

# TOYOPEARL® MX-Trp-650M Resin Selectivity

## TOYOPEARL APPLICATION NOTE

TOYOPEARL MX-Trp-650M, a new, mixed-mode chromatography resin combining a weak cation exchange and a hydrophobic ligand is the latest addition to the TOYOPEARL product line. This resin, capable of being run much the same as a standard cation exchanger or in a more traditional HIC mode, truly lives up to the mixed-mode moniker.

### Introduction

Chromatographic resins with high capacities and selectivities differing from those seen with traditional hydrophobic interaction and ion exchange media are now in demand. Mixed-mode media offers an alternative to traditional single-mode chromatography media. The polymethacrylic base bead (TOYOPEARL HW-65) is chemically modified with the amino acid tryptophan, which combines a weak cationic group with a hydrophobic functional group. The resulting resin exhibits dynamic binding capacities of approximately 90 mg/mL for human IgG. TOYOPEARL MX-Trp-650M offers chromatographers selectivity with binding capacities that are similar to traditional cation exchange resins.

### Experimental Conditions

To evaluate the selectivity of TOYOPEARL MX-Trp-650M in comparison to a traditional weak cation exchange (TOYOPEARL GigaCap® CM-650M) and a traditional strong cation exchange (TOYOPEARL GigaCap S-650M) resin, 6.6 mm ID × 15.5 ± 1.0 cm columns were packed with new resin. A three protein mixture (trypsinogen, cytochrome C, and lysozyme) was loaded onto the column in 20 mmol/L sodium phosphate buffer (pH 7.0) and eluted with a linear salt gradient (Figure 1). Resolution between the peaks was measured and recorded for comparison (Table 1).

Further selectivity comparisons were done at decreasing pH levels for all three resins with the same protein mixture at pH 6.0 (20 mmol/L sodium acetate) and pH 5.0 (20 mmol/L sodium citrate) and were compared to the initial screening at pH 7.0 (Figures 2-4). Resolution between the peaks was likewise measured and recorded for comparison (Tables 2-4).

To examine the role the hydrophobic region of the tryptophan ligand can play in protein separations on TOYOPEARL MX-Trp-650M, the following experiments were carried out using this resin as a traditional HIC media. First, the resin was tested to determine if it was possible to be used in HIC mode by loading lysozyme onto the column in 10 mmol/L sodium citrate, 1.8 mol/L ammonium sulfate, pH 5.0. The bound lysozyme was eluted with a decreasing linear gradient of 10 mmol/L sodium citrate, pH 5.0 (Figure 5). Having established that the TOYOPEARL MX-Trp-650M resin could function as a traditional HIC resin, binding capacity and basic selectivity experiments were carried out.

