Amsphere™

# COLUMN PACKING PROTOCOL FOR AMSPHERE A3

This packing protocol is for small columns (1–20 mL, ID = 0.5–1 cm). The recommended packing method is by flow.

## Preparation of the slurry

The slurry of Amsphere A3 is provided in a storage buffer solution containing 16% ethanol in 50 mM sodium phosphate, pH 7.5. Decant the storage solution once the resin has settled, and add 0.1 M sodium chloride to obtain a slurry concentration of 50% (v/v) (buffers other than 0.1 M sodium chloride can be used but could influence the packing quality).

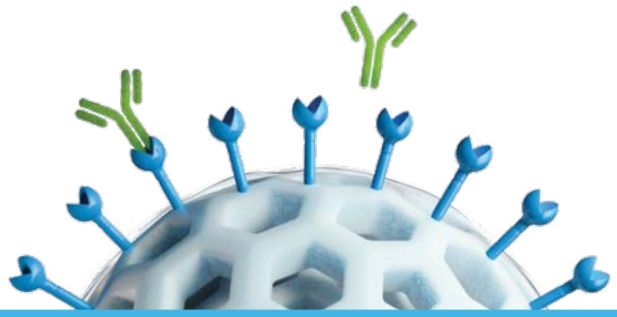
**Caution:** Do not mix or re-slurry with a magnetic stir bar.

**Note:** Column packing with water or 1 M sodium chloride is possible but not recommended.

The volume of the slurry needed to pack the bed can be calculated using the following formula:

Volume slurry = volume packed bed × (100/slurry (%)) × compression factor

Compression factor of Amsphere A3: 1.10 (±0.02)



### Amsphere™ A3 Construct

Amsphere A3 is a new protein A resin designed with a surface modified base bead and novel, alkali-resistant ligand.

**Protein A ligand**

- High DBC via controlled conformation and orientation
- High alkaline stability from protein engineering

**Surface modification**

- Low HCP levels by surface hydrophilization

**Base bead formulation**

- High DBC at high flow rate
- Excellent pressure and flow properties via rigid crosslinking

## Column Packing

**Table 1:** Recommended packing conditions (For 20 cm bed height)

| Column | Internal diameter | 1.0 cm | 0.5 cm |
|---|---|---|---|
| | Bed height | 20 cm | |
| Packing buffer | | 0.1 M sodium chloride | |
| Equilibration buffer | | 20 mM sodium phosphate; 150 mM sodium chloride pH 7.5 | |
| Packing velocity | | 600–750 cm/h | 900–1200 cm/h |
| Maximum operation velocity (When resin is packed at the flow rate above) | | 500–600 cm/h | 600–800 cm/h |

***Degassing of all buffers is recommended prior to use.***

1. Assemble the column according to instructions. If the column does not allow for 50% slurry, or 2X of the packing buffer compared to the settled bed volume, please attach a packing reservoir tube.

2. Add 0.5 mL of the packing buffer to the empty column and then pour slurry into the column (avoid air bubbles).

3. Pack the column by a downward flow. Flow rate should be 120–200% of the operating flow rate or refer to the recommended packing condition.

4. Let the flow run until bed has consolidated.

5. Measure the bed height.

6. Stop the flow and disconnect the packing reservoir if used from the column. Install the top adapter according to instructions and lower the adapter until it touches the resin bed. Make sure that no air is trapped or that the pressure does not increase. Do not exceed the pressure specifications of the media or the column.

7. Flow the column according to your maximum operational velocity for 2–5 CV to condition the bed.

**Before evaluation:** Flush 2–5 CV of equilibration buffer, e.g., 20 mM sodium phosphate, 150 mM sodium chloride, 7.5 pH at 120–200% of the operating flow rate.

**Caution:** Sometimes replacing buffer causes the bed height to reduce slightly. When this occurs, adjust the adapter to the new bed height.

**Before use:** Wash the column with 5 CV of elution buffer. Then flush the column with equilibration buffer until the pH and conductivity become stable.

## Evaluation of Column Packing Efficiency

Packing evaluation can be represented with the number of theoretical plates (N) and peak asymmetry ($A_s$).

**Table 2**

| |
|---|
| $N = 5.14 (V_R/W½h)^2$ |
| VR = Peak elution volume or time |
| W½h = Peak width at peak's half height in volume or time |
| $A_s = b/a$ |
| a = Front half-width of the peak measured at 10% of the peak height |
| b = Back half-width of the peak measured at 10% of the peak height |

To evaluate $A_s$, use (i) 1 M sodium chloride as the test marker for conductivity and (ii) acetone (or other substance with low molecular weight that is not retained in the media) as the test marker for UV (absorbance at 280 nm). Equilibrate the column with 150 mM sodium chloride before evaluation. The injection volume of the test marker should be 1–3% of column volume.

Note: The acceptable value range of As is usually between 0.7–1.5, but is application dependent.

## Example of Column Packing

Below is an example of column packing using the protocol on the previous page.

**Materials and Method**

| | |
|---|---|
| Column | Tricorn™ 5/200 (4 mL) |
| Packing solution | 0.1 M sodium chloride |
| Equilibration buffer | 20 mM sodium phosphate, 150 mM sodium chloride, 7.5 pH |
| Packing flow rate | 3.9 mL/min (1200 cm/hr) |
| Equipment | ÄKTA™ system |

**Evaluation Condition**

| | |
|---|---|
| Loading | Equilibration buffer |
| Inject solution | 1% acetone in 1 M sodium chloride |
| Inject solution | 100 µL |
| Flow rate | 1.0 mL/min (300 cm/hr) |

**Evaluation Results**

Chromatogram and evaluation results can be seen below.

Bed height for the column after packing was 20.6 cm and volume was 4.03 mL.
Asymmetry and plates per meter were within specification (see below).

**Asymmetry**

**Figure 1:** Example of packing evaluation

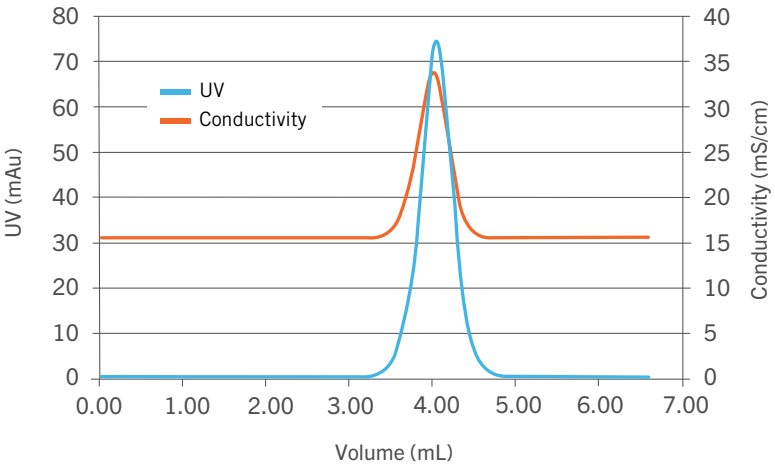



**Table 3**

| | UV | Conductivity |
|---|---|---|
| Asymmetry | 0.98 | 0.87 |
| N (/m) | 2481 | 2262 |

## Conclusion

Good packing was observed.

## Storage

For storing a column packed with Amsphere A3, equilibrate the column with 16–20% ethanol, and store between 2–8 °C. Instead of 16–20% ethanol, 2% benzyl alcohol can be used. After long-term storage, it is recommended to do a blank run including CIP before use. Please refer to General Instructions for details.



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