**Mixed Mode Chromatography Media**

# Cellufine™ MAX AminoButyl

## DESCRIPTION

Mixed mode chromatography media are well-known to have unique selectivity differences from traditional IEX or HIC resins. JNC corporation has developed a new mix-mode resin, Cellufine MAX AminoButyl, for virus or virus like particles (VLPs) purification. This resin has both salt tolerant primary amine and hydrophobic butyl group as ligands. Especially ligand density of butyl group is optimized for adsorption/desorption of VLPs having strong hydrophobicity. This mixed mode resin is built on the cross-linked Cellufine cellulose base bead that is very stable, resistant to base CIP and can be operated under high flow modes with minimal back pressure.

### Properties of Cellufine MAX AminoButyl

| Properties | Values |
|---|---|
| Base bead matrix | Highly cross-linked cellulose |
| Particle size | 90 µm average (40 to 130 µm) |
| Microscopic test (%) | < 5 |
| Ligand | A primary amine and a butyl group |
| N contents as amine group | 20 to 30 µmol/mL |
| Elution volume (mL)<br>α-chymotrypsinogen A (HIC mode)<br>Pepsin (IEX mode) | <br>12 to 17<br>12 to 17 |
| CIP | 0.5 M NaOH |
| Operating pressure | < 0.3 MPa |
| Recommended cleaning conditions | Ethanol (70%), Isopropanol (30%), Guanidine HCl (6M) and Urea (6M) |

## Packing to the column

1. Calculate volume required for the desired bed dimension.
   (a) Packed bed volume = column cross sectional area ($cm^2$) x bed height (cm)
   (b) Required sedimented gel volume ≒ Packed bed volume x 1.5
   (c) The slurry concentration in the bottle of Cellufine MAX AminoButyl is approximately 50 % in 20 % ethanol.
2. (If necessary, Cellufine MAX AminoButyl can be packed with 20 % ethanol.) Wash the gel with water or the appropriate buffer.
3. (If necessary) Prepare a 40 to 60 % (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer. Equilibrate at ambient temperature for one hour.

4. Gently stir. If required place under vacuum to degas.
5. Assemble column
   (a) A column is prepared according to the instructions from the column supplier.
   (b) The bed support should be wetted in a packing solution or 20 % ethanol before use to remove air.
   (c) Pour the packing solution into the column tube and it check that solution flows out from the column exit. Shut the exit valve when approximately 0.5 to 1 cm height of the solution remains.
6. Carefully pour the slurry into the column without creating air bubbles. Depending on the volume, a filler tube may be necessary.
7. Mount the top adapter on the top of column. (Be careful to not entrain air)
8. Open the column outlet and begin pumping elution buffer at 0.25 to 0.3 MPa for 30 to 60 min with flow packing. The final column volume should be close to the target calculated by the suitable compression factor (*Cf*) of the resin.

   ***Cf of Cellufine MAX AminoButyl = 1.15 – 1.30***

   *Cf = gravity settled bed height/flow packed bed height.*
9. Caution: do not excess the operation pressure limit for the selected column.
10. Mark the gel bed height. Stop the pump and shut the column outlet valve.
11. Disconnect the top adapter line from the pump. Loosen the adaptor seal and move the top adapter down to mark the gel bed reached at packing.
12. After the bed stabilizes, lock the adapter and reconnect the line from the pump. Equilibrate with 5 to 10 column volumes of adsorption buffer before sample loading.

### Evaluation of packing

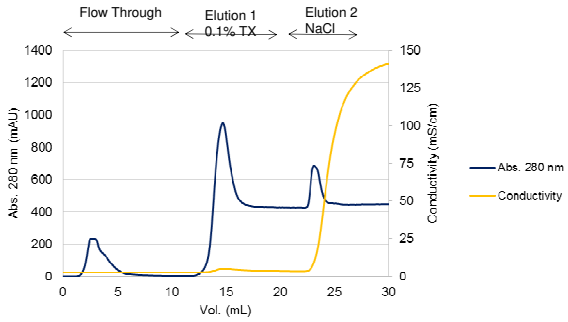
See appendix 1

## Operating Guidelines

### General Operation

Cellufine MAX AminoButyl has two different properties based on anion exchange mode and HIC mode. Because of these properties, the adsorption/desorption performance of the resin is strongly affected by pH and conductivity (ionic strength). Operation condition with the resin should be carefully optimized before use.

Figure 1. Partial purification of r HBsAg with Cellufine MAX AminoButyl




Column: 6.6 mm x 30 mmL (R.T.= 2 min)
Equilibration and wash: 20 mM Phosphate buffer (pH7.0)
Elution (1): 20 mM Phosphate buffer (pH7.0) + 0.1 % TritonX
Elution (2): 20 mM Phosphate buffer (pH7.0) + 2 M NaCl

| | VLP | | Protein | |
|---|---|---|---|---|
| | nU | % | ug | % |
| Load | 4260 | 100 | 2320 | 100 |
| Flow through | 480 | 11 | 350 | 13 |
| Elution 1 | 2060 | 48 | 770 | 30 |
| Elution 2 | 172 | 4 | 1190 | 46 |

Anion exchange mode is recommended to use for virus particles or VLPs purification with Cellufine MAX AminoButyl. Target molecules are adsorbed to the resin under low ionic strength condition or normal buffer such as phosphate buffer. Target molecules can be eluted by not only salt but also detergents easily. Figure 1 showed the results of partial purification of r-HBsAg VLPs. The VLPs were

adsorbed to the resin under 20 mM phosphate bugger and eluted by 0.1 % detergent (Triton X).