Figure 1. Initial Selectivity Screening

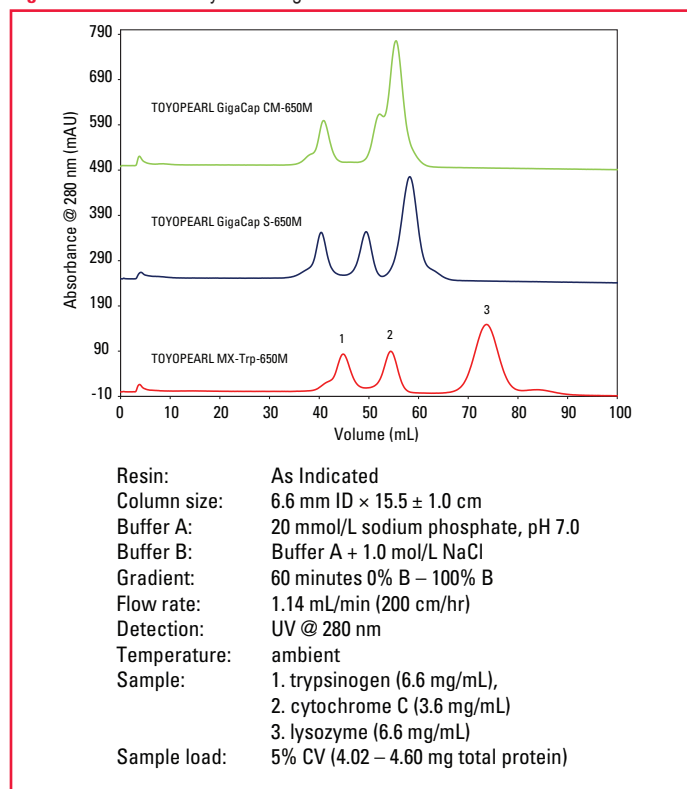
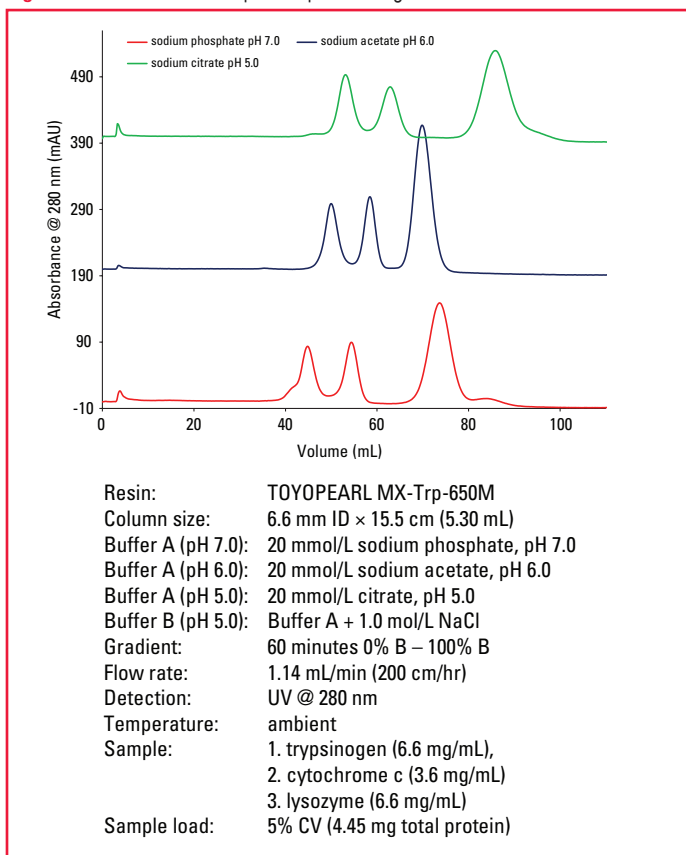
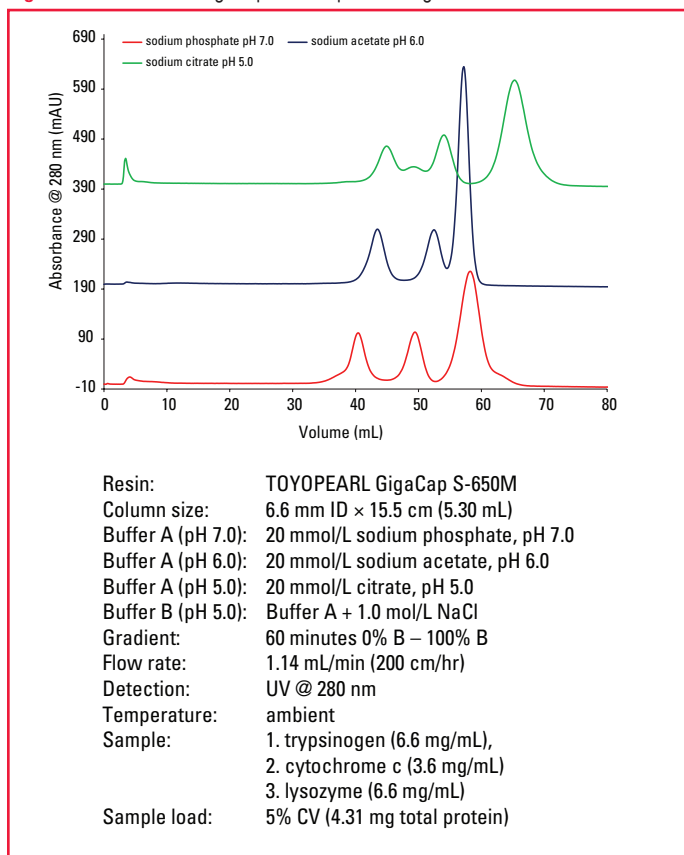


Table 1. Initial Selectivity Screening Peak Resolutions

Resin	Peak Resolution	
	Trypsinogen/Cytochrome C	Cytochrome C/Lysozyme
TOYOPEARL MX-Trp-650M	0.81	1.50
TOYOPEARL GigaCap S-650M	0.94	0.82
TOYOPEARL GigaCap CM-650M	1.40	0.43

For the determination of dynamic binding capacity, TOYOPEARL MX-Trp-650M was packed in a 6.6 mm ID × 8.5 cm (2.74 mL) column. Lysozyme (0.5 mg/mL) in 20 mmol/L sodium citrate, 1.8 mol/L ammonium sulfate, pH 5.0, was loaded onto the column at 200 cm/hr. The dynamic binding capacity was measured at 10% breakthrough by absorbance at 280 nm. Comparison of resin selectivity in HIC mode and weak cation mode was done using a three protein mix (ribonuclease A, α-chymotrypsinogen, and lysozyme) at pH 5.0 with sodium citrate as the mobile phase buffering salt (Figure 6-7).



