

TOYOPEARL® NH₂-750F - SALT TOLERANT ANION EXCHANGE RESIN

INTRODUCTION

Ion Exchange Chromatography (IEC) is one of the most frequently used chromatographic modes for the separation and purification of biomolecules. Compared with other chromatographic modes, modern ion exchange media offer high dynamic binding capacities and a straightforward method development. IEC is used at all stages and scales of purification of therapeutic proteins: from laboratory scale purification to industrial scale downstream processing. Today, modern ion exchange resins offer extremely high binding capacities. The interest is now shifting towards salt-tolerant ion exchange media that enable capturing out of a biological feedstock at physiological conditions or direct processing of target fractions without dilution.

Tosoh Bioscience has developed a new salt tolerant anion exchange resin, TOYOPEARL NH₂-750F, offering a high binding capacity across a range of pH values and conductivities. A TOYOPEARL HW-75 polymeric bead has been functionalized with a primary amine-containing ligand resulting in a resin with increased salt tolerance and selectivity different than that of quaternary amine anion exchange resins currently available. This new resin is ideal for process scale applications from the capture of proteins from biological feedstock (mammalian cell culture, plasma, bacterial feedstock, etc.) without dilution to the intermediate or final purification of monoclonal antibodies (mAbs) where aggregates and other impurities are removed from the target of interest.

TOYOPEARL NH₂-750F is capable of post-protein A removal of aggregates in both, flow-through and bind-elute modes.

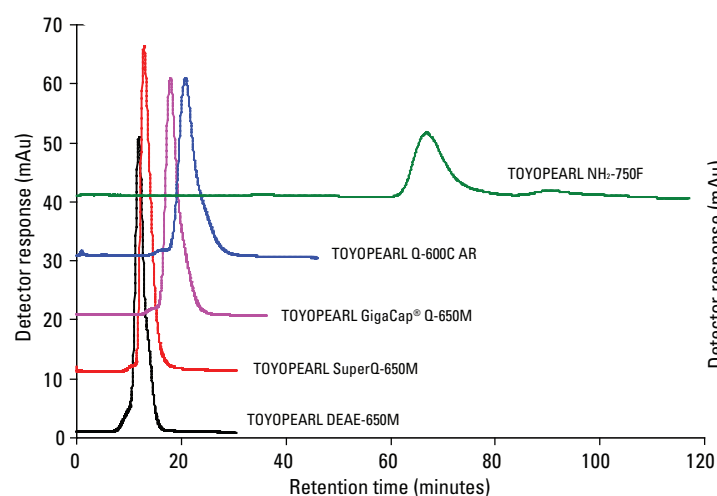
HIGHLIGHTS

- High protein binding capacity at elevated ion strength
- Unique selectivity, differing from conventional AEX media
- Binds targets at pH close to isoelectric point
- Aggregate removal in flow-through or bind/elute mode

TOYOPEARL NH₂-750F offers static binding capacities approaching 70 g/L for bovine serum albumin across a range of pH values and conductivities. Increased salt tolerance of TOYOPEARL NH₂-750F as compared to other TOYOPEARL anion exchange resins can be seen in Figure 1. While BSA is starting to elute at 0.14 mol/L NaCl for most conventional ion exchangers, the BSA peak begins to elute from the TOYOPEARL NH₂-750F column at a concentration of approximately 1 mol/L NaCl.

Retention can be affected by mobile phase pH (Figure 2) without greatly changing the selectivity of the resin. BSA binding occurs even at pH values similar to the isoelectric point, indicating a multimodal binding mechanism of the resin. This allows for a large design space in which to develop a separation method.

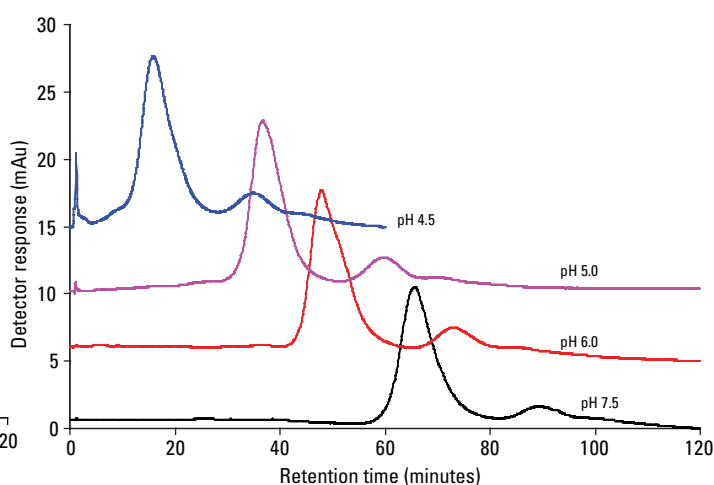
COMPARISON OF SALT TOLERANCE OF AEX RESINS



➤ **Figure 1** Resins: various

Resins: various; Column size: 5 mm ID × 5 cm L; Mobile phase: A: 20 mmol/L Tris-HCl, pH 8.0, B: mobile phase A + 2.0 mol/L NaCl; Gradient: 0 -100% B (120 min); Flow rate: 300 cm/h (1.0 mL/min); Detection: UV @ 280 nm; Temperature: ambient; Sample: BSA (1.0 g/L)

