



TOYOPEARL INSTRUCTION MANUAL



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➤ **TOSOH HISTORY**

- 1935 FOUNDING OF TOYO SODA MANUFACTURING CO., LTD.
- 1936 OPERATION OF NANYO MANUFACTURING COMPLEX BEGINS
- 1971 SCIENTIFIC INSTRUMENTS DIVISION FORMED, FIRST GPC COLUMN USING TSK-GEL DEVELOPED BY TOSOH
- 1974 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COLUMN PLANT IS COMPLETED
- 1979 TOSOH DEVELOPS TOYOPEARL MEDIA
- 1983 TOSOH DEVELOPS HYDROPHOBIC INTERACTION MEDIA
- 1987 TOSOH US OPERATIONS FORMED IN MONTGOMERYVILLE
- 1989 TOSOH GMBH OPERATIONS FORMED IN STUTTGART
- 1995 TOSOH NANYO GEL FACILITY RECEIVES ISO 9001
- 2000 IN NOVEMBER FORMER TOSOH US OPERATIONS BECOMES TOSOH BIOSEP LLC, A 100% SUBSIDIARY OF TOSOH CORPORATION
- 2001 IN JANUARY FORMER TOSOH GMBH EUROPEAN OPERATIONS BECOMES TOSOH BIOSEP GMBH, A 100% SUBSIDIARY OF TOSOH CORPORATION
- 2002/2003 TOSOH CORPORATION ANNOUNCES THAT ALL TOSOH AFFILIATED SCIENTIFIC AND DIAGNOSTIC SYSTEM RELATED COMPANIES IN EUROPE, WILL BE UNIFIED UNDER THE NEW NAME TOSOH BIOSCIENCE.
- 2008 ECOSEC , THE 7TH GENERATION GPC SYSTEM IS INTRODUCED GLOBALLY
- 2009 20TH ANNIVERSARY OF TOSOH BIOSCIENCE GMBH, STUTTGART

Toyopearl® Instruction Manual

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PACKING

Introduction

Toyopearl chromatographic resins are macroporous polymeric packings for bioprocess chromatography. They are applicable for the laboratory and process scale purifications of globular proteins, peptides, nucleic acids, and other biologically derived materials. These resins are a modified methacrylate polymer which gives the resin a hydrophilic surface due to the presence of ether and hydroxyl groups. It also confers upon the resin excellent pressure/flow characteristics and pH stability.

I. Packing

1. Preparation for Packing

1.1 General Considerations for Packing Toyopearl

It is best to pack Toyopearl resins by the application of pressure from 0.5 to 3 bar (7 to 45 psi) across the bed length. Although it is not recommended, Toyopearl resins can be packed by simple gravitational settling.

The equipment components (shown in Fig. 1) required to successfully pack Toyopearl resins are:

a pump, a pressure gauge, a level, glasses, acrylic or PEEK or stainless steel column and a packing reservoir (optional).

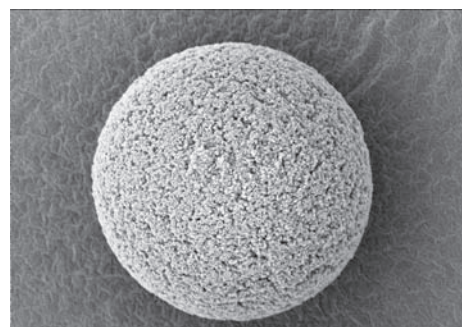
≡ **FIGURE 1**

Equipment required for packing



≡ **FIGURE 2**

Toyopearl base particle



1.2 Removal of Fines

Tosoh Bioscience recommends that fines be removed. Fines in the gel slurry may obstruct screens or sintered filters and may eventually increase the pressure drop across the column. The following decantation process is required to remove fines from the resin slurry.

a) The settled resin in the shipping containers should be suspended by vigorous agitation or stirring with a rod or paddle (do not use a magnetic stirrer; it will grind 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.

