

# DEVELOPMENT OF A HIGH CAPACITY MIXED MODE RESIN FOR HIGH CONDUCTIVITY MAB FEEDSTOCKS

## INTRODUCTION

Cation exchange resins are used in many protein purifications. The high capacity of these resins is very important to process throughput particularly as protein expression levels reach titers greater than 10 g/L.

In many cases the feedstock may need to be diluted prior to loading onto a cation exchange resin to maintain the high protein dynamic binding capacity (DBC) reported by the manufacturer. The additional water, buffer volumes and process time, adds cost to the loading step.

The mixed mode resin TOYOPEARL® MX-Trp-650M shows both high DBC and tolerance to feedstocks with conductivities up to 17 mS/cm. It has been engineered to have excellent elution kinetics.

The data shows that TOYOPEARL MX-Trp-650M resin:

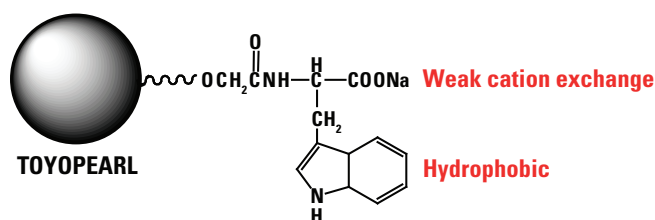
- Tolerates high conductivity feedstocks with minimal dilution required during loading.
- Exhibits mAb DBCs as high as 90-100 g/L.
- Results in improved resolution and peak shape compared to agarose materials.
- Minimizes process pool volumes with both fast binding and fast elution kinetics.

TOYOPEARL MX-Trp-650M is the optimal resin for protein capture steps where the selectivity and protein clearance needed is lower than with affinity resins. This novel resin an excellent choice for intermediate purifications.

## RESIN STRUCTURE

TOYOPEARL MX-Trp-650M uses tryptophan as the active ligand (Figure 1). This amino acid has both weak carboxyl cation exchange and indole hydrophobic functional groups.

## TOYOPEARL MX-Trp-650M STRUCTURE



Product name: TOYOPEARL MX-Trp-650M  
 Particle size: 50-100 µm

Figure 1

The 50-100 µm particle size of this resin is very useful for capture or intermediate purification steps.

## DBC AT HIGH FEEDSTOCK CONDUCTIVITIES

Table 1 shows the DBC of TOYOPEARL MX-Trp-650M at two feedstock conductivities: 12 mS/cm and 17 mS/cm. For comparison purposes, data for a Brand M agarose resin is also shown.

## DYNAMIC BINDING CAPACITIES AT HIGH CONDUCTIVITIES

RESIN	PARTICLE SIZE (µm)	ION EXCHANGE CAPACITY (meq)	DBC (g/L)	RECOVERY %
TOYOPEARL MX-Trp-650M (12mS/cm)	50-100	0.12	95	97
TOYOPEARL MX-Trp-650M (17mS/cm)	50-100	0.12	48	96
Brand M (Agarose 12mS/cm)	75 (median)	0.24	14	86
Brand M (Agarose 17mS/cm)	75 (median)	0.24	11	85

Table 1

Resins: TOYOPEARL MX-Trp-650M, Brand M; Column size: 6 mm ID × 4 cm L; Mobile phase: Buffer (12 mS/cm): 0.05 mol/L acetate buffer (pH 4.3, 4.7, 5.0) + 0.10 mol/L NaCl; Buffer (17 mS/cm): 0.05 mol/L acetate buffer (pH 4.3, 4.7, 5.0) + 0.15 mol/L NaCl; Flow rate: 1.0 mL/min (212 cm/hr); Detection: UV @ 280nm; Sample: human polyclonal IgG (1mg/mL); DBC calculated from 10% height of breakthrough curve.

