UNOsphere™ Q and S Ion Exchange Media

Instruction Manual

Please read these instructions prior to using UNOsphere ion exchange media. If you have any questions or comments regarding these instructions, please contact your Bio-Rad Laboratories representative.

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Section 1
Introduction

UNOsphere Q and S ion exchange media are hydrophilic spherical polymeric beads designed for the separation of proteins, nucleic acids, viruses, plasmids, and other macromolecules. The UNOsphere beads are designed for high capacity, low backpressure, and high productivity.

Section 2
Technical Description

Table 1. Characteristics of UNOsphere Media.

	UNOsphere Q	**UNOsphere S**
Type of ion exchanger	Strong anion	Strong cation
Functional group	$-N^+(CH_3)_3$	$-SO_3^-$
Total ionic capacity	120 µeq/ml	260 µeq/ml
Dynamic binding capacity*		
150 cm/hr	180 mg/ml	60 mg/ml
600 cm/hr	125 mg/ml	30 mg/ml
Shipping counterion	Cl^-	Na^+
Median particle size	120 µm	80 µm
Recommended linear flow rate range**	50–1,200 cm/hr	50–1,200 cm/hr
Chemical stability		
1.0 M NaOH (20°C)	≤2,000 hr	≤2,000 hr
1.0 M HCl (20°C)	≤200 hr	≤200 hr
Volume changes		
pH 4–10	<5%	<5%
0.01–1.0 M NaCl	<5%	<5%
Gel bed compression ratio***	25% ± 3%	25% ± 3%
pH stability	1–14	1–14
Autoclavability (121ºC, 30 min)	Yes	Yes
Antimicrobial agent	20% ethanol	20% ethanol
Regeneration	70% ethanol or 1–2 M NaCl	70% ethanol or 1–2 M NaCl
Storage conditions	20% ethanol or 0.1 M NaOH	20% ethanol or 0.1 M NaOH

*10% breakthrough capacity determined with 4.5 mg/ml human IgG (UNOsphere S) and 2.0 mg/ml BSA (UNOsphere Q) in a 1.1 x 20 cm column.

**UNOsphere media packed to a 20 cm bed height and run at 1,200 cm/hr generate less than 2 bar backpressure.

***A compression ratio is for axially compressed columns packed at a constant flow rate (100–1,200 cm/hr) or constant pressure of 1–2 bar. Gel bed will rebound to its original volume 15–20 min after the pump stops. Columns with small ID may not rebound to original volume due to Wall effects.

Section 3
Preparation

UNOsphere ion exchange media are supplied fully hydrated in 100 mM NaCl in 20% ethanol as a 50% (v/v) slurry. For column packing, removal of the shipping buffer is recommended.

Small volumes of UNOsphere media are easily washed in a Büchner funnel with 4–5 volumes of packing buffer. For large volumes, cycling through 3–4 settling and decanting steps using the packing buffer in the shipping container is recommended.

Complete removal of fines from UNOsphere media is not required due to the narrow particle-size range. If fines have been generated during handling, resuspend sediment and remove the milky supernatant before sedimentation is complete. Repeat several times.

Section 4
Column Packing

Polymeric UNOsphere media may be packed using pressure, volumetric flow, or vacuum packing methods. To pack highly efficient columns, it is recommended to use a 20–50% slurry volume.

Packing Small Columns

This slurry packing method was designed to pack 25 ml of UNOsphere in a conventional column with an internal diameter of 5–15 mm. All buffers should be degassed. Since a relatively large volume of slurry is required, it is recommended that a packing reservoir be used.

1. Prepare degassed 1.0 M NaCl, 20–50 mM buffer salt (see Table 2) referred to herein as the packing buffer.
2. UNOsphere media are shipped as a 50% slurry. Measure 50 ml of suspended slurry into a 100 ml graduated cylinder. Allow the resin bed to settle. Decant the shipping solution away from the resin bed.
3. Add 50 ml of degassed packing buffer to resin.
4. Seal the cylinder and rotate it to suspend the resin. Caution: Do not mix with a magnetic stir bar, as damage may occur. Larger amounts of slurry may be mixed with a marine impeller at low to moderate speed.
5. Add 10 ml of packing buffer to the column. Pour in 75 ml of resin slurry.
6. Insert the column flow adaptor and flow-pack at a linear velocity of 1,200 cm/hr with packing buffer for at least 10 min. Note the compressed bed height, stop the flow, and adjust the flow adaptor to compress the bed 0.1–1.0 cm.