## Sample Preparation and Load

Prepare sample by centrifugation or microfiltration to remove insoluble material. Samples should be prepared to a concentration of 1 to 20 mg/mL, in binding buffer or at comparable conditions of ionic strength and pH. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography.

## Recommended Buffers

Adsorption buffer: 0.01 to 0.05 M phosphate or Tris-HCl (pH 6 to 9), acetate or citrate (pH 3 to 5) + 0 to 0.15 M NaCl.

Elution buffer: Detergent or 0.1 to 2.0 M sodium chloride in adsorption buffer.

Other common buffer systems may be used.

## Regeneration and equilibration

After separation, wash the binding material with over 5-bed volumes of high ionic strength solution (1 to 2 M NaCl). After washing the column, pump an additional 5-bed volume of adsorption buffer, or until the column eluate will be stable pH and conductivity values.

## Depyrogenation

Wash the column with 5-bed volumes of 0.2 M NaOH let stand for 16 hours, and then wash with endotoxin-free water or equilibration buffer.

0.2 M NaOH - 20 % ethanol is effective for endotoxin removal. Moreover 0.2 M NaOH - 90 % ethanol can rapidly decrease LPS, with contact for at least 2 hours.

## Chemical and Physical Stability

Stable in:

Most salts (NaCl, $(NH_4)_2SO_4$, etc.), Alcohol (30 % (v/v) IPA, 70 % (v/v) ethanol), Urea (6 M) and Guanidine-HCl (6 M)

pH 2 to 12 at 20 ºC, 1week

## Cleaning-in-place (CIP)

0.5 M NaOH solution is recommended for CIP of Cellufine MAX AminoButyl. When CIP is not enough, the packed column soaked in 0.1 M (up to 0.5 M) NaOH for overnight may be useful. Otherwise, washing by alkaline or acetic solution (for example 0.5 M NaOH or acetic acid) including 0.1 to 0.5 % of nonionic detergent or organic solvents (for example, 30 % ethanol or 27 % IPA) including alkaline may be useful.

## Flow Rate

Cellufine MAX AminoButyl is based on highly cross-linked cellulose gel, and are stable at high flow velocities.

1,000 cm/h in a 2.2 cm diameter column with 20 cm bed height at < 0.3 MPa.

Over 500 cm/h 30 cm diameter column with 20 cm bed height at < 0.3 MPa.

## Storage

Store unopened container at ambient temperature. Do not freeze.

Short-term storage for bulk and column (2 weeks or less) can be at a room temperature with pH 2 to pH 12. It is possible to store under alkaline condition at less than 20 °C as recommendation.

Longer storage should be in neutral buffer containing 20 % ethanol, at 2 to 25 °C. Do not freeze.

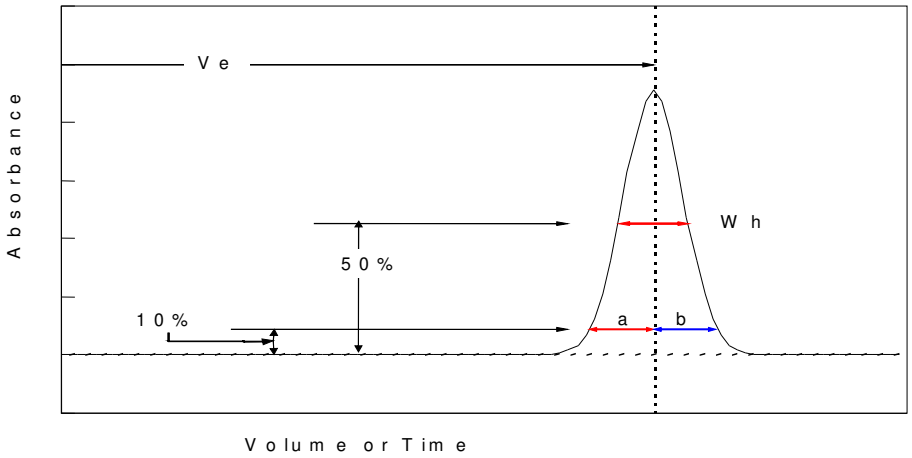
# Appendix 1: Evaluation of column packing of Cellufine

Evaluation methods for the packing status of a column can employ indices, such as the number of (theoretical) plates (N), the height equivalents to a theoretical plate (HETP), and a symmetry factor (As).

The selected evaluation indices are affected by the measurement condition. For example, they vary with changes in the difference between the diameter/height of a column, the difference in piping, solvent sample volume, the flow velocity, temperature, etc.

Therefore, it is necessary to use the same measurement conditions each time.

| Conditions | |
|---|---|
| Sample volume | 1%（MAX 2.5 %）of　column bed volume |
| Sample concentration | 1-2 %(V/V) 1 M NaCl |
| Flow rate | ~30cm/h ( X mL/hr/column cross section ) |
| Detector | UV, conductivity |




**Formula**

**HETP = L/N**

**$N = 5.54 \times (Ve/Wh)^2$**

**As = b/a**

| | |
|---|---|
| L | Column length [ cm or m ] |
| Ve | Elution time or volume |
| Wh | Half of width of peak |
| a,b | Peak width of 10% peak height (a)front, (b)rear |
| Note | Ve,Wh and a, b should have same dimensional units |

(Note)

Generally, a larger value of N is good. (Likewise a smaller value of HETP is good.)

The asymmetry factor value (As) should be close to 1. Generally, acceptable symmetry values range from 0.8 -1.6.