INFLUENCE OF pH ON BSA ELUTION



➤ **Figure 2** Resins: TOYOPEARL NH₂-750F; Column size: 5 mm ID × 5 cm L; Mobile phase:

A: 20 mmol/L N-methyl piperazine, pH 4.5 and 5.0; 20 mmol/L Bis-Tris, pH 6.0; 20 mmol/L Tris-HCl, pH 7.5 B: mobile phase A + 2.0 mol/L NaCl; Gradient: 0 -100% B (120 min); Flow rate: 300 cm/hr (1.0 mL/min); Detection: UV @ 280 nm; Temperature: ambient; Sample: BSA (1 mg, pl 4.7-4.9)



TOYOPEARL NH₂-750F is effective at removing aggregates from mAbs, as demonstrated in Figure 3. SEC analysis of the peaks (data not shown) shows that fraction 1 contains pure monomer. High molecular weight aggregates are completely removed from the mAb peak.

TOYOPEARL NH₂-750F offers good caustic stability and exhibits excellent pressure-flow characteristics (Figure 4).

SEPARATION OF AGGREGATES FROM IgG₁ MONOMER

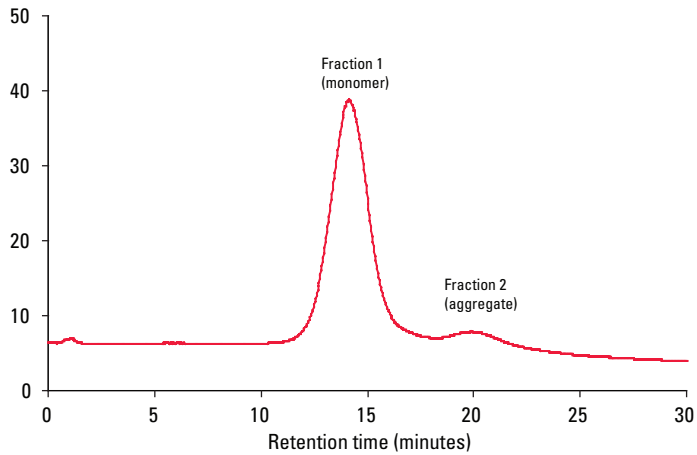


Figure 3

Resin: TOYOPEARL NH₂-750F; Column size: 5 mm ID × 5 cm L; Mobile phase: A: 20 mmol/L Tris-HCl, pH 8.0; B: mobile phase A + 1.0 mol/L NaCl; Gradient: 0 -100% B (60 min); Flow rate: 300 cm/h (1.0 mL/min); Detection: UV @ 280 nm; Temperature: ambient; Sample: mAb (IgG₁) 0.5 g/L

PRESSURE – FLOW CHARACTERISTICS OF TOYOPEARL NH₂-750F

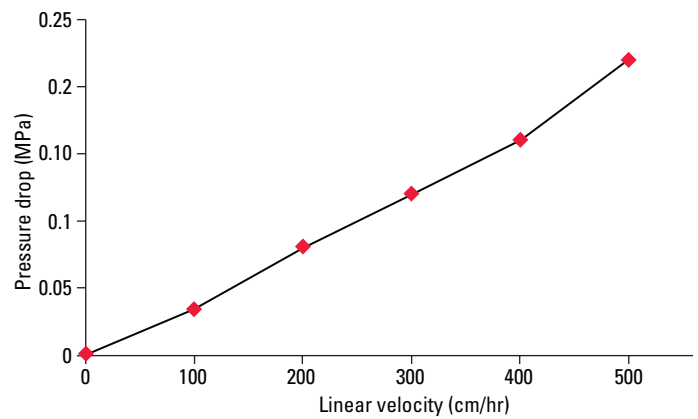


Figure 4

Resin: TOYOPEARL NH₂-750F; Column size: 4.4 cm ID × 29 cm L; Mobile phase: 0.1 mol/L NaCl; Flow rate: multiple

Ordering Information

TOYOPEARL NH₂-750F

Part-No	Description	Resin volume	Pore size	Particle size
TOYOPEARL				
0023438	TOYOPEARL NH ₂ -750F	100 mL	> 100 nm	45 µm
0023439	TOYOPEARL NH ₂ -750F	250 mL	> 100 nm	45 µm
0023440	TOYOPEARL NH ₂ -750F	1 L	> 100 nm	45 µm
0023441	TOYOPEARL NH ₂ -750F	5 L	> 100 nm	45 µm
0023442	TOYOPEARL NH ₂ -750F	50 L	> 100 nm	45 µm

ToyoScreen

0023443	ToyoScreen NH ₂ -750F	1 mL x 6	> 100 nm	45 µm
0023444	ToyoScreen NH ₂ -750F	5 mL x 6	> 100 nm	45 µm

MiniChrom

0045108	MiniChrom TOYOPEARL NH ₂ -750F	5 mL	> 100 nm	45 µm
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RoboColumns

0045021	ToyoScreen RoboColumn NH ₂ -750F	200 µl x 8	> 100 nm	45 µm
0045022	ToyoScreen RoboColumn NH ₂ -750F	600 µl x 8	> 100 nm	45 µm

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