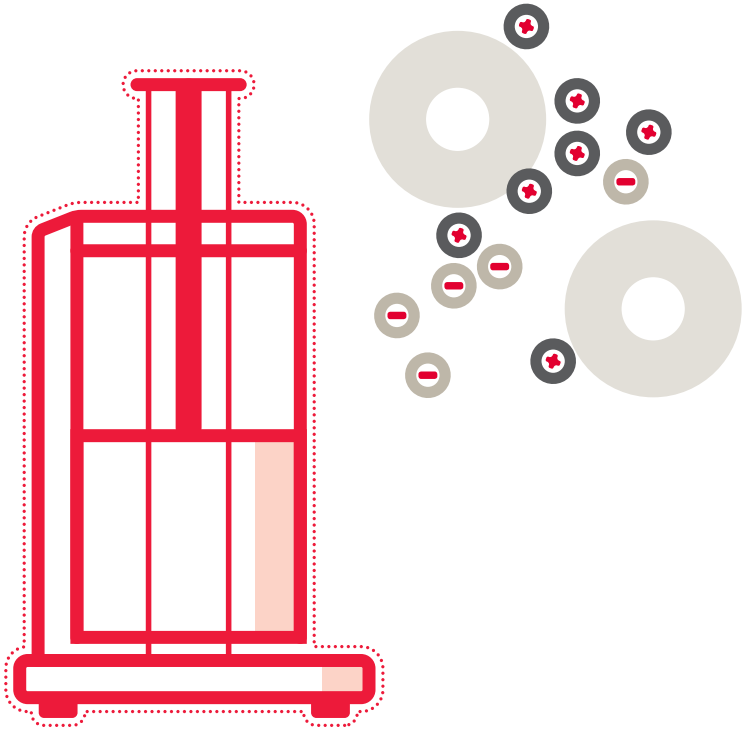
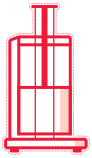
TOSOH

TOYOPEARL® ION EXCHANGER

Instruction Manual
TOYOPEARL NH_2-750F



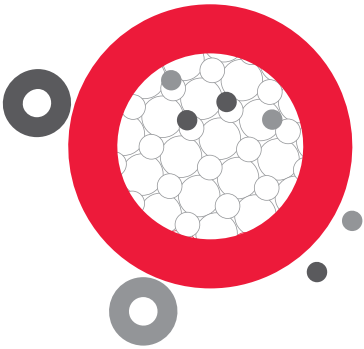
TOSOH BIOSCIENCE



You have plenty of options to get support and insights for your chromatography projects!

➨ ANY QUESTIONS?

Our technical experts are happy to discuss your specific separation needs:
+49 (0)6155-70437-36 or
techsupport.tbg@tosoh.com



➨ LOOKING FOR INSTRUCTION MANUALS OR APPLICATION NOTES?

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Instruction Manual
TOYOPEARL NH2-750F

TABLE OF CONTENTS

Safety Precautions

To help protect you and/or your property from potential damage and ensure personal safety, please read this manual thoroughly before using the product and refer to the Safety Data Sheet for specific safety information.

1. INTRODUCTION

TOYOPEARL NH_2-750F is a salt tolerant anion exchange resin capable of aggregate removal in both flow-through and binding and elution modes. The resin is composed of polymethacrylate beads that have been functionalized with proprietary primary amine (NH_2) strong anion exchange groups.

TOYOPEARL NH_2-750F is ideal for process scale applications ranging from the purifications 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_2-750F is available in 45 µm particle size (F-grade) and exhibits typical binding capacities of 70 g/L for BSA. It maintains its high capacity at increased linear velocities, offers good caustic stability (Figure 1, and has good pressure-flow characteristics.

The specifications of the resin can be found in Table 1.