7. Attach the column to your chromatography system, and purge the column with starting buffer at linear velocities up to 1,200 cm/hr. If the bed compresses, repeat steps 6 and 7.

Packing Large Columns

In large columns, UNOsphere should be packed using a 20–50% slurry at 1–2 bar (constant pressure systems) or at 100–1,200 cm/hr (constant flow systems). It is recommended to never use a packed column at a pressure or flow rate that is >75% of the maximum pressure and flow rate achieved during its initial packing. Given the industrial column hardware and packing skids, we recommend using your standard operating procedures for column packing.

Section 5
Column Packing Evaluation

Once column packing is complete, equilibrate the column with up to 5 column volumes (CV) of starting buffer. To test the effectiveness of column packing, inject a sample of a low molecular weight, unretained compound (e.g., acetone or 1 M NaCl). If acetone is used as the test marker (use an absorbance monitor set at 280 nm), the starting buffer must have a salt concentration less than 100 mM. If 1 M NaCl is the test marker (use a conductivity monitor), then the testing buffer salt concentration should be between 100 and 200 mM. The sample volume should be 2–5% of the total column volume. The column testing should be operated using the same linear velocity used to load and elute the sample.

To obtain comparable Height Equivalent to a Theoretical Plate (HETP) values between columns, the same conditions must be applied. Minimum theoretical plate values should be between 1,000 and 3,000 plates/m for linear velocities of 50–500 cm/hr.

HETP = L/N

$N = 5.54(V_e/W_{½h})^2$

L = Bed height (cm)
N = Number of theoretical plates
V_e = Peak elution volume or time
$W_{½h}$ = Peak width at peak's half height in volume or time
V_e and $W_{½h}$ should always be in the same units.

Peaks should be symmetrical, and the asymmetry factor as close as possible to 1. Values of 0.8 to 1.5 are acceptable. A change in the shape of the peak is usually the first indication of deteriorating performance.

Peak asymmetry factor calculation:

$A_S = b/a$

a = Front section of peak width at 10% of peak height bisected by line denoting V_e.
b = Latter section of peak width at 10% of peak height bisected by line denoting V_e.
A_S = 0.8–1.8 is acceptable.

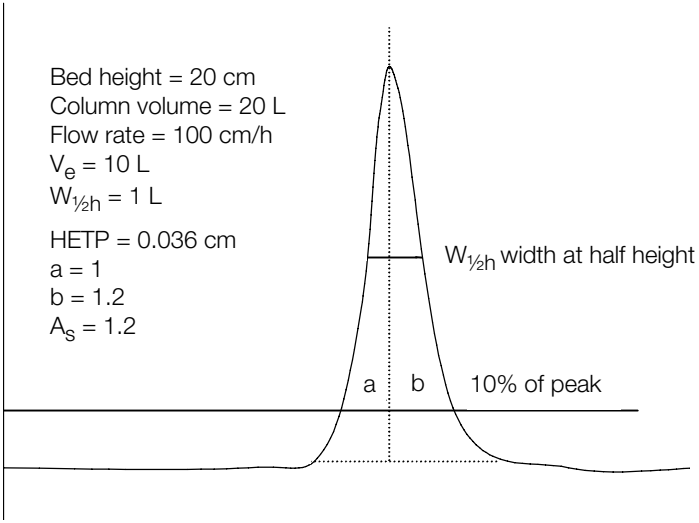

Fig. 1. A simulated chromatography profile from which HETP and A_S values are calculated.

Section 6
Operation and Maintenance

UNOsphere media were designed to achieve the highest productivity (grams of drug per operational hour per liter of media) possible. UNOsphere media should be run at the highest linear velocities and loading capacities allowed by the column and chromatography system. A linear flow rate of 600 cm/hr and a 20 cm bed is a recommended starting point. The purification may be optimized by changing the pH or flow rate, changing the ionic strength of the elution buffer, modifying the gradient profile, or experimenting with different buffer salts.

