Not autoclavable

UNOsphere SUPrA Resin is also available in bottles. Refer to Ordering Information in section 6 of this manual. See Table 2 for specifications. Go to **bio-rad.com/ResinsandColumns** for more information.

Table 2. Technical description of UNOsphere SUPrA Affinity Chromatography Resin.

Composition	Highly crosslinked polyacrylamide polymer
Particle size range	53–61 µm
Ligand	Recombinant Protein A
Coupling chemistry	Epoxy
Dynamic binding capacity*	150 cm/hr 30 ± 3 mg/ml 300 cm/hr 25 ± 2 mg/ml 450 cm/hr 20 ± 2 mg/ml
Chemical stability**	10 mM hydrochloric acid 6 M guanidine hydrochloride 0.1 M arginine, pH 2.8 0.1 M citrate, pH 2.8 0.1 M glycine, pH 2.8
Working pH range	3–11
Cleaning-in-place (CIP)	6 M guanidine hydrochloride 10 mM hydrochloric acid 0.1 M sodium hydroxide 1 M acetic acid/20% ethanol
Recommended mobile phase velocity range	100–600 cm/hr
Temperature stability	4–40°C
Delivery conditions	50% slurry in 20% ethanol
Storage conditions	4–8°C

* Minimum 20 mg/ml at 300 cm/hr; 10% breakthrough capacity determined with 1.0 mg/ml polyclonal human IgG in 1.1 x 10 cm column.
** No significant change in chromatographic performance after 24-hour storage at room temperature.

Section 3
Preparing a Column and Subsequent Purification

After connecting the column to a liquid chromatography system, prepare it as follows in Section 3.1.

3.1 Screening Buffers and Conditions for UNOsphere SUPrA Buffers

A1: 0.02 M sodium phosphate, 0.02 M sodium citrate, pH 7.5.

A2: 0.02 M boric acid, 0.02 M sodium phosphate, 0.02 M sodium citrate, 1.0 M sodium sulfate, pH 9.0.

B: 0.02 M sodium citrate, 0.1 M sodium chloride, pH 2.5.

Conditions

Equilibrate column: with 10 column volumes (CV) buffer A1 or A2 (see note below).

Inject: MAb sample either as is or diluted 1:10 in buffer A1 or A2 (see below).

Wash: buffer A1 or A2 until effluent absorbance returns to baseline.

Elute: in a 10 CV linear gradient to 100%B, or desired % buffer B.

Strip: with 5 CV buffer B.

Use buffer A1 for binding human and guinea pig IgG. Use buffer A2 for all others. The recommended column equilibration interval is excessive under most conditions, but should be used as a default until specific equilibration requirements are established for your particular system. In addition, be aware of solubility limitations of antibodies that require high salt concentrations (A2 buffer) for binding.

Characterize product solubility thoroughly under loading conditions. If the antibody fails to remain fully soluble for the longest possible duration from equilibration to completion of sample load when adjusted to load conditions, then load by using online dilution technique.

Initial selectivity screening should be conducted with a linear gradient. Knowledge of a monoclonal Ab's subclass may suggest a particular range of conditions, but variation from one monoclonal to another is sufficient to risk incomplete or no elution.

The choice of citrate for the low pH buffer is predicated on the broad pH range achievable with phosphate/citrate systems. If a higher pH range is required, add boric acid to the binding buffer. (Note: if using CHT Ceramic Hydroxyapatite Media as the subsequent polishing column, replace citrate, once scouting is finished, with glycine or other nonchelating salt for further process development. Citrate buffers are incompatible with CHT.)

Section 4
Scaling Up

For quick scale-up, two or three columns of the same type can be connected in series. Backpressure will increase with columns in series, so care should be taken to maintain pressures ≤72 psi. EconoFit UNOsphere SUPrA Columns are available in 1 and 5 ml formats. The UNOsphere SUPrA Resin is also available in larger amounts, from 25 ml bottles to bulk quantities.

In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory to process scale. Ask your local Bio-Rad representative or go to **bio-rad.com/ResinsandColumns** for more information.

Section 5
Care of the Column

5.1 Cleaning the Column

During operation it is recommended that the column bed be cleaned periodically to remove bound substances that can adversely impact the separation performance of the column. The accumulated substances fall into two general categories: a) difficult-to-remove precipitated or denatured substances, and b) substances that are hydrophobically bound to the column bed. To ensure that all bound substances are released and washed out of the column, the following cleaning-in-place (CIP) protocols are recommended.

CIP Protocols

The following protocols are suggested to remove precipitated or denatured substances from the bed.

Wash the bed with 2–5 CV in reverse flow with one of the following solutions:

- 6 M guanidine hydrochloride
- 10 mM hydrochloric acid
- 0.1 M sodium hydroxide
- 1 M acetic acid/20% ethanol

Follow with a reverse flow wash with at least 5 CV of binding buffer, neutral pH, 7–8.

To remove any hydrophobically bound substances from the bed, wash the column with 2–5 CV of a nonionic surfactant/detergent in reverse flow, followed by a reverse-flow wash with at least 5 CV of neutral pH binding buffer.

Suggested contact time per cycle is 15 min at room temperature.

5.2 Sanitization

To reduce the potential for microbial contamination of the column, the column can be periodically washed with a solution consisting of 0.1 M sodium hydroxide. Allow to stand for 1 hour, then wash with buffer until a neutral pH is reached.

5.3 Storage

To store UNOsphere SUPrA Resin for long periods, equilibrate the media with a 20% ethanol/water solution and store at 4ºC.

5.4 Autoclaving

EconoFit Columns are not autoclavable.

Section 6
Ordering Information

Catalog #	Description
EconoFit UNOsphere SUPrA Columns	
12009322	**EconoFit UNOsphere SUPrA Columns**, 5 x 1 ml columns
12009323	**EconoFit UNOsphere SUPrA Column**, 1 x 5 ml column
UNOsphere SUPrA Resins	
1560218	**UNOsphere SUPrA Affinity Chromatography Media**, 25 ml bottle
1560219	**UNOsphere SUPrA Affinity Chromatography Media**, 100 ml bottle
156-0220	**UNOsphere SUPrA Affinity Chromatography Media**, 500 ml bottle

Section 7
Bibliography

Gagnon P (1996). Purification Tools for Monoclonal Antibodies (Singapore: Validated Biosystems, Inc.).

Gagnon P (1997). Avoiding Instrument-Associated Aberrations in Purification Scale-Up and Scale-Down. BioPharm 10, 42–45.

Harris ELV and Angal S (1989). Protein Purification Methods: A Practical Approach (Oxford, UK: IRL Press).

Scopes RK (1987). Protein Purification: Principles and Practice, Second Edition (New York, NY: Springer-Verlag).

Snyer LR and Kirkland JJ (1979). Introduction to Modern Liquid Chromatography, Second Edition (New York: Wiley).

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