Example for Fine Removal:

5 liter resin ordered = 7 - 8 liter of suspension in total
(65 - 70 % slurry concentration)
Fill in a 20 liter vessel and fill up with 12 liter water.

b) Allow the resin to settle. Settling time is dependent on the vessel height, the slurry concentration, the solvent, and the resin particle size. The average settling times for Toyopearl resins in water in a typical measurement cylinder are:

| Toyopearl | Pore Size | Minutes |
|-----------------------|-----------|---------|
| Coarse ("C") Grade | 100 µm | 15-30 |
| Medium ("M") Grade | 65 µm | 30-45 |
| Fine ("F") Grade | 45 µm | 45-60 |
| Superfine ("S") Grade | 35 µm | 60-90 |

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In larger tanks sedimentation of particles takes longer:

| | 50 % slurry | 25% slurry |
|--|--|--|
| in water | 65 µm particles need 3 - 4 hours per meter SD | 65 µm particles need 1,5 - 2,5 hours per meter SD |
| | 35 µm particles need 5 - 7 hours per meter SD | 35 µm particles need 2 - 3,5 hours per meter |
| in 1 M NaCl | 65 µm particles need 3 - 5 hours per meter SD | 65 µm particles need 2 - 3 hours per meter SD |
| | 35 µm particles need 12 - 16 hours per meter SD | 35 µm particles need 3 - 7 hours per meter SD |
| | | |
| in 1.8 M (NH₄)₂SO₄ | 65 µm particles need 6 - 9 hours per meter SD | 65 µm particles need 4 - 8 hours per meter SD |
| in 20 % ethanol | 65 µm particles need 6 hours per meter SD | |

SD = Sedimentation Distance

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

FIGURE 3 + 4



d) Add three times the resin volume of either distilled water or packing buffer to the decantation vessel, and re-suspend the resin by gentle overhead stirring. Do not use a magnetic stir bar; it will grind the resin, generating fines.

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

1.3 Buffer Equilibration

When choosing a packing buffer, it is best to choose empirically since the optimal buffer will vary with your specific application. In general, the highest ionic strength mobile phase to be used in the separation (including the cleaning and sanitization steps) is a suitable starting point. Some typical packing buffers are listed in Table 1.

TABLE 1

Typical packing buffer

| | |
|--|---|
| SEC | |
| HW-40, HW-50, HW-55, HW-65 and HW-75 | 0.1 M Na ₂ SO ₄ , NaNO ₃ , or NaCl in 50 mM phosphate or Tris buffer |
| IEC | |
| DEAE-type, QAE, Q-type, CM-type, SP-type, MegaCap II-SP | 1 M NaCl in 50 mM phosphate, Tris, or acetate buffer |
| HIC | |
| Ether-650, Phenyl-type, Butyl-type, Hexyl-650, PPG-600, | 2 M Na ₂ SO ₄ , (NH ₄) ₂ SO ₄ , or NaCl in 50 mM phosphate buffer |
| AFC | |
| AF-Tresyl and AF-Epoxy-650 | 0.5 M NaCl in 0.1 M NaHCO ₃ or phosphate buffer |
| AF-Formyl-650, AF-Amino-650, and AF-Carboxy-650, Protein A | 1 M NaCl in 100 mM phosphate or NaHCO ₃ buffer |
| AF-Chelate-650, AF-Blue HC-650 and AF-Red-650 | 0.5 M NaCl or 0.2 M glycine in 20 mM phosphate or Tris buffer |

1.4 Slurry Preparation

After de-fining the resin, the slurry concentration can be adjusted for packing the column. The slurry concentration is calculated as the volume of settled gel divided by the total volume of the slurry, and the slurry concentration is adjusted as follows:

