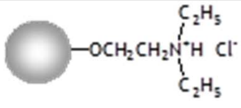
# Cellufine™ A-500

## Column Packing of Cellufine A-500 ion exchange chromatography resin

Cellufine A-500 is a conventional weak anion exchange resin which is easy and robust to use. The IEX chemistry is shown in Figure 1. The spherical cross-linked beads of exclusion limit up to 500 kDa are made from Cellulose having unique crystalline molecular structure which imparts chemical stability and mechanical strength. Thus, Cellufine A-500 has excellent column packing characteristics and can be operated at high flow rates with low back pressure. Cellufine A-500 media is comparable to conventional media, such as cross-linked agarose. This Cellufine resin shows good flow properties and has been packed in all column sizes up to 2 meter diameter.

Figure 1, Cellufine IEX Ligand Structure

| | |
|---|---|
| Cellufine<br>A-200, A-500, A-800 |  |



The following packing modes can be used with Cellufine IEX for up to 30 cm in diameter columns.

**Flow packing** – at a flow rate at least 50% greater than the maximum operating flow rate for your chromatography operation while not exceeding a final packing pressure of 0.3MPa at the column inlet. For large columns with a slurry inlet adaptor place the top flow adaptor at a height that will accommodate the slurry volume. Fill the column with a suitable packing buffer and then pump the slurry into the column using the slurry nozzle adaptor. Use packing buffer to chase the remaining resin from the pump and slurry nozzle. Avoid introducing air into the line. Pump packing buffer at flow rates/pressures up to the limits of the column. Bring the top adaptor down to contact the flow packed bed.

***Note:*** *the top adaptor should only enter the packed bed by 0.5 cm.*

**Flow packing followed by axial compression** – flow packing as described above followed by axial compression to further consolidate the packed bed. After about 2 CVs at the 50% higher flow rate, lower the top adapter to contact the packed bed as described above. Then apply additional axial compression to achieve the desired final column volume while not exceeding the final pressure of 0.3MPa at the column inlet.

**Axial compression** – Pack at flow rates/pressures not to exceed 0.3MPaat the column inlet. Proceed directly with axial compression by lowering the top adaptor to pack the resin bed up to the flow pressure limit of the column.

**Pack-in-place/Stall pack** – Pack at flow rates/pressures up to the limits of the column. Lock the top adapter into place at the desired bed height and pump resin into the column until the column is full or the pump stalls. Slurry should be transferred at a flow rate equal to 500–1000 cm/hour with the final packing pressure not to exceed 0.3MPa. A constant pressure pack can also be employed if a slurry tank is available that can be pressurized to 0.3MPa.

***Note:*** *If the column is not packed at a high enough flow or pressure, then flowing a more viscous solution (like a cleaning solution) over the column at the same flow rate will result in further bed compaction.*

## Detailed Flow Packing Procedure in Columns fitted with Flow Adaptors

1) ***For column volumes < 1 l***; transfer sufficient slurry for the target column volume (CV) into a filter funnel (glass fitted) and wash with at least 5 volumes of water for a total of 3 x to remove the storage solution. If necessary, repeat with packing buffer if different from water.

2) ***For column volumes > 1 L***; decant the storage buffer from above the settled resin in the shipping container and replace with water. Then re-suspend the resin and allow to settle again to wash away the storage buffer. Repeat 2-3x or consider packing in the storage buffer and washing the column on-line.

3) After final wash add sufficient packing buffer to suspend the washed resin into a 50-60% slurry.

4) Transfer some of the slurry into a 50-mL measuring cylinder and allow to settle overnight or a minimum of 4h. Measure bed height of a gravity settled bed and calculate the slurry% from;

% = Gravity settled bed height/Total slurry volume

5) Adjust to a 50 % slurry concentration of resin with packing buffer.