**Figure 2.** TOYOPEARL MX-Trp-650M pH Scouting**Figure 3.** TOYOPEARL GigaCap S-650M pH Scouting**Table 2.** TOYOPEARL MX-Trp-650M pH Scouting Peak Resolutions

	Trypsinogen Retention (mL)	Cytochrome C Retention (mL)	Trypsinogen/ Cytochrome C Resolution (Rs)	Lysozyme Retention (mL)	Cytochrome C/ Lysozyme Resolution (Rs)
Phosphate pH 7.0	44.88	54.36	0.81	73.63	1.50
Acetate pH 6.0	50.01	58.45	0.89	69.87	1.04
Citrate pH 5.0	53.08	62.94	1.07	85.97	1.57

**Table 3.** TOYOPEARL GigaCap S-650M pH Scouting Peak Resolutions

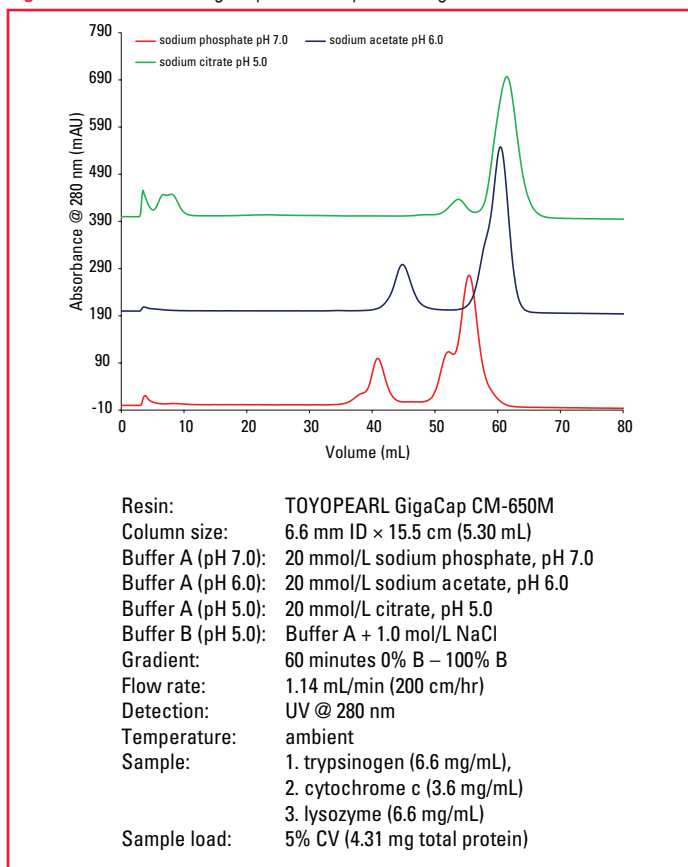
	Trypsinogen Retention (mL)	Cytochrome C Retention (mL)	Trypsinogen/ Cytochrome C Resolution (Rs)	Lysozyme Retention (mL)	Cytochrome C/ Lysozyme Resolution (Rs)
Phosphate pH 7.0	40.38	49.46	0.94	58.27	0.82
Acetate pH 6.0	43.44	52.46	1.16	57.20	0.75
Citrate pH 5.0	44.96	54.05	1.23	65.29	1.00

## Results

The order of elution of the proteins for each of the chromatograms is as follows: trypsinogen, cytochrome C, and lysozyme. Two of the three resins were able to separate all three proteins (Figure 1). The third resin, TOYOPEARL GigaCap CM-650M, showed reduced selectivity compared to the TOYOPEARL MX-Trp-650M and the TOYOPEARL GigaCap S-650M resins (Table 1). As the pH was decreased, the proteins were generally more strongly retained, requiring greater concentrations of NaCl to desorb them (Figure 2-4) while maintaining selectivity and resolution (Table 2-3). The TOYOPEARL GigaCap CM-650M was the exception to this observation (Table 4).