- Resuspend the resin slurry in the de-fining vessel and transfer the homogeneous slurry to a graduated cylinder
- Allow the slurry to settle overnight (>12 hours) for best results.
- Determine the settled resin volume, and adjust the slurry concentration to 30 - 50 % by adding or removing packing buffer.
- For packing a column of a given volume, use the following amounts of settled resin:

| | |
|--|--|
| HW-40, HW-50, HW-55, HW-65, and HW-75F | use approximately 1.1 x the column volume |
| Ether-650, Phenyl-type, Butyl-type, Hexyl-650, PPG-600, DEAE-type, Q-type, CM-650, SP-type, Giga Cap-type and all affinity | use approximately 1.2 x the column volume |
| QAE-550C and SP-550C Toyopearl MegaCap II SP 550EC | use approximately 1.25 x the column volume |

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1.5 Alternative Slurry Preparation

- Re-suspend the resin slurry in the de-fining vessel and transfer the homogeneous slurry to a Büchner funnel or equivalent.
- Filter the slurry under suction until the slurry becomes a wetcake (all excess liquid has been removed).
- Weigh out the appropriate amount of resin wetcake (1 g of wetcake \approx 1 ml of gravity settled gel) using the above table.
- Transfer the wetcake to a beaker and add enough packing buffer to make a slurry concentration of 30 - 50 %.

FIGURE 5
Settled resin in water



PACKING

2. Packing Procedures

Do not pack Toyopearl like traditional soft gels. For best results Toyopearl should be packed at a higher flow rate and pressure!

TABLE 2

Packing and operating velocities for Toyopearl resins

LABORATORY SCALE

| | Resin Type | Column Size (cm ID x cm L) | Grade | Packing velocity flow rate (cm/hr) | Operating velocity (cm/hr) | (ml/min) |
|-----------|--|-------------------------------|-----------------|---------------------------------------|-------------------------------|------------|
| SEC | HW-40 | 2.2 x 60 | S (30 µm) | 30 - 40 | 10 - 25 | 0.6 - 1.6 |
| | | | F (45 µm) | 60 - 80 | 25 - 50 | 1.6 - 3.2 |
| | | | C (75 µm) | 120 - 160 | 50 - 100 | 3.2 - 6.4 |
| | HW-50 | 2.2 x 60 | S (30 µm) | 25 - 35 | 10 - 20 | 0.6 - 1.3 |
| | | | F (45 µm) | 50 - 70 | 25 - 35 | 1.6 - 2.2 |
| | HW-55 | 2.2 x 60 | S (30 µm) | 25 - 35 | 10 - 20 | 0.6 - 1.3 |
| F (45 µm) | | | 50 - 70 | 25 - 35 | 1.6 - 2.2 | |
| HW-65 | 2.2 x 60 | S (30 µm) | 20 - 75 | 10 - 15 | 0.6 - 1.0 | |
| | | F (45 µm) | 40 - 150 | 15 - 30 | 1.0 - 1.9 | |
| HW-75 | 2.2 x 60 | F (45 µm) | 40 - 150 | 15 - 30 | 1.0 - 1.9 | |
| | | | | | | |
| IEC* | DEAE-650, SuperQ-650 CM-650, SP-650 Giga Cap S,CM,Q | 2.2 x 20 | S (35 µm) | 400 - 600 | 45 - 65 | 3.0 - 4.0 |
| | | | M (65 µm) | 800 - 1000 | 80 - 130 | 5.0 - 8.0 |
| | | | C (100 µm) | 800 - 1200 | 80 - 600 | 5.0 - 40 |
| | SP-550 QAE-550 | 2.2 x 20 | C (100 µm) | 700 - 1000 | 80 - 240 | 5.0 - 15 |
| | Toyopearl MegaCap II SP-550 | | EC (100-300 µm) | 800 - 1200 | 80 - 500 | 5.0 - 30.0 |
| HIC* | Ether-650, Hexyl-650, Butyl-600, Phenyl-650, PPG-600, Butyl- 650, SuperButyl-550, PPG-600, Phenyl-600 | 2.2 x 20 | S (35 µm) | 400 - 600 | 45 - 65 | 3.0 - 4.0 |
| | | | M (65 µm) | 800 - 1000 | 80 - 130 | 5.0 - 8.0 |
| | | | C (100 µm) | 800 - 1200 | 80 - 500 | 5.0 - 30 |
| | | | | 700 - 1000 | 80 - 240 | 5.0 - 15 |
| AFC* | AF-Amino-650, AF-Tresyl-650 AF-Carboxy-650, AF-Blue-650 AF-Formyl-650, AF-Chelate-650 AF-Epoxy-650, AF-Blue-650 | 2.2 x 10 | M (65 µm) | 800 - 1000 | 30 - 130 | 2.0 - 8.0 |
| | | | | | | |

* Not all resins are available in all particle sizes.