6) Calculate the volume of slurry required to pack the column using the following equation;

Volume 50% slurry = (Target CV x 2) x (Cf)

Where Cf is the resin compression factor derived from:

Cf = gravity settled bed height/flow packed bed height

For example, for a 100 ml CV you will need (100 x 2) x 1.15 = 230 mL, for a resin compression factor of 1.15.

7) **For manual column packing**; assemble the column hardware with the bottom flow adaptor in place. Prime the bottom frit assembly to remove air with packing buffer from a syringe or pump for a large diameter column. Leave about 1 cm in the bottom of the column.

8) If necessary add a bed height adapter to the top of the column to accommodate the full volume of the slurry.

***Note:*** *in manual packing the full volume of slurry will be poured into the column in one step to ensure a uniform packed bed.*

9) Close the bottom outlet of the column.

10) Pour the slurry into column in one operation and avoid trapping air in the resin slurry.

**Note:** *pour the slurry down the column wall or a glass pipet will avoid introduction of air into the slurry bed.*

11) Open a bottom outlet and allow the bed to settle until 2-3 cm of clear liquid is seen above the resin bed.

12) Stop the outlet flow and carefully fill the column with packing buffer up to the top without disturbing the settling resin bed.

13) Prime the upper flow adapter with an attached syringe filled with packing buffer in an upright position to remove any air trapped in the assembly.

**Note:** *use of a three-way valve on the top adaptor will facilitate this priming operation and will ensure air is not introduced into the column at this stage.*

14) Assemble the top flow adaptor on to the column minimizing any trapped air bubbles in the head of the column using the three-way valve.

15) Initiate flow with the packing buffer at 200 cm/h for 30 to 60 min flow pack the resin bed.

**Note**: *the column back pressure* should be in the range 0.15 – 0.25 MPa at this flow rate. This is a higher flow rate than normal operation of the column to ensure a stable bed packing.*

** This is the pressure drop across the column when the column is filled with resin. Allowance should be made for the system back pressure where an empty buffer filled column of the same size is place in-line. Backpressure is best measured with a gauge on the inlet side of the column.*

16) After the bed height, has stabilized close the outlet and allow flow from the top of the column (DO not remove the flow adaptor) and slowly move the top flow adaptor down displacing packing buffer through the three-way inlet valve. Bring the top adaptor down to contact the settle resin bed.

17) Open the outlet valve and re-start flow at 200 cm/h. If the bed settles and shrinks away from the top adaptor, adjust the top adaptor down to accommodate the new bed height.

18) At the final bed height, calculate the column volume. If the bed volume is higher than expected, axial compression can be applied by lowering the top adaptor. The final column volume should be close to the target. If the volume is lower than expected, the original volume of slurry may have been lower or the resin may have packed down more on flow since its compression factor may have been higher than used in the calculation (see step 6)

19) **For automated or very large scale column packing**; assemble the column hardware with the top and bottom flow adaptors in place. Prime the bottom frit assembly to remove air with packing buffer from a pump.

20) Lower the top adaptor to the desired bed height for the packing mode being employed (see above).

21) Fill the column with packing buffer and expel any air in the top adaptor.

22) Close the bottom outlet valve.

23) Prepare the required volume (see step 6) of the resin slurry and re-suspend in the slurry tank.

24) Prime the slurry pump and packing nozzle with slurry.

**Note:** *allow for this volume of slurry in your calculation.*

25) Connect the packing nozzle to the column, open the top inlet valve and initiate flow of slurry into the column.

26) Pump packing buffer at flow rates or pressures up to the limits of the column.

27) Bring the top adaptor down to contact the flow packed bed; A) for flow packing continue flow as described in step 15 above and fix the top adaptor position when the bed height has stabilized. B) for axial compression, the top adaptor is lowered till the target bed height



(calculated as in step 6 allowing form the resin compression factor Cf) is reached. C) for stall packing the top adaptor is set to the target bed height and resin is pumped into the column until high backpressure is reached and the slurry pump stalls.

