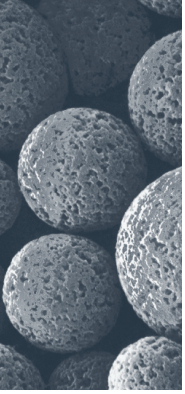

CHROMATOGRAPHY

UNOsphere™ Q Anion Exchange Support

- Hydrophilic polymeric beads engineered for high mechanical stability and low backpressures
- Efficient capture of biopharmaceutical molecules from crude feedstreams
- Ultrahigh binding capacities at fast linear velocities
- Robust polymer designed to withstand repeated clean-in-place cycles
- Biopharmaceutical manufacturing quantities available
- Fully supported for regulatory submission

Achieve High Productivity Using UNOsphere Q Anion Exchange Support

Be Productive

In the bioprocess industry, the isolation of biomolecules from crude feedstock is one of the most demanding chromatographic steps in the downstream process. Biopharmaceutical manufacturers are under increasing economic pressure to reduce drug production costs. These factors require the media used in the capture step to have very high binding capacities at fast linear velocities, while maintaining low column backpressure.

Based on a single-step polymerization process, UNOsphere is a new-generation polymeric support that delivers high productivity in the capture step.

UNOsphere Polymer Technology

The genesis of UNOsphere support is based on the single-step polymerization process used to prepare continuous-bed matrix UNO® ion exchange columns. Incorporation of a quaternary amine ligand into the matrix during polymerization leads to consistent batch-to-batch reproducibility. UNOsphere beads are macroporous (>2,000 Å), leading to fast binding kinetics and high binding capacities. Careful selection of monomers and crosslinkers provides unrivaled base stability and bead rigidity.

Properties of UNOsphere Q Support

Most production chromatography systems have maximum pressure limits of 3 bar. The median particle size of UNOsphere Q support is 120 µm, which generates a backpressure of less than 1 bar at 1,200 cm/hr (Figure 1).

The highly macroporous nature of UNOsphere Q support provides high binding capacities that range from 125 to 180 mg BSA per ml of support in the linear velocity range of 150–1,200 cm/hr.

Harsh conditions, such as clean-in-place and corrosive buffer systems, may affect the long-term stability of chromatographic media. The robustness of UNOsphere Q support allows it to survive these conditions with minimal loss of performance.

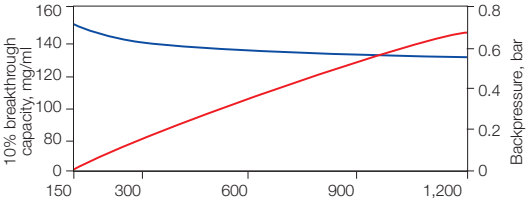

Fig. 1. Binding and backpressure properties of UNOsphere Q support. A 5 mg/ml sample of BSA in 10 mM Tris, pH 8.5, was loaded onto a 1.1 x 20 cm column. Red, backpressure; blue, 10% breakthrough capacity.

Plasmid Capture Performance

UNOsphere Q support is designed for high-efficiency capture of biomolecules from crude feedstreams. Figure 2 shows the capture of 0.61 mg of plasmid DNA (5.9 kb, derived from pUC19) from clarified bacterial lysate using UNOsphere Q support. Most of the RNA was removed in this capture step (Figure 3), and the eluted plasmid could be digested by EcoRI restriction enzyme (Figure 4). Residual contaminants, such as RNA, cDNA, endotoxins, and host cell proteins, may be removed using CHT™ ceramic hydroxyapatite to produce gene therapy–grade plasmid.

BIO-RAD

Technical Assistance

Regulatory support files are available upon request. Bio-Rad Laboratories is an ISO 9001 registered corporation. For additional information and technical assistance, contact your local Bio-Rad office. In the USA and Canada, call 1(800)-4BIORAD.

For more information on Bio-Rad's complete line of process chromatography media, visit us on the Web at **www.bio-rad.com/unosphere/**

For more information about UNO columns, request bulletin 2116. For more information about the chromatographic performance of UNOsphere support, see the bibliography section; note that the authors refer to UNOsphere as BRX.