PROCESS SCALE

The packing velocity in process scale columns should be at least 1.5 x the operating velocity

Please call our product specialists for your individual discussion.

PACKING

The following descriptions are valid for packing under flow. If you have other equipment, or pack greater than 5 liters, please call our Technical Specialists. We have experience with many different column designs and brands.

TABLE 3
Features of packing methods

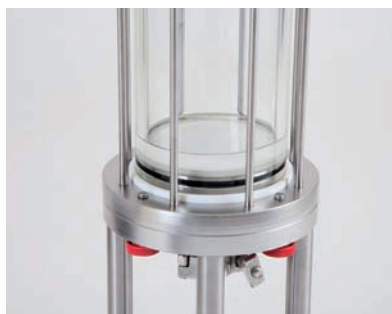
| Packing Method | Constant Pressure | Constant Velocity | Assisted Gravity |
|-------------------------|-------------------|-------------------|------------------|
| Packing Velocity | fast | fast | slow |
| Flow Rate Range | up to high | up to high | limited to low |
| Pump | constant pressure | constant velocity | peristaltic pump |
| Pressure Gauge | needed | needed | not needed |

2.1 Constant Velocity / Semi-Constant Pressure Methods

a) If used, place the packing reservoir on the column. The total volume of the column and the reservoir should be sufficient to allow the entire resin slurry to be poured in one operation.

b) Ensure that the column is leveled prior to packing. Wet the bottom frit or screen in the column with buffer. Allow the buffer to drain a few seconds to remove any air bubbles. Plug the outlet of the column and leave 1 - 2 cm of buffer in the bottom of the column.

FIGURE 6
Bubble free, liquid covered bottom frit



c) Resuspend the resin slurry to assure homogeneity.

FIGURE 7
Homogenise slurry



d) Carefully pour the resin slurry slowly down along the inside wall of the column. Prevent air from being trapped in the resin slurry.

e) After the resin slurry is transferred to the column, rinse the inside walls of the column using a squirt bottle containing packing buffer.

FIGURE 8
Pouring the resin



f) Immediately place the flow adapter of the column onto the resin slurry. There should be no trapped air between the flow adapter and the buffer.

g) Open the column outlet, and start the pump. Start slowly to flow packing buffer through the column.

FIGURE 9
Adjusting column



h) Two different Packing Methods can be applied:

Constant Velocity Method

Slowly increase to the final flow rate. This prevents hydraulic shock to the forming bed and prevents uneven packing of the column bed. The flow rate can be ramped up in several incremental changes. These increments will be determined by the size of the column and target flow rate. Some examples are listed in [Table 4](#).

PACKING

Pressure Method

Slowly ramp up to the target pressure. This prevents hydraulic shock to the forming bed, and therefore prevents uneven packing of the column. The pressure can be maintained by manually decreasing the flow rate to keep a constant pressure on the forming bed. The optimal packing pressure for Toyopearl resins is around 3 bar (44 psi) across the bed length.

➤ TABLE 4

Typical packing buffer

| Column Size (ID x L) | Media Type | Target Flow Rate (ml/min) | Increment (ml/min) | Hold Time (min) |
|----------------------|------------|---------------------------|--------------------|-----------------|
| 2.2 cm x 60 cm | HW-55S | 2 | 0.5 | 0.5 |
| 9 cm x 30 cm | QAE-550C | 300 | 50 | 2 |
| 25 cm x 30 cm | DEAE-650M | 2,000 | 400 | 3 |

i) After the bed has fully formed, shut off the pump, and close the column outlet.