PRESSURE – FLOW CHARACTERISTICS OF TOYOPEARL NH_2-750F

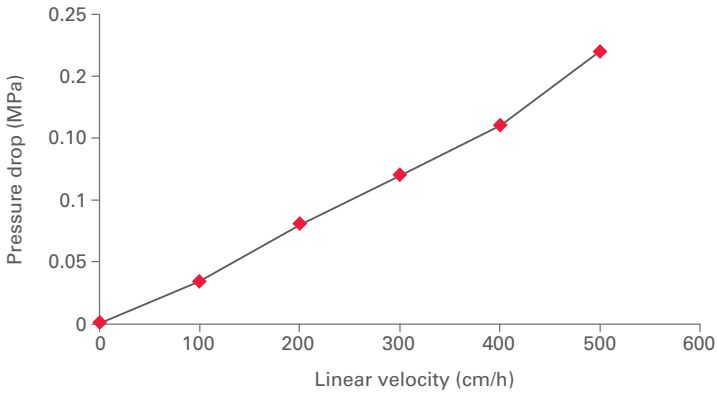


Figure 1

Resin: TOYOPEARL NH_2-750F; Column size: 4.4 cm ID × 20 cm L; Mobile phase: 0.1 mol/L NaCl

Operating Conditions	Packing pressure	Typically 0.3 MPa
	Shipping solvent	20 % (v/v) ethanol
	Shipping formulation	72 % (v/v) slurry in shipping solvent (*)
	Pressure limiting factor	Depend on column hardware (typically 0.7 MPa)
	Operating linear flowrate	Typically 10 – 600 cm/h
	Long-term storage conditions	20 % (v/v) ethanol (counter ion should be replaced with Cl^-)
	Cleaning-in-place/Sanitization	0.1 - 0.5 mol/L NaOH or 0.1 mol/L HCl
Specifications	Particle size distribution (min. 80 % within range)	30 – 60 µm
	Protein adsorption capacity (of human antibody)	Min. 70 g/L
	Bacterial count	Max. 100 CFU/mL
	Endotoxin concentration	Max. 10.0 EU/mL
	Eluable matter	Max. 0.2 % (for dry gel)
	Foreign substance (colored particle)	Max. 6
Additional Information	Appearance	White resin slurry which settles upon standing
	Mean pore diameter (base resin)	100 nm *

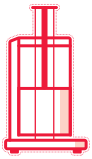
**The value is for reference only, not guaranteed.*

Table 1

Lot-specific data are included in the Certificate of Analysis (COA) shipped with the product.
For detailed test procedures please refer to the appropriate Regulatory Support File.

Remarks:

- The quantity of gel listed on the container represents the volume of gravity settled resin, and not the total liquid volume.
- This document gives some general procedures for packing and using the resin in specific equipment and in small scale. If you have other equipment or pack larger volumes, please call our Technical Specialists. They have experience with many different column designs, brands, and dimensions, and can share specific packing data.



2. PROCEDURE FOR CHROMATOGRAPHY

Note: To speed-up your development process, you can use Resin Seeker 96-well plates, RoboColumns®, and SkillPak™ pre-packed columns.

2-1 Removal of Fines

a) The settled resin in the shipping containers should be suspended by gentle agitation or stirring with a rod or paddle. Do not use a magnetic stir bar; it grinds the resin, generating fines. Once suspended, transfer the required amount of suspension (approximately 4 volumes suspension = 3 volumes resin) into a container of sufficient volume to hold 4 times the volume of resin being prepared. Add distilled water or buffer to 4 times the resin volume and stir thoroughly.

b) Once the resin has settled, carefully decant the supernatant.

c) Add three times the resin volume of either distilled water or packing buffer to the decantation vessel, and resuspend the resin by gentle overhead stirring.

d) Repeat steps b) and c) at least two more times.

2-2 Preparation of Gel Slurry and Packing

The following descriptions are valid for packing under flow. If you have other equipment or pack larger volumes, please call our Technical Specialists.

REMOVAL OF FINES

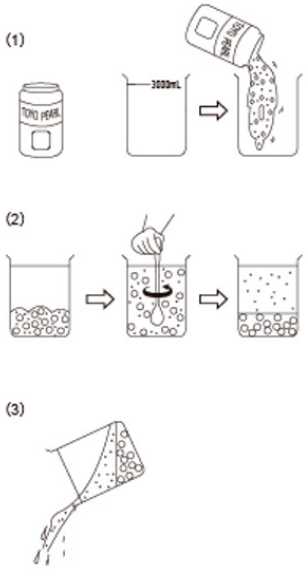


Figure 2

After removing fines from the gel by decantation (Section 2-1), wash the gel with packing buffer. The packing buffer should contain 0.5-1 mol/L NaCl. Ideally, the packing buffer should be adjusted to pH <5 (e.g. with 50 mM acetate) to achieve the best packing results. Transfer the gel into a beaker and add the packing buffer to make an approximately 30-50 % (V/V) (recommended) slurry.