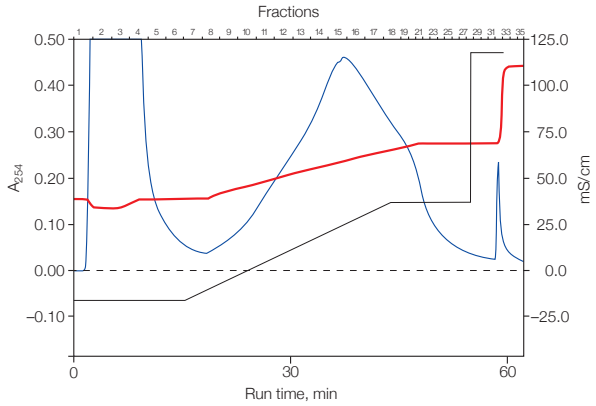

Fig. 2. Purification of plasmid DNA on a UNOsphere Q column. Clarified bacterial lysate (10 ml, adjusted to pH 8.0) was loaded onto a 0.5 x 11 cm column (2.1 ml) in buffer A (10 mM sodium phosphate, 0.3 M NaCl, 1 mM EDTA, pH 8.0). The sample was eluted with a 0–40% gradient of buffer B (10 mM sodium phosphate, 1.0 M NaCl, 1 mM EDTA, pH 8.0) at a flow rate of 2 ml/min (600 cm/hr). The column was washed with 10 column volumes of 40% buffer B, followed by 100% buffer B for 5 column volumes. The effluent was monitored at 254 nm. Each fraction was 5 ml. Blue, A_{254}; red, conductivity; black, theoretical gradient.

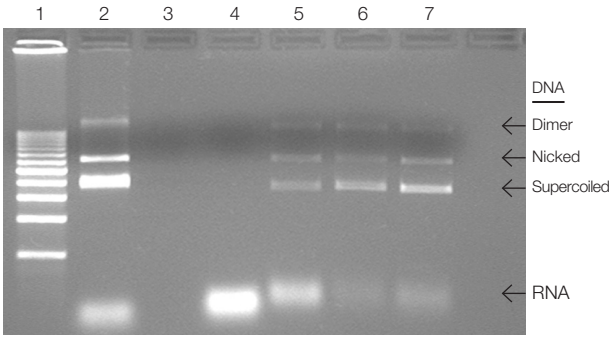

Fig. 3. Analysis of plasmid DNA purified on UNOsphere Q support. Fractions from the chromatography run shown in Figure 2 were separated on a 0.8% agarose gel. Lane 1, 1 kb marker (catalog #170-8204); lane 2, crude lysate; lane 3, flowthrough (fractions 2–6); lane 4, fractions 9–18; lane 5, fractions 19–30; lane 6, fractions 31–35; lane 7, fraction 36.

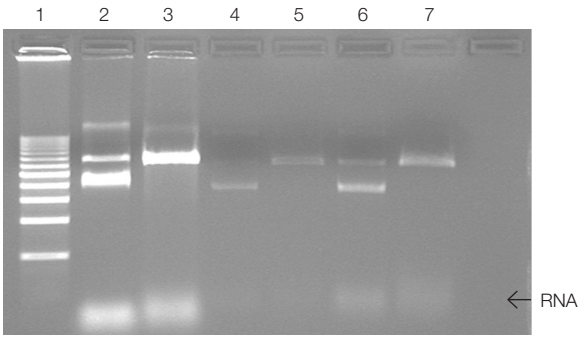

Fig. 4. Restriction enzyme analysis of plasmid DNA purified on UNOsphere Q support. Lane 1, 1 kb marker; lane 2, undigested clarified lysate; lane 3, EcoRI-digested clarified lysate; lane 4, undigested fractions 31–35; lane 5, digested fractions 31–35; lane 6, undigested fraction 36; lane 7, digested fraction 36.

Properties of UNOsphere Q Support

Type of ion exchanger	Strong anion
Functional group	$–N(CH_3)_3^+$
Total ionic capacity	120 µeq/ml
Dynamic binding capacity*	
at 150 cm/hr	180 mg BSA/ml
at 600 cm/hr	125 mg BSA/ml
Median particle size	120 µm
Recommended linear flow rate range**	50–1,200 cm/hr
Chemical stability	
in 1.0 M NaOH (20°C)	Up to 10,000 hr
in 1.0 M HCl (20°C)	Up to 200 hr
pH stability range	pH 1–14
Regeneration conditions	1–2 M NaCl
Storage conditions	20% ethanol 0.1 M NaOH for 30 days

* 10% breakthrough capacity determined with 2.0 mg/ml BSA in a 1.1 x 10 cm column.

** UNOsphere Q support packed into a 20 cm bed height and run at 1,200 cm/hr generates less than 2 bar backpressure.

Bibliography

Hunter AK and Carta G, Protein adsorption on novel acrylamido-based polymeric ion exchangers. I. Morphology and equilibrium adsorption, J Chromatogr A 897, 65–80 (2000)

Hunter AK and Carta G, Protein adsorption on novel acrylamido-based polymeric ion exchangers. II. Adsorption rates and column behavior, J Chromatogr A 897, 81–97 (2000)

Ordering Information

Catalog #	Description
156-0101	UNOsphere Q Support, 25 ml
156-0103	UNOsphere Q Support, 100 ml
156-0105	UNOsphere Q Support, 500 ml
156-0107	UNOsphere Q Support, 10 L

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