## DBC AT VARYING pH

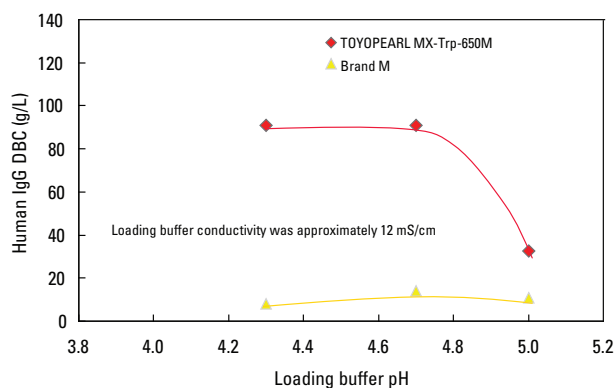


Figure 2

Resins: TOYOPEARL MX-Trp-650M, Brand M; Column size: 6 mm ID × 4 cm L; Mobile phase: Buffer A: 0.05 mol/L acetate buffer (pH 4.3, 4.7, 5.0) + 0.10 mol/L NaCl (12 mS/cm); Buffer B: 0.1 mol/L Tris-HCl buffer (pH 8.5) + 0.3 mol/L NaCl; Flow rate: 1.0 mL/min (212 cm/hr); Detection: UV @ 280nm; Sample: human polyclonal IgG (1mg/mL) DBC calculated from 10% height of breakthrough curve.

For the 12 mS/cm and 17 mS/cm measurements, the TOYOPEARL MX-Trp-650M resin shows almost 7× higher and 4× higher DBC, respectively, than Brand M.

DBC is maintained in 12 mS/cm loading buffers up to pH 4.8 (Figure 2).

### MASS TRANSFER PARAMETERS

The mass transfer properties of a resin influence the economics of the loading and elution stages of a capture step, and the degree of resolution for intermediate purification. In keeping with the exceptional target binding and eluting properties of Tosoh's newer TOYOPEARL GigaCap® resins, the TOYOPEARL MX-Trp-650M also shows a narrow elution peak width to complement its higher capacity (Figure 3).

The mass transfer properties also contribute to minimal peak broadening when doing chromatography. Figure 4 shows the excellent peak shape for the new TOYOPEARL MX-Trp-650M and the much broader tailing associated with the Brand M agarose material.

### RESIN CLEAN IN PLACE (CIP)

The new TOYOPEARL MX-Trp-650M resin has excellent stability to 0.5 mol/L NaOH and can be run for many CIP cycles without losing its high capacity (Figure 5).

### CONCLUSION

TOYOPEARL MX-Trp-650M resin is a high capacity and high performance mixed mode resin for intermediate purification. The new product has 3-5 × the DBC of typical mixed mode resins so reductions in process buffer and pool volumes can be expected. The ability of this novel resin to bind protein from high conductivity feedstock minimizes the amount of water needed for dilution before loading the column.

