UNOsphere™ *rapid S* Cation Exchange Media

Instruction Manual

Catalog #s
156-0211
156-0213
156-0215
156-0217

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 *rapid S* ion exchange media is a hydrophilic spherical polymeric bead 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 ***rapid S***
Type of ion exchanger	Strong cation
Functional group	$–SO_3^-$
Total ionic capacity	140 µeq/ml
Dynamic binding capacity*	
150 cm/hr	60 mg/ml
600 cm/hr	30 mg/ml
Shipping counterion	Na^+
Median particle size	100 µm
Recommended linear flow rate range	50–800 cm/hr
Chemical stability	
1.0 M NaOH (20°C)	≤800 hr
1.0 M HCl (20°C)	≤200 hr
Gel bed compression ratio	17–18%
pH stability	1–14 short term 2–13 long term
Autoclavability (121ºC, 30 min)	Yes
Antimicrobial agent	20% ethanol
Regeneration	70% ethanol or 1–2 M NaCl
Storage conditions	20% ethanol or 0.1 M NaOH

*10% breakthrough capacity determined with 4.5 mg/ml human IgG.

Section 3
Preparation

UNOsphere *rapid S* media is supplied fully hydrated 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 *rapid S* 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 *rapid S* should be packed using a 20–50% slurry, ideally using a combination of axial and flow compression. The best overall performance of UNOsphere *rapid S* will be obtained when a compression ratio of 17–18% is used. After achieving the desired compression it is recommended to flow condition the column with fresh packing buffer for 3 column volumes of up flow followed by 3 column volumes of down flow at 100 cm/hr. After flow conditioning it is recommended to evaluate the column efficiency using your standard operating procedures or the procedure described in section 5 below.

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 100–200 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 1,000–3,000 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 0.8–1.2 are acceptable.

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

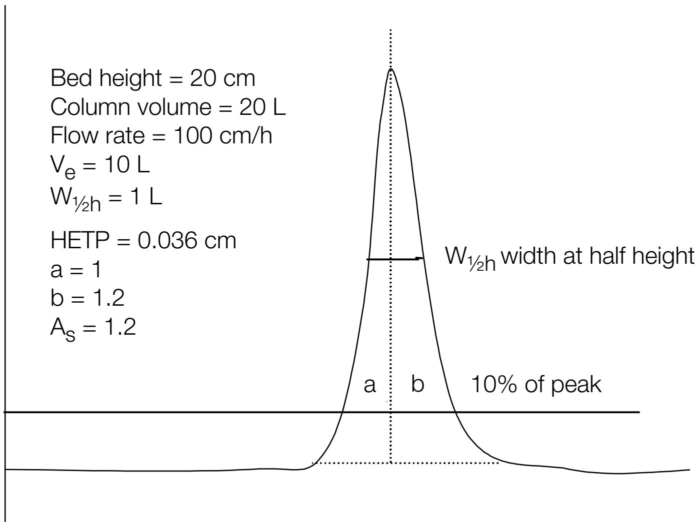

Fig. 1. A simulated chromatography profile from which HETP and A_S values are calculated.

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 400 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.

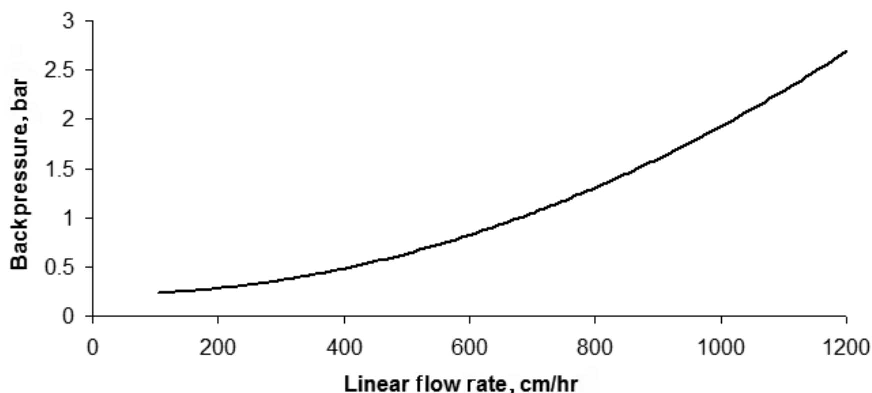

Fig. 2. UNOsphere *rapid S* pressure/flow performance for a 20 cm diameter x 20 cm bed height column, packed to 17% axial compression.

All buffers commonly used for ion exchange chromatography can be used with UNOsphere *rapid S* media (see Table 2). The use of buffering ions that have the same charge as the functional group on the ion exchanger will produce the best results.

Table 2. Common Buffers for Cation Exchange Chromatography.

Buffer	Buffering Range
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

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 (containing 50–100 mM buffer).
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 *rapid S* media. If you need assistance validating the use of UNOsphere *rapid S* in a production process, contact your local Bio-Rad representative.

Section 11
Ordering Information

Catalog #	Description
156-0211	UNOsphere *rapid S* Support, 25 ml
156-0213	UNOsphere *rapid S* Support, 100 ml
156-0215	UNOsphere *rapid S* Support, 500 ml
156-0217	UNOsphere *rapid S* Support, 10 L

Larger volumes and special packaging for industrial applications are available upon request.

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