➤ FIGURE 10

Clear supernatant of sedimenting resin



j) The entire bed should reside in the lower column section if using a packing reservoir. Using a pipette or pump, siphon the supernatant from the upper reservoir. Remove the upper reservoir and the coupling ring.

k) Carefully place the flow adapter into the column, approximately 2 - 3 cm away from the consolidated bed. Avoid introduction of air into the column.

l) Secure the flow adapter in place, begin the pump as described in **Step h** (Pressure Method), and open the column outlet.

➤ FIGURE 11

Flow adapter in place



m) The bed will compress further. When compression is complete and pressure is stable, stop the pump and close the column outlet.

n) Carefully loosen the flow adapter seal and lower the adapter near to the resin bed. Take care not to disturb the resin bed when moving the flow adapter.

➤ FIGURE 12 + 13

Bubble free (recommendable)



Air disturbs homogenous settling procedure



o) Repeat **Steps l) - n)**, until there is no further compression of the resin bed from the flow adapter (< 0.5 cm). It will usually take 2-3 iterations until the bed is stable.

p) In the final step lower the adapter 1 - 5 mm into the bed.

q) The column is now ready for efficiency evaluation. (see page 8)

PACKING

2.2 Alternative Packing Method, Assisted Gravity

Due to hardware constraints, it may not be possible to use a reservoir when packing Toyopearl resin. The following method was developed to pack the resin without a packing reservoir.

a) Adjust the resin slurry concentration to 50 %, and gently resuspend the resin with overhead stirring. Do not use a magnetic stirrer!

b) As shown in [Figure 14](#), attach a peristaltic pump to the bottom outlet of the column.

c) Ensure that the column is leveled prior to packing.

d) With the pump running in the upflow direction, backflow packing buffer into the column until it is about 50 % full. Stop the pump.

e) With the pump running at the desired flow rate in the downflow direction, slowly add the homogeneous resin slurry to the column. Pour the slurry down along the inner wall of the column to prevent the formation of air bubbles.

f) When the bed is almost entirely formed, and with approximately 2-3 cm of buffer above the bed, shut off the pump and column outlet valve.

g) Gently rinse down the inside walls of the column with a squirt bottle containing packing buffer.

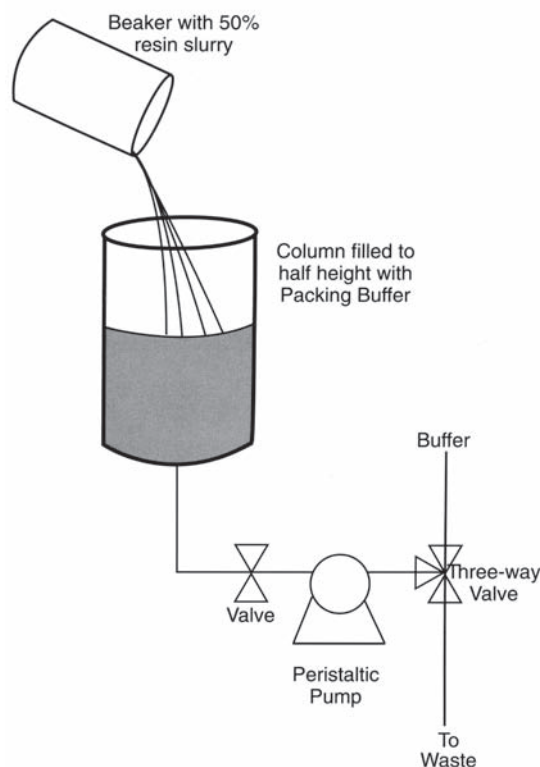
h) Carefully place the flow adapter into the column, with the adapter just touching the packing buffer.

i) With the adapter firmly in place, place the pump in front of the column. Eliminate air in tubing.

j) Start the pump at a low flow rate; open the bottom valve.

k) Slowly ramp up to the target flow rate. This prevents hydraulic shock to the forming bed, and therefore prevents uneven packing of the column. The flow rate can be ramped up in several ml/minute increments over the initial phase of the packing. The size and duration of these increments will be determined by the size of the column which is being packed (see [Table 4](#)).