### NARROW ELUTION PEAK WIDTHS

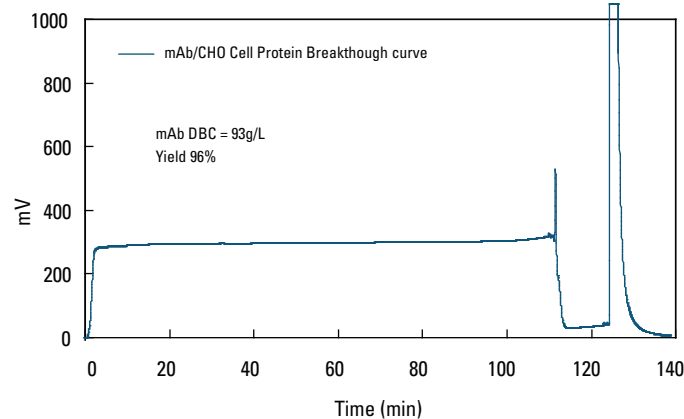


Figure 3

Resin: TOYOPEARL MX-Trp-650M; Column size: 6 mm ID × 4 cm L; Mobile phase: Buffer A: 0.05 mol/L acetate buffer (pH 4.7) + 0.1 mol/L NaCl (12 mS/cm); Buffer B: 0.1 mol/L Tris-HCl buffer (pH 8.5 + 0.3 mol/L NaCl; Flow rate: A: 1.0 mL/min (212 cm/h); B: 2.0 mL/min (started at 124 min); Detection: UV @ 280nm; Sample: CHO cell culture media, monoclonal antibody (1 mg/mL) diluted with Buffer A

### GOOD RESOLUTION FOR INTERMEDIATE PURIFICATION

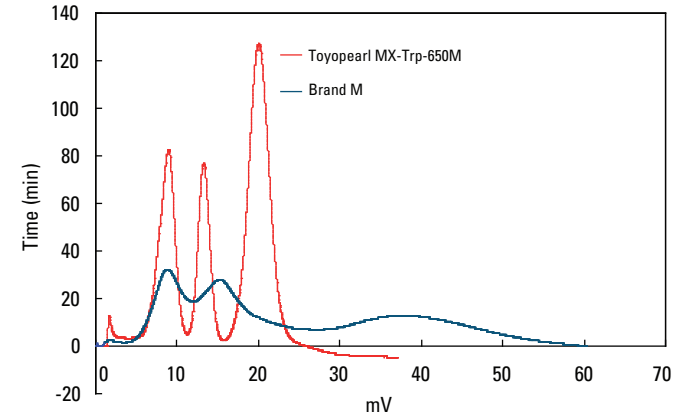


Figure 4

Resins: TOYOPEARL MX-Trp-650M, Brand M; Column size: 7.5 mm ID × 7.5 cm L; Mobile phase: Buffer A: 20 mmol/L phosphate (pH 7.0); Buffer B: 20 mmol/L phosphate + 1.0 mol/L NaCl (pH 7.0); Gradient: 30 min. linear gradient from buffer A to buffer B; Flow rate: 1.0 mL/min; Detection: UV @ 280nm; Sample: trypsinogen (6.6 mg/mL) cytochrome C (3.6 mg/mL) lysozyme (6.6 mg/mL); Sample vol.: 25 µL

### STABILITY IN 0.5 mol/L NaOH

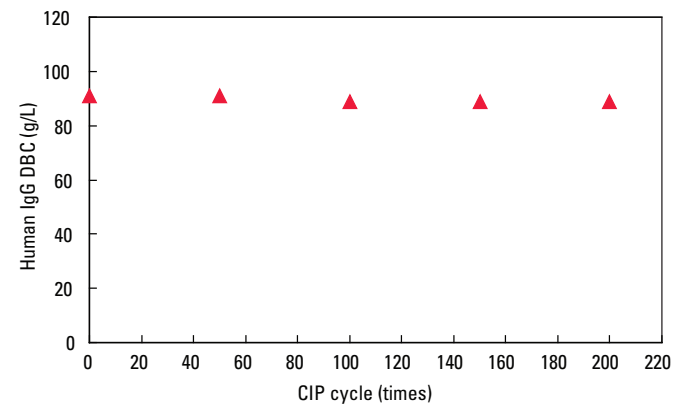


Figure 5

Alkaline cleaning (CIP) conditions  
 3CV: 0.5 mol/L NaOH,  
 5CV: 0.1 mol/L Tris-HCl pH 8.5 + 0.3 mol/L NaCl  
 Flow rate: 1  
 DBC Measurement  
 Column Size: 6 mm ID × 4 cm L; Binding buffer: 0.05 mol/L acetate buffer (pH 4.7) + 0.1 mol/L NaCl; Flow rate: 1 mL/min.; Detection: UV @ 280 nm; Sample: polyclonal human IgG; Sample Load: 1 mg/mL

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