28) Open outlet valve and re-initiate packing buffer flow through the top inlet valve. Continue flow until the bed height has stabilized (see step 18 for troubleshooting if the bed height is not at the target desired).

29) Check and evaluate the status of packing by measuring HETP and peak symmetry(As) by injection of a small volume (1% of column volume) of an un-retained material (1-2% acetone or 1M NaCl at a 30 cm/h flow rate. Calculate As, N (# of theoretical plates / M column length) from the resulting peak monitoring at 280 nm or by conductivity for NaCl.

## Characteristics of Cellufine IEX Media

The basic characteristics of Cellufine Anion IEX media are summarized in Table 1. All Cellufine IEX media are based on 90 μm (average) cross-linked cellulose beads. Figure 2 shows a typical particle-size distribution for the standard Cellufine A-500 anion exchange resin.

Table 1, Characteristics of Cellufine IEX media

| Properties | A-200 | A-500 | A-800 |
|---|---|---|---|
| Base Matrix | Cross-linked cellulose | | |
| Particle Size (μM) | 40-130 | | |
| IEX chemistry | Weak Anion - DEAE | | |
| Exclusion limit (kDa) | > 30 | > 500 | >1000 |
| Operating Pressure | Up to 0.3 MPa | | |
| IEC capacity (meq/mL resin) | 0.13 – 0.18 | 0.13 – 0.17 | 0.05-0.08 |
| BSA Protein* Binding Capacity (mg/mL resin) | 45 | 58 | 82 |
| IgG Protein** Binding Capacity (mg/mL resin) | 38 | 40 | 68 |

* Anion IEX measured with 1 mg/mL BSA in 50 mM Tris HCl, 50 mM NaCl pH 8.5

** and 1mg/mL IgG in Tris HCL, 50 mM NaCl pH 9.5.

Flow rate 150cm/h in a 5 mmID x 5 cmL column. DBC was estimated at the 10% point on the breakthrough curve.

Figure 2, Particle Size Distribution
Cellufine A-500

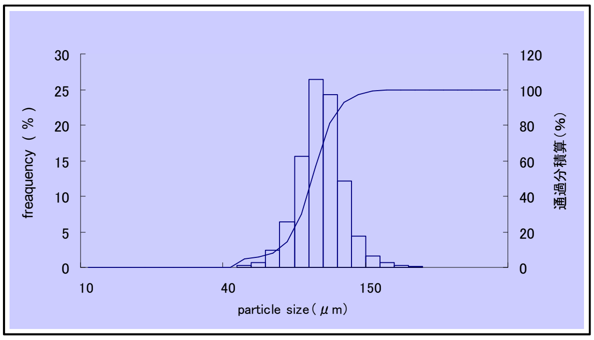
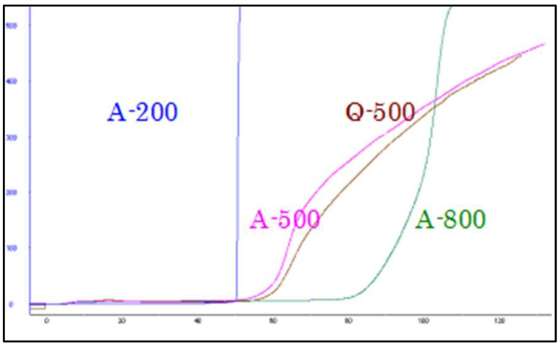



Figure 3, Breakthrough DBC curves for Cellufine
Anion IEX resins




Column: 5 mmID × 5 cmL
Flow rate: 150 cm/h
Sample: 1 mg/ ml
Buffer: 50 mM Tris-HCl (pH 8.5) for A-200, A-500 and A-800
50 mM Tris-HCl (pH 8.0) + 50 mM NaCl for Q-500

**Cellufine A-200** has the smallest pore size in Cellufine IEX resin range. Since the adsorption and desorption of protein occurs on its surface, a typical breakthrough curve is very sharp as shown in Fig. 2. Cellufine A-200 can be used in a flow through polishing mode. **Cellufine A-500** type resins have excellent flow properties for use in commercial processes. These are resins have larger pores, making their IEX surface chemistries available for larger molecule, such as antibodies at 150 kDa molecular weight. **Cellufine A-800** has very large pores, suitable for purification of very large proteins, such as thyroglobulin at a molecular weight of 660kDa.

