# UNOsphere™ Q & 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.

BIO-RAD

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*4110109 Rev B*

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# Section 1. Description

The UNOsphere Q & 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.

*UNOsphere media were designed to achieve the highest productivity (grams of drug per operational hour per liter of support) possible. UNOsphere media should be run at the highest flow rates and loading capacities while staying within the pressure limits of the column and chromatography system.*

# Section 2. Characteristics

**Table 1. Characteristics of UNOsphere Ion Exchangers**

| | **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 | 280 µeq/ml |
| Dynamic binding capacity[1] | | |
| 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[2] | 50-1200 cm/hr | 50-1200 cm/hr |
| Chemical stability | | |
| 1.0 M NaOH (20°C) | up to 2,000 hrs | up to 2,000 hrs |
| 1.0 M HCl (20°C) | up to 200 hrs | up to 200 hrs |
| Volume changes[3] | | |
| pH 4-10 | <5% | <5% |
| 0.01-1.0 M NaCl | <5% | <5% |
| pH stability | 1-14 | 1-14 |
| Autoclavable | yes | yes |
| Antimicrobial agent | 20% ethanol | 20% ethanol |
| Regeneration | 70% ethanol | 70% ethanol |
| Storage conditions | 20% ethanol or 0.1 M NaOH | 20% ethanol or 0.1 M NaOH |

[1]10% breakthrough capacity determined with 4.5 mg/ml human IgG (S) and 2.0 mg/ml BSA (Q) in a 1.1 x 20 cm column.
[2]UNOsphere packed into a 20 cm bed height and run at 1,200 cm/hr generates less than 2 bar backpressure.

# 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/decanting steps using the packing buffer in the shipping container is recommended.

Removal of fines from UNOsphere media is not required due to the support's narrow particle size range.

# Section 4. Column Packing

UNOsphere is a polymeric media that 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 into 5 mm to 15 mm ID conventional type columns. 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 referred to herein as the packing buffer.
2. UNOsphere is shipped as a 50% slurry, so 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 stirring bar, as damage may occur. Larger amounts of slurry may be mixed with marine type impellers 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 adapter and flow pack at a linear velocity of 1200 cm/hr with packing buffer for at least 10 minutes. Note the compressed bed height, stop the flow, and adjust the flow adaptor to compress the bed 0.1-1 cm.
7. Attach the column to your chromatography system and purge column with starting buffer at linear velocities up to 1200 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 1,200 cm/hr (constant flow systems). Given the variety of industrial column hardware and packing skids used throughout the world, we recommend packing using your standard operating procedures for column packing.

# Section 5. Column Testing

Once column packing is completed, equilibrate the column with up to 5 column volumes 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 probe (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 probe (use a conductivity monitor), then the testing buffer salt concentration should be between 100-200 mM. The sample volume should be 2-5% of the total column volume. The column testing should be operated using the same as the linear velocity used to load and elute the sample.

To obtain comparable HETP values between columns, the same conditions must be applied. Minimum theoretical plate values should be between 1,000-3,000 plates/m for linear velocities of 50-500 cm/hr.

# Section 6. Operating Conditions

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 while staying within the pressure limits of 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.

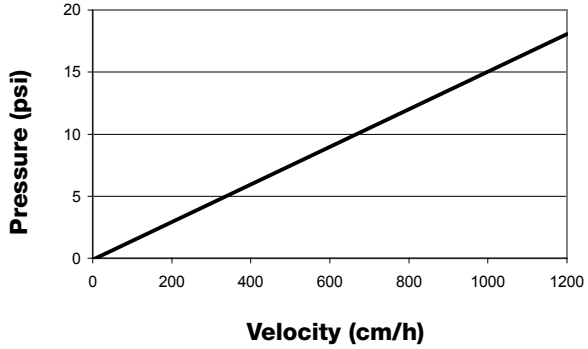


Fig. 1. UNOsphere Q and S pressure/flow chart for a 1.1 x 20 cm column using deionized water as eluent. Note: Backpressure may vary from different column hardware.

All buffers commonly used for anion or cation exchange chromatography can be used with the respective 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**

| Type of Ion Exchanger | Buffer | Buffering Range |
|---|---|---|
| **Cation** | | |
| | 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 |
| | Pivalic acid | 4.7-5.4 |
| | TES | 7.2-7.8 |
| | Tricine | 7.8-8.9 |
| **Anion** | | |
| | 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 | 7.3-8.0 |
| | Tris | 7.5-8.5 |

# 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 cleaning-in-place (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% isopropanol, 1 N HCl, 1 N NaOH, 6 M guanidinium 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 minutes.
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 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. Product Information

| Catalog # | Product 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.