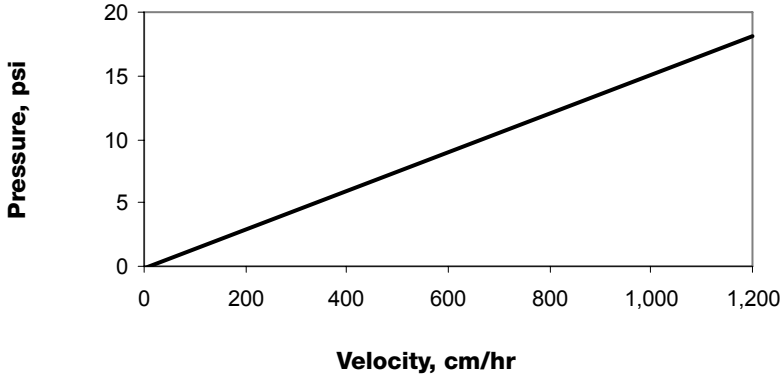

Fig. 2. UNOsphere Q and S pressure/flow chart for a 11 x 200 mm column using deionized water as eluent. Note: Backpressure may vary for different column hardware.

All buffers commonly used for anion or cation exchange chromatography can be used with the respective UNOsphere ion exchange media (see Table 2). The chemical stability and broad operating pH range of these ion exchangers allow the use of a variety of buffers. The use of buffering ions that have the same charge as the functional group on the ion exchanger (e.g., phosphate with a cation exchanger and Tris with an anion exchanger) will produce the best results.

Table 2. Common Buffers for Ion Exchange Chromatography.

Buffer	Buffering Range
Cation Exchanger	
Acetic acid	4.8–5.2
Citric acid	4.2–5.2
HEPES	6.8–8.2
Lactic acid	3.6–4.3
MES	5.5–6.7
MOPS	6.5–7.9
Phosphate	6.7–7.6
PIPES	6.1–7.5
TES	6.8–8.2
Tricine	7.8–8.9
Anion Exchanger	
Bicine	7.6–9.0
Bis-Tris	5.8–7.2
Diethanolamine	8.4–8.8
Diethylamine	9.5–11.5
L-histidine	5.5–6.0
Imidazole	6.6–7.1
Pyridine	4.9–5.6
Tricine	7.4–8.8
Triethanolamine	7.3–8.3
Tris	7.5–8.0

Section 7
Regeneration

After each run, the packed bed should be washed with 2–4 bed volumes of 1–2 M NaCl to remove reversibly bound material. Samples may be loaded onto the column after reequilibration in starting buffer.

Section 8
Cleaning-in-Place (CIP) and Sanitation

If a column no longer yields reproducible results, the media may require thorough CIP and sanitation to remove strongly bound contaminants. Acceptable CIP agents include 25% acetic acid, 8 M urea, 1% Triton X-100, 6 M potassium thiocyanate, 70% ethanol, 30% isopropyl alcohol, 1 N HCl, 1 N NaOH, and 6 M guanidine hydrochloride.

1. Sanitize the support in the column with 2–4 bed volumes of 1.0 M NaOH at 50–100 cm/hr while maintaining a minimum contact time of 40 min.
2. To reequilibrate the column, wash the column with 2–4 bed volumes of 0.5–2 M NaCl solution (should contain 50–100 mM buffer salt).
3. If lipid removal is required, the column may be washed with a 20–50% ethanol solution at 50 cm/hr.

Section 9
Storage

For long-term storage, UNOsphere media should be equilibrated with 20% ethanol.

Section 10
Regulatory Support

Regulatory support files are available for UNOsphere ion exchange media. If you need assistance validating the use of UNOsphere supports in a production process, contact your local Bio-Rad representative.

Section 11
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
156-0111	UNOsphere S Support, 25 ml
156-0113	UNOsphere S Support, 100 ml
156-0115	UNOsphere S Support, 500 ml
156-0117	UNOsphere S Support, 10 L

Larger volumes and special packaging are available upon request.

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