## Example of flow-packing a 3.2 cm I.D column

- Column: 3.2 cm ID x 25 cm L (Millipore Vantage Column)
- Packing buffer: Pure water (25℃)
- Packing condition: 50 %(v/v) slurry and flow packed at 54 ml/min (403 cm/hr) till bed height was stable.
- Axial Compression; Manual-packing till bed height of 20 cm (target bed volume = 161 mL) was achieved.

The packing and pressure/flow properties of Cellufine A-500 in a 3.2 cm diameter column packed by different Cf with flow packing procedure are summarized in Table 2 and Figure 3. 2 % acetone was used as test probe to evaluate column packing (flow rate of 4 ml/min).

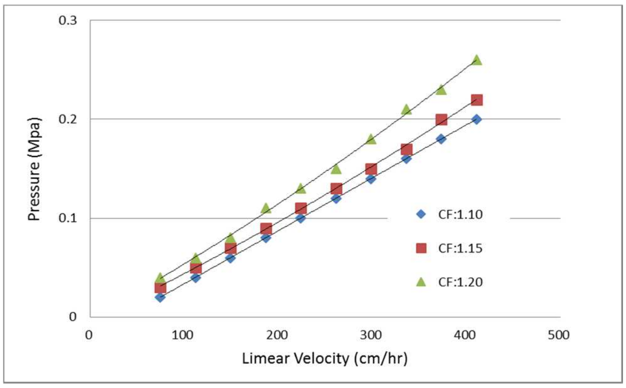




Table 2, Summary of peak properties at a range of resin compression factors

| Cf | N($m^{-1}$) | $A_s$ |
|---|---|---|
| 1.10 | 2,600 | 1.27 |
| 1.15 | 3,200 | 1.17 |
| 1.20 | 2,900 | 1.29 |

Figure 3, Pressure/Flow in a 3.2 cm ID column




In this column format Cellufine A-500 showed good flow properties over a range of packing compression factors (Cf) from 1.10 to 1.20 while keeping the back pressure < 0.3MPa.

## Example of manual packing a 4.4 cm I.D column

- Column: 4.4 cm ID x 25 cm L (Millipore Vantage Column)
- Packing buffer: Pure water (25℃)
- Packing condition: 50 % (v/v) slurry and compressed bed-height corresponding to Cf = 1.15 (24.7 cm → 21.5 cm) by manual-packing (after opening the outlet valve and bringing the top flow adaptor down to 21.5 cm bed height).
- 2%acetone was used as test probe to evaluate column packing (flow rate of 4 ml/min).

Table 3, Packing test of Cellufine A-500 in a 4.4 cm diameter column

| Cf | N($m^{-1}$) | $A_s$ |
|---|---|---|
| 1.15 | 6100 | 0.86 |

## Example of flow packing a 30 cm I.D column

- Column: 30 cm ID x 50 cm L (Easy Column300)
- Packing buffer: Pure water (25℃)
- Packing condition: 50 % (v/v) slurry and flow packed at 0.15, 0.20 and 0.25 MPa for 30 min (after open the column outlet valve)
- Evaluation of column packing:

1. Equilibrate the column at 75 cm/hr (0.83 L /min) for 1CV.

2. Inject 150 mL of 2 % acetone (Mobile Phase: pure water) at a flow rate of 30 cm/h,
3. Elute at the same flow rate for 1.3 CV.

