Technical Data Sheet
MIX MODE; Cellufine MAX AminoButyl

JNC offers unique mix-mode resin, Cellufine MAX AminoButyl, which is developed mainly for concentration of molecules having strong hydrophobicity such as VLPs (virus like particles). We developed a new, highly cross-linked base matrix and have applied to Cellufine MAX series. Cellufine MAX AminoButyl is designed on the basis of optimized Cellufine MAX Butyl to improve recovery of target molecules.

Partial structure of Cellufine MAX AminoButyl

Ligand structure and specification of Cellufine MAX AminoButyl is described in Figure 1 and Table 1 respectively.

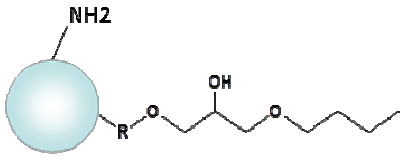
Cellufine MAX AminoButyl	

Fig.1. Ligand Structure of Cellufine AminoButyl

Table 1. Specification of Cellufine MAX AminoButyl

Item	
N contents (μ mol/ml)	20 - 33
Elution volume (ml) α-chymotrypsinogen A (HIC mode) Pepsin (IEX mode)	 12.0 - 17.0 12.0 - 17.0
Microscopic test (%)	< 5

Characteristics of Cellufine MAX AminoButyl

The basic characteristics of Cellufine MAX AminoButyl are shown in Table 2. The media are based on 90 μm (average) highly cross-linked cellulose beads for suitable use in bio-pharmaceuticals manufacturing processes.

Table 2. Characteristics of Cellufine MAX AminoButyl

Matrix	Highly cross-linked cellulose
Particle size	90μm (40 – 130 μm)
Ligand	Butyl + primary Amine
Protein adsorption (1) in 2M $(NH_4)_2SO_4$	α-Chymotripsinogen A; + Ribonuclease A; − Lysozyme; − +; adsorb, -; not adsorb
Protein adsorption (2) in 20 mM Tris-HCl (pH7.5)	Transferrin; − BSA; + Pepsin; + +; adsorb, -; not adsorb

As base matrix is highly cross-linked cellulose particles, Cellufine MAX AminoButyl shows superior flow property like figure 2.

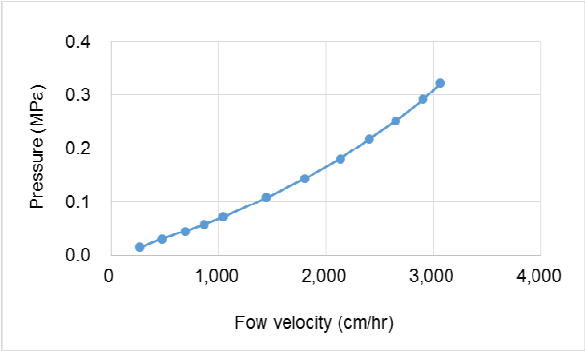


Fig.2. Pressure flow curve of Cellufine MAX AminoButyl

(Condition: Column; 2.2 cm ID x 20 cm, Mobile phase; Pure water (24±1℃)

Ligand density of butyl substance in Cellufine MAX AminoButyl is quite low in comparison with Cellufine MAX Butyl. Figure 3 and 4 show typical model protein separation with Cellufine MAX Butyl and Cellufine MAX AminoButyl under condition of hydrophobic mode. Only α-Chymotripsinogen A is adsorbed to Cellufine MAX AminoButyl in high salt solution such as 2 M ammonium sulfate.