The initial functionality screening of TOYOPEARL MX-Trp-650M indicates that the resin was capable of operating as either a salt tolerant cation exchanger or as a traditional hydrophobic interaction chromatography resin (Figure 5). The lysozyme can be bound at high salt concentration and desorb as the conductivity is increased or it can be bound at low salt and desorb as the conductivity is increased.

**Figure 4.** TOYOPEARL GigaCap CM-650M pH Scouting

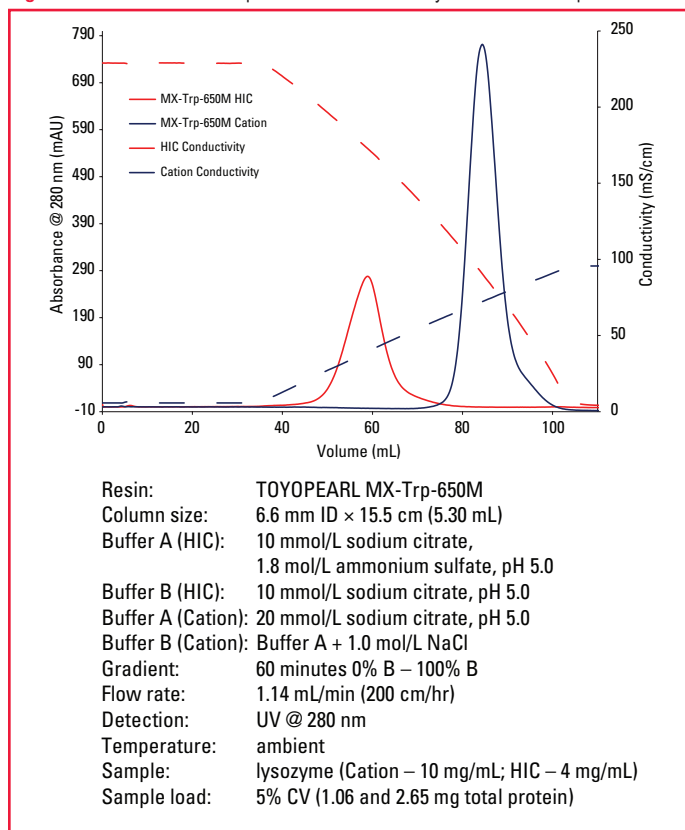


**Table 4.** TOYOPEARL GigaCap CM-650M pH Scouting Peak Resolutions

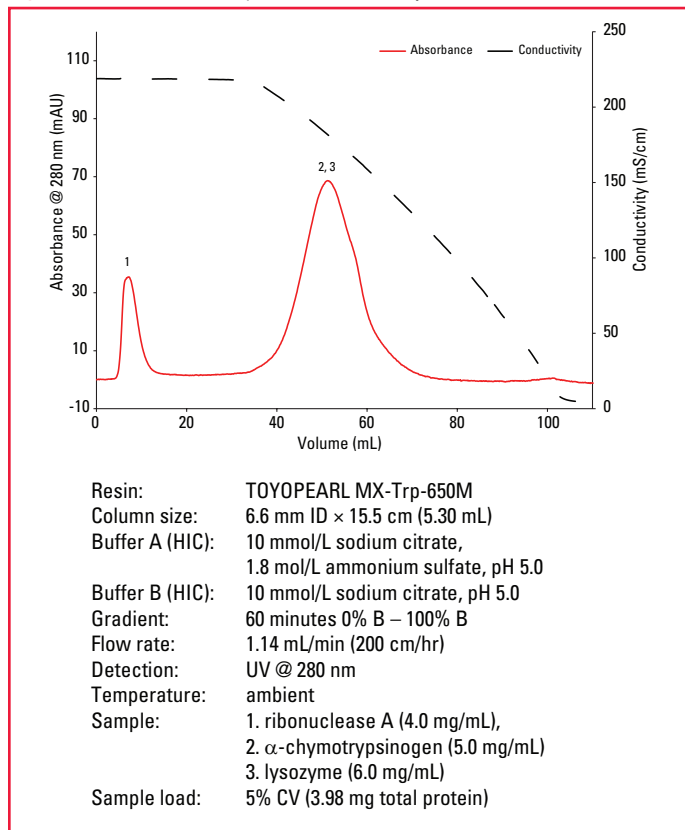
	Trypsinogen Retention (mL)	Cytochrome C Retention (mL)	Trypsinogen/Cytochrome C Resolution (Rs)	Lysozyme Retention (mL)	Cytochrome C/Lysozyme Resolution (Rs)
Phosphate pH 7.0	40.89	52.20	1.40	55.45	0.43
Acetate pH 6.0	44.81	60.46	1.18	60.46	0
Citrate pH 5.0	53.71	61.46	0.84	61.46	0