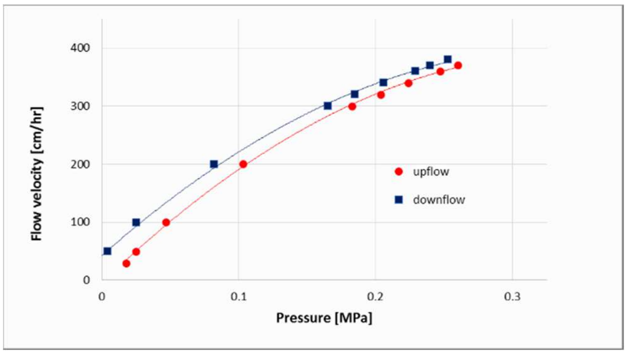
The packing and pressure/flow properties of Cellufine A-500 in a 30-cm diameter column packed under different pressure with flow packing procedure are summarized in Table 4 and Figure 4

Table 4, Packing properties for a 30 cm ID column

| Pressure (MPa) | Cf | N($m^{-1}$) | $A_s$ | RPH* |
|---|---|---|---|---|
| 0.15 | 1.125 | 4,600 | 1.25 | 2.41 |
| 0.20 | 1.150 | 5,300 | 1.20 | 2.12 |
| 0.25 | 1.175 | 5,500 | 1.37 | 2.03 |

* Reduction of theoretical plate height

Figure 4, Pressure/Flow properties for a flow packed 30 cm ID column




This data suggests that an optimal compression factor for packing Cellufine A-500 in a 30 cm ID column was 1.125 to 1.175. In Figure 4 above, a pressure - flow velocity curve of Cellufine A-500 packed in a 30 cm I.D. column is shown with flow in both directions.

## Conclusion

Cellulose is well-known as a natural product having unique crystalline molecular structure which imparts chemicals stability and mechanical strength on spherical chromatography beads. Cellufine IEX resins can be easily packed into a range of large volume column sizes under flow, axial and stall packing formats. Cellufine A-500 resin exhibits compression factors (Cf) in the range 1.125 to 1.200, while giving high volumetric flow up to 400 cm/h linear velocity while keeping the backpressure < 0.3MPa.

## Ordering Information

| Description | Quantity | Catalogue No. |
|---|---|---|
| Cellufine A-200 | 5 x 1 ml cartridge | 19611-51 |
| | 100 mL | 676980327 |
| | 500 mL | 19611 |
| | 5 L | 19612 |
| | 10 L | 676980335 |
| Cellufine A-500 | 5 x 1 ml cartridge | 19805-51 |
| | 1 x 5 mL cartridge | 19805-15 |
| | 100 mL | 675980327 |
| | 500 mL | 19805 |
| | 5 L | 19806 |
| | 10 L | 675980335 |
| Cellufine A-800 | 5 x 1 ml cartridge | 19865-51 |
| | 1 x 5 mL cartridge | 19865-55 |
| | 100 mL | 673980327 |
| | 500 mL | 19800 |
| | 5 L | 19801 |
| | 10 L | 673980335 |
| Cellufine Q-500 | 5 x 1 ml cartridge | 19907-51 |
| | 1 x 5 mL cartridge | 19907-55 |
| | 100 mL | 675982327 |
| | 500 mL | 19907 |
| | 5 L | 19908 |
| | 10 L | 675982335 |
| Cellufine C-500 | 5 x 1 ml cartridge | 19800-51 |
| | 1 x 5 mL cartridge | 19800-55 |
| | 100 mL | 675983327 |
| | 500 mL | 19865 |
| | 5 L | 19866 |
| | 10 L | 675983335 |
| Cellufine S-500 | 5 x 1 ml cartridge | 21200-55 |
| | 1 x 5 mL cartridge | 21200-15 |
| | 100 mL | 21200 |
| | 500 mL | 21201 |
| | 5 L | 21202 |
| | 10 L | 21203 |

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