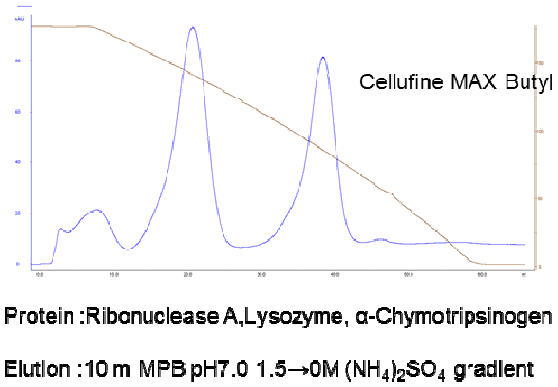


Fig.3. Typical model protein separation curve of Cellufine MAX Butyl

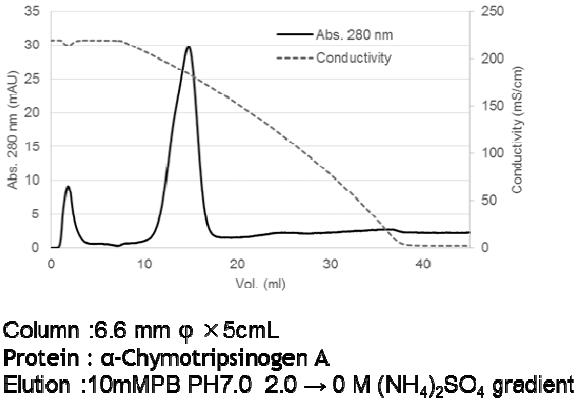


Fig.4. Typical model protein separation curve of Cellufine MAX AminoButyl

In general, surface of VLPs is well known to have strong hydrophobicity. Cellufine MAX AminoButyl is designed in order to occur effective adsorption-desorption in the surface of VLPs. Further Cellufine AminoButyl has unique characteristic to add primary amine as a ligand. Primary amine is used as an anion exchange ligand. Because of this ligand effect, the resin can bind a target molecule in low conductivity condition or standard buffer such as phosphate buffer. Thus target molecule is eluted by not only salt but also detergents.

Application of Cellufine MAX AminoButyl

Purification of r-HBsAg (recombinant Hepatitis B surface antigen) VLPs from yeast with Cellufine AminoButyl

Partial purified r-HBsAg VLPs solution was loaded on packed Cellufine MAX AminoButyl column (16 mm I.D. x 500 mm H) and then column was washed by 20 mM phosphate buffer (pH 7.0) sufficiently. At first, phosphate buffer (pH 7.0) containing 0.1 % Triton X was used as elution solution (Elution 1). And then molecules was eluted by 2 M NaCl (Elution 2). Each fraction was recovered and analyzed.

The figure below showed a chromatogram of purification of r-HBsAg VLPs with Cellufine AminoButyl. r-HBsAg VLPs was detected by ELISA assay.

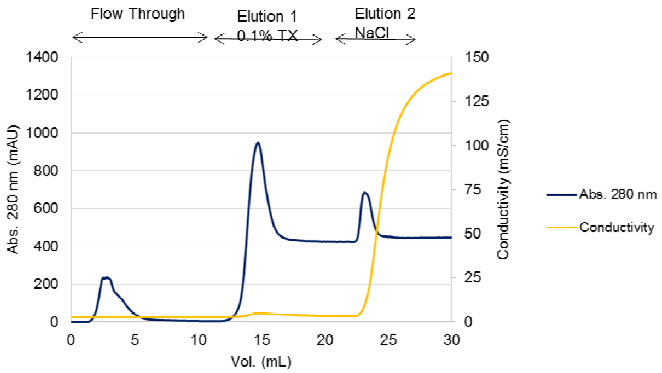


Table below showed the results in this test.

	VLP		Protein	
	nU	%	μg	%
Load	4,260	100	2,320	100
Flow through	480	11	350	13
Elution 1	2,060	48	770	30
Elution 2	172	4	1,190	46

Most r-HBsAg was obtained in elution 1. Results of protein assay in fraction suggested further purified r-HBsAg was concentrated by detergent elution. The results suggested Cellufine MAX AminoButyl is useful to purify VLPs.

Chemical Stability and Cleaning-In-Place

Cellulose is well-known as a natural product having chemical and physical stability. Thus, since Cellufine is derived from cellulose, it also is stable to chemicals and caustic and acidic solutions. CIP of all Cellufine media can be carried out with 0.5 M NaOH solution. Used media should be stored in 20 % ethanol at 2-25 ℃ after cleaning.

- ✓ Ethanol (70%)
- ✓ Isopropyl alcohol (30%)
- ✓ Guanidine hydrochloride (6M)
- ✓ Urea (6M)
- ✓ NaOH (0.5M)
- ✓ Detergents
- ✓ Autoclave (121 ℃, 20 min)