FIGURE 14
Assisted cavity packing method



l) After bed consolidation is complete, stop the pump and shut the bottom outlet.

m) Loosen the seal on the flow adapter, and gently place the flow adapter onto the resin bed. Be careful not to allow resin past the column seal.

n) Repeat **steps l)** through **m)** until there is no further bed compression from the flow adapter (< 0.5 cm).

o) In the final step lower the adapter 1 - 5 mm into the bed.

p) The column is now ready for an efficiency evaluation. (see [page 9](#))

PACKING

3. Equilibration and Efficiency Evaluation

Once the packing operation is completed, equilibrate the column with 5 - 10 column volumes of low ionic strength buffer. Test the effectiveness of the packing procedure by injecting a sample (0.25 - 1% of the column volume) of a low molecular weight, unretained compound (i.e. acetone, Vitamin B12, sodium chloride), and determine the column plate count and asymmetry as shown in Figure 15. Columns packed according to the above procedures, and operated at linear velocities of 50 – 250 cm/h (depending on the particle size) should have the minimum plate counts listed in Table 5, and asymmetries between 0.8 - 1.5 when tested.

TABLE 5

Typical packing buffer

| Mode | Column ID (cm) | S Grade (plates/M) | F Grade (plates/M) | M Grade (plates/M) | C Grade (plates/M) |
|------|----------------|--------------------|--------------------|--------------------|--------------------|
| SEC | 2.2 | 5,000 | 3,500 | - | 3,000 |
| | 5.5 | 5,000 | 3,300 | - | - |
| | 10.8 | 5,000 | 2,500 | - | - |
| | 21.0 | 4,000 | 2,200 | - | 1,500 |
| | 31.0 | - | 2,000 | - | 1,200 |
| | 40.0 | - | 1,800 | - | 1,000 |
| IEC | 2.2 | 6,000 | - | 4,000 | 2,000 |
| | 5.5 | 6,000 | - | 4,000 | - |
| | 10.8 | 6,000 | - | 4,000 | - |
| | 21.0 | 4,000 | - | 2,600 | 2,000 |
| | 31.0 | - | - | 2,000 | 1,000 |
| | 40.0 | - | - | 1,500 | 750 |
| HIC | 2.2 | 6,000 | - | 4,000 | 2,000 |
| | 5.5 | 6,000 | - | 4,000 | - |
| | 10.8 | 6,000 | - | 4,000 | - |
| | 21.0 | 4,000 | - | 2,600 | 2,000 |
| | 31.0 | - | - | 2,000 | 1,000 |
| | 40.0 | - | - | 1,500 | 750 |
| AFC | 2.2 | - | - | 4,000 | - |
| | 5.5 | - | - | 4,000 | - |
| | 10.8 | - | - | 4,000 | - |
| | 21.0 | - | - | 2,600 | - |
| | 31.0 | - | - | 2,000 | - |
| | 40.0 | - | - | 1,500 | - |

If there is a large deviation from expected plate height number and asymmetry factors, please repeat the packing procedure.

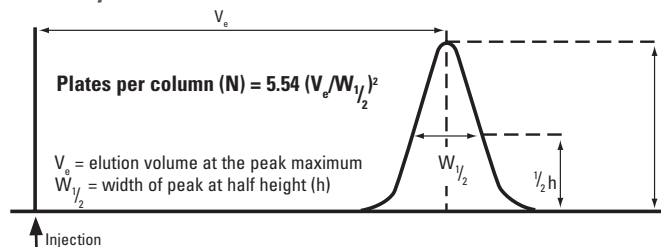
If column diameters > 40 cm are utilized, the number of plates/M can slightly decrease

For further details call our Technical Specialists.

