



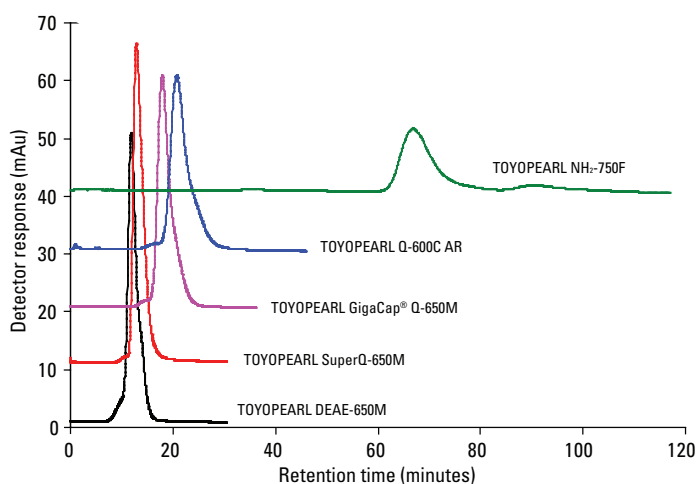
# TOYOPEARL® NH<sub>2</sub>-750F

## SALT TOLERANT ANION EXCHANGE RESIN

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 the salt tolerant anion exchange resin, TOYOPEARL NH<sub>2</sub>-750F, offering 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 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 NH<sub>2</sub>-750F is capable of post-protein A removal of aggregates in both, flow-through and bind/elute modes.

### COMPARISON OF SALT TOLERANCE OF AEX RESINS



➤ **Figure 1**

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)

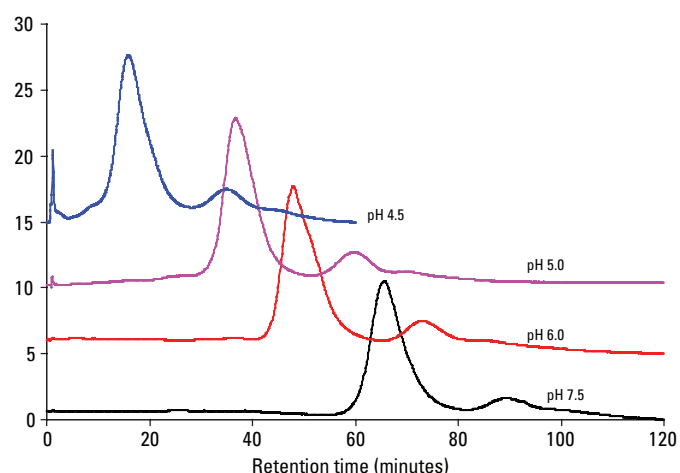
### HIGHLIGHTS

- 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 NH<sub>2</sub>-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 NH<sub>2</sub>-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 NH<sub>2</sub>-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.

### INFLUENCE OF pH ON BSA ELUTION



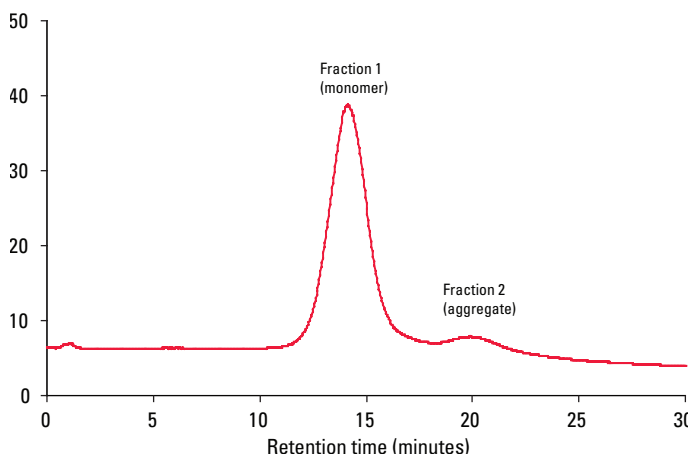
➤ **Figure 2**

Resins: TOYOPEARL NH<sub>2</sub>-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 NH<sub>2</sub>-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 NH<sub>2</sub>-750F offers good caustic stability and exhibits excellent pressure-flow characteristics (Figure 4).

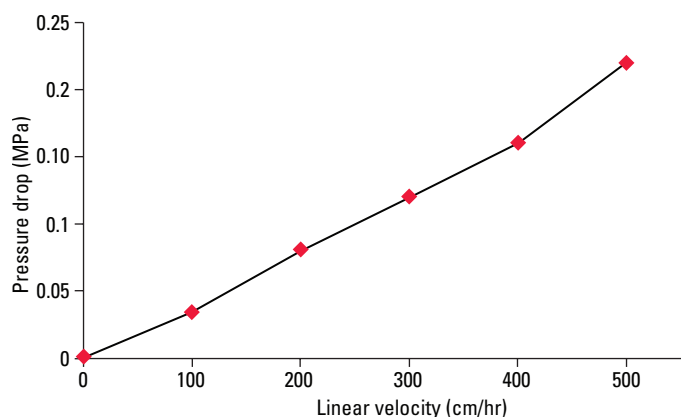
SEPARATION OF AGGREGATES FROM IgG<sub>1</sub> MONOMER



➤ Figure 3

Resin: TOYOPEARL NH<sub>2</sub>-750F; Column size: 5 mm ID x 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 (IgG<sub>1</sub>) 0.5 g/L

PRESSURE – FLOW CHARACTERISTICS OF TOYOPEARL NH<sub>2</sub>-750F



➤ Figure 4

Resin: TOYOPEARL NH<sub>2</sub>-750F; Column size: 4.4 cm ID x 29 cm L;  
 Mobile phase: 0.1 mol/L NaCl; Flow rate: multiple

### Ordering Information

#### TOYOPEARL NH<sub>2</sub>-750F

| Part-No            | Description                                 | Resin volume | Pore size | Particle size |
|--------------------|---|--------------|-----------|---------------|
| <b>TOYOPEARL</b>   |   |              |           |               |
| 0023438            | TOYOPEARL NH <sub>2</sub> -750F             | 100 mL       | > 100 nm  | 45 µm         |
| 0023439            | TOYOPEARL NH <sub>2</sub> -750F             | 250 mL       | > 100 nm  | 45 µm         |
| 0023440            | TOYOPEARL NH <sub>2</sub> -750F             | 1 L          | > 100 nm  | 45 µm         |
| 0023441            | TOYOPEARL NH <sub>2</sub> -750F             | 5 L          | > 100 nm  | 45 µm         |
| 0023442            | TOYOPEARL NH <sub>2</sub> -750F             | 50 L         | > 100 nm  | 45 µm         |
| <b>ToyoScreen</b>  |   |              |           |               |
| 0023443            | ToyoScreen NH <sub>2</sub> -750F            | 1 mL x 6     | > 100 nm  | 45 µm         |
| 0023444            | ToyoScreen NH <sub>2</sub> -750F            | 5 mL x 6     | > 100 nm  | 45 µm         |
| <b>MiniChrom</b>   |   |              |           |               |
| 0045108            | TOYOPEARL MiniChrom NH <sub>2</sub> -750F   | 5 mL         | > 100 nm  | 45 µm         |
| <b>RoboColumns</b> |   |              |           |               |
| 0045021            | ToyoScreen RoboColumn NH <sub>2</sub> -750F | 200 µL x 8   | > 100 nm  | 45 µm         |
| 0045022            | ToyoScreen RoboColumn NH <sub>2</sub> -750F | 600 µL x 8   | > 100 nm  | 45 µm         |