Packing the column under pressure (ca. 0.05-0.3 MPa) is recommended. In this case, a pump and a reservoir or column tube extension are necessary to pack the column. The packing flow rate is typically 1.5-2 fold the flow rate applied later in the process. Initial packing using a gravity-settled bed can be applied; however, applying pressure from flow rate or dynamic axial compression results in the best-packed columns.

2-3 Equilibration and Performance Testing

Once the packing operation is completed, equilibrate the column with 5 - 10 column volumes of buffer containing 0.5 M NaCl. Test the effectiveness of the packing procedure by injecting 0.25 – 1% of the column volume of 2-5 M NaCl and determine the column plate count and asymmetry (please note that the best results are obtained by injecting 0.25 – 0.5%).

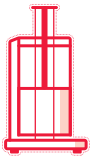
2-4 Sample Loading and Elution

Equilibrate the column with 5 to 10 column volumes of an appropriate starting buffer solution such as sodium acetate, TRIS, or BIS-TRIS. Buffers containing multivalent anions such as sodium sulfate, sodium phosphate, or sodium citrate are not recommended. Multivalent anions strongly decrease binding capacity. The sample being purified is typically adsorbed onto the column using a low conductivity buffer and is desorbed from the column using an increasing salt gradient.

2-5 Cleaning

The chromatographic resin in a packed column can be cleaned easily by flowing the cleaning buffers through the column. For severe fouling, a potential clogging of the column may be prevented by running the cleaning buffers in reverse flow.

- General cleaning method
  First, wash the gel with 1-2 mol/L NaCl solution. Then equilibrate the gel with the loading buffer.
- Severe contamination
  Wash the gel with 0.1-0.5 mol/L NaOH, followed by washing with 1-2 mol/L NaCl solution. Then equilibrate the gel with the loading buffer.
- Extremely severe contamination
  Wash with 0.1 - 0.5 M NaOH, then distilled water, then 0.1 - 0.5 M HCl, and then 0.1 - 0.5 M NaCl. Equilibrate with the starting buffer.



3. STORAGE

TOYOPEARL NH_2-750F should be stored in the 20 % ethanol solution at 4 °C to 35 °C after exposure to HCl solution to change the counterion from OH^- to Cl^- (as described in the two protocols below) because the OH^- group on the resin becomes less stable if the gel is stored in a low ionic strength solution.

1. Wash the column with 1 column volume of 0.1 mol/L HCl.
2. Wash the column with 5-10 column volumes of distilled water.

4. REMARKS

4-1 Removal of Fines

As described in Section 2, remove fines before use. When the fines are not removed completely, there is a possibility that micro-particles may leach from the column during chromatography. Leaching of micro-particles, however, should stop after a short period.

4-2 Clogging of Filter

Increasing of pressure-drop or decreasing flowrate is typically caused by the filter (frit) clogging. When this happens, remove the chromatographic resin from the column and clean the fitting and screens. Once the hardware is completely clean, repack the chromatographic resin into the column as described above.

4-3 Adsorption of Protein

When the protein or other small molecule is not adsorbed onto the column with the initial buffer, the sample should be dialyzed or desalted to reduce the conductivity. Alternatively, raise the pH of the binding buffer.

4-4 Operation recommendations in process columns

- Prefer using self-packer type columns from Verdot-IPS2, DAC columns and traditional flow packed columns.
- Do not pump or spray the resin at high flow, which may damage the particles.
- A mean pore size of the frit/net/mesh of 10-15 um is recommendable.
- Do not stir vigorously to resuspend sedimented particles (e.g. disk stirrer). Take a flat blade ‘ impeller instead and re-slurry gently at moderate speed.

ORDERING INFORMATION

P/N	Description	Dimension
0023438	TOYOPEARL NH_2-750F	100 mL
0023439	TOYOPEARL NH_2-750F	250 mL
0023440	TOYOPEARL NH_2-750F	1 L
0023441	TOYOPEARL NH_2-750F	5 L
0023442	TOYOPEARL NH_2-750F	50 L
0045021	RoboColumn NH_2-750F	200 µL x 8
0045022	RoboColumn NH_2-750F	600 µL x 8
0045209	SkillPak* NH_2-750F 5 x 1 mL col.	7 mm ID x 2.5 cm L
0045245	SkillPak NH_2-750F 5 mL col.	8 mm ID x 10 cm L

** SkillPak columns are designed for fast method development, resin screening, or sample concentration*

TRADEMARK INFORMATION

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