FIGURE 15

How to calculate efficiency & asymmetry Factor

Efficiency



Asymmetry

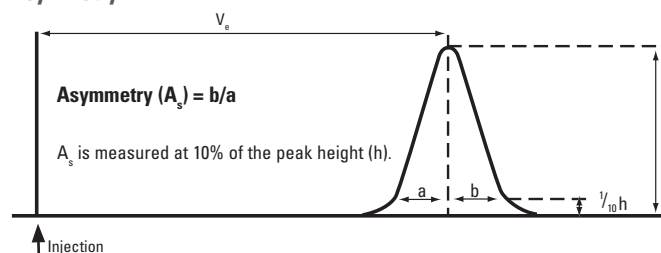


TABLE 6

Troubleshooting performance evaluation

| $A_s < 0.8$ | $A_s > 1.4$ |
|---|---|
| Overpacking the column. Packing at too high pressure. Column bed cracking. | Column not packed "tight" enough. Clogged screens or frits at top or bottom of the column. Small void at top of column. Air pockets in column hardware void spaces. Poor injection technique. |
| High HETP* | Low HETP* |
| Injection sample or detector too far from column. Injection volume too high. Column not packed efficiently. | Probe molecule retained on column due to interaction with functional group or backbone. |

*HETP (Height Equivalence of a Theoretical Plate)

COLUMN OPERATION

II. Column Operation

1. Chromatographic Separation

1.1 Size Exclusion Chromatography (SEC)

Equilibrate the resin with 5 - 10 column volumes of an appropriate buffer solution (see Table 1). Size exclusion separations on Toyopearl HW columns are performed under isocratic conditions using buffered salt solutions of moderate ionic strength. Sample volumes are usually 1 - 3% of the column packed bed volume. If retention times are shorter or longer than expected, changes in the mobile phase may be necessary. Please refer to Table 7 for suggested mobile phase changes.

≡ TABLE 7

Non ideal SEC behavior

| Observation | Cause/Solution |
|--|---|
| Retention time is shorter than expected | <p>Sample can be partially or totally excluded from column, confirm MW of sample and use a resin with higher exclusion limit if necessary.</p> <p>Anionic molecules can be repulsed by ionic exclusion, increase the ionic strength of the mobile phase.</p> |
| Retention time is longer than expected | <p>Cationic molecules can be retarded by ionic attraction, increase the ionic strength of the mobile phase.</p> <p>Hydrophobic molecules can be retarded by hydrophobic attraction, decrease the ionic strength of the mobile phase or add a small percentage (10-20 %) of an organic solvent such as methanol, ethanol, or acetonitrile.</p> |

1.2 Ion Exchange Chromatography (IEC)

Equilibrate the column with 5 to 10 column volumes of an appropriate starting buffer solution (Table 8). The elution is performed by increasing the salt concentration or changing the pH of the eluent.

If the ion-exchanger fails to adsorb the desired protein, change the pH of the equilibration buffer to enhance the electrostatic interaction between the protein and the ion-exchanger, or decrease the salt concentration in the equilibration buffer.

≡ TABLE 8

Examples for buffers used in IEC

| Resin Type | Buffer | Buffering Range |
|--------------------------|-----------------|-----------------|
| Cation Exchangers | Acetic acid | 4.8 - 5.2 |
| | Citric acid | 4.2 - 5.2 |
| | MES | 5.5 - 6.7 |
| | Phosphate | 6.7 - 7.6 |
| | HEPES | 7.6 - 8.2 |
| Anion Exchangers | L-Histidine | 5.5 - 6.0 |
| | Imidazole | 6.6 - 7.1 |
| | Triethanolamine | 7.3 - 7.7 |
| | Tris-HCl | 7.5 - 8.0 |
| | Diethanolamine | 8.4 - 8.8 |

1.3 Hydrophobic Interaction Chromatography (HIC)

Equilibrate the column with an appropriate buffer solution containing a concentrated (generally 1 M to 3 M) neutral salt such as one listed in Table 9. High ionic strength enhances the hydrophobic interaction between proteins and the resin and thus facilitates adsorption. Before introducing a sample onto the column, make at least one blank analysis and equilibrate the column in the initial mobile phase.