At 212 cm/hr, TOYOPEARL MX-Trp-650M has a dynamic binding capacity of 3.80 mg/mL for lysozyme at pH 5.0. While this capacity is very low, it does demonstrate that the resin has the ability to function as a traditional HIC resin. TOYOPEARL MX-Trp-650M exhibits different selectivities depending on the mode being used. When run as a cation exchanger, the resin is able to resolve all three proteins into individual peaks. When used in HIC mode, the resin is only able to resolve one of the proteins from the other two in the mixture under the conditions tested (Figure 6-7).

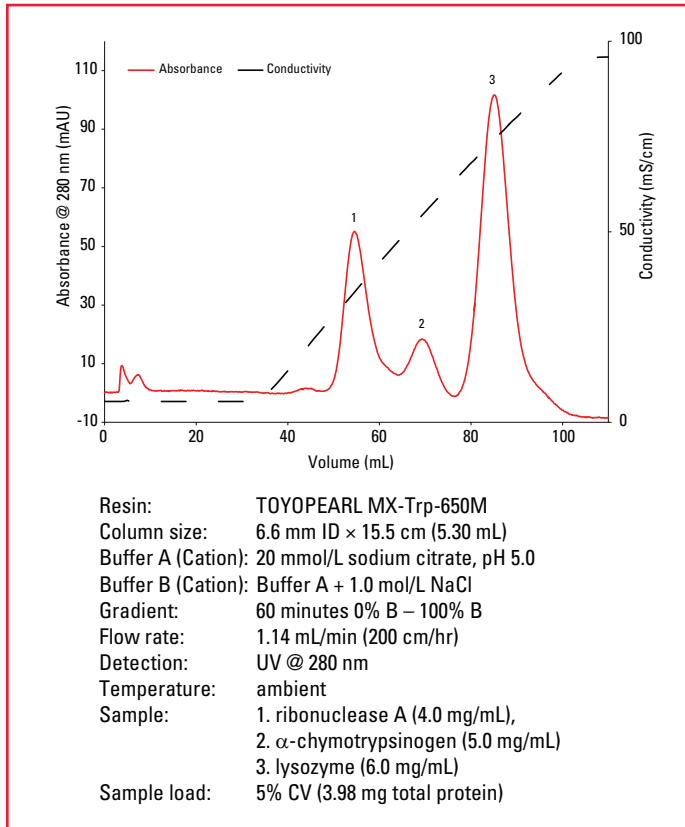
**Figure 5.** TOYOPEARL MX-Trp-650M HIC Functionality with Cation Comparison



**Figure 6.** TOYOPEARL MX-Trp-650M HIC Selectivity



**Figure 7.** TOYOPEARL MX-Trp-650M Cation Selectivity



## Conclusions

TOYOPEARL MX-Trp-650M is capable of functioning as both a salt tolerant cation exchanger and in a more traditional hydrophobic interaction chromatography mode. This dual-mode functionality suggests that there may be a conductivity band between 60 mS/cm and 130 mS/cm at pH 5.0 where protein will not bind to the resin. While TOYOPEARL MX-Trp-650M can bind proteins in HIC mode and does exhibit a degree of selectivity while being used in this mode, its low dynamic binding capacity of 3.8 mg/mL limits the effectiveness the resin may have operating as a traditional HIC resin. The selectivity of TOYOPEARL MX-Trp-650M resin when used as a weak cation exchanger is different from that of a traditional weak cation exchange resin (TOYOPEARL GigaCap CM-650M), and in fact has a similar selectivity to that of a strong cation exchanger (TOYOPEARL GigaCap S-650M).

Tosoh Bioscience, TOYOPEARL and TOYOPEARL GigaCap are registered trademarks of Tosoh Corporation.



**TOSOH BIOSCIENCE**

TOSOH

TOSOH BIOSCIENCE LLC  
3604 Horizon Drive, Suite 100  
King of Prussia, PA 19406  
Tel: 800-366-4875  
email: info.tbl@tosoh.com  
www.tosohbioscience.com

AN44  
0113