Elute adsorbed proteins by decreasing the concentration of salt in the eluent. Proteins with lower hydrophobicity are eluted earlier and at higher salt concentrations than more hydrophobic proteins. If the desired protein is not eluted by this method, add a small percentage of organic solvent or nonionic detergent, change the eluent pH, or lower the temperature. See Table 10 for suggestions on what organic solvents, detergents, or chaotropes to use.

If sample profiles are inconsistent, first increase the column equilibration step by using an additional 3 to 10 column volumes of starting eluent. If the desired protein is not adsorbed on the column, increase the concentration of salt in the starting buffer or adjust the pH of the buffer closer to the isoelectric point of the protein.

≡ TABLE 9

Neutral salts used in HIC

| Salt (listed in decreasing order of strength)* | Comments |
|--|---|
| Sodium Citrate | May exhibit high UV absorbency, prone to microbial growth |
| Ammonium Sulfate | Not stable above pH 8, low UV interference, resists microbial growth, most commonly used salt for HIC |
| Sodium Sulfate | Solubility is low (1.5 M at 25 °C) |
| Sodium Chloride | Halide salt can be corrosive to stainless steel, inexpensive |
| Potassium Chloride | Halide salt can be corrosive to stainless steel |

* - based on the Hofmeister series of lyotropic salts

COLUMN OPERATION

TABLE 10

Mobile phase additives for HIC

| Organic Additives | Detergents | Chaotropic Agents |
|-------------------|-----------------|-----------------------------|
| ethanol | Triton X-100 | guanidine hydrochloride |
| methanol | octyl glucoside | tetraethylammonium chloride |
| isopropanol | Tween 20 | urea |
| n-butanol | SDS | potassium thiocyanate |
| acetonitrile | CHAPS | |
| ethylene glycol | Emulgen 911 | |
| | CTAB | |
| | Lubrol PX | |

1.4 Affinity Chromatography (AFC)

Included among the Toyopearl affinity resins are both group specific ligand resins (Chelate, Red and Blue-HC), and resins with surface chemistries that allow attachment of custom ligands by the end user.

Contact Tosoh Bioscience Technical Service for information concerning coupling chemistries for the attachment of ligands to Formyl, Carboxy, Amino, Epoxy and Tresyl Toyopearl.

Equilibration

AF Red, AF Blue-HC and Chelate resins should be equilibrated with 3 - 5 column volumes of the appropriate starting buffer, such as phosphate or Tris, with little or no salt.

The dye affinity chromatographic resins may release a small amount of conjugated dye during storage. Be sure to wash the dye affinity columns before each use to remove the released dye. Wash a column containing new resin with 1 M sodium chloride or 1 M potassium chloride. Use 2 M potassium chloride or 4 M urea for washing used resin. Equilibrate a column containing old or new resin with an appropriate starting buffer, such as 20 mM phosphate at pH 7.5.

Loading and Elution

After applying the sample, wash the column with 3 - 5 column volumes of starting buffer to remove unadsorbed impurities. Two kinds of elution methods are commonly used in affinity chromatography: nonspecific and specific.

Nonspecific elution generally is achieved by increasing the salt concentration in the eluent. Most proteins are eluted with a solution containing 2 M sodium chloride or 3 M potassium chloride. Proteins not eluted with these eluents can be eluted with solutions listed in [Table 11](#).

In **specific elution**, an enzyme is eluted with a solution containing its substrate or coenzyme. A substrate or coenzyme concentration below 10 mM usually is sufficient for elution.

TABLE 11

Eluents for exhaustive elution from AF Toyopearl resins

| | |
|---------|---|
| Choice: | 2 M KCl or 3 M NaCl |
| Choice: | 1 % Triton X-100 / 1 M NaSCN / 75% ethylene glycol / 4 M urea or 0.1 M NaOH / 4.2 M (NH ₄) ₂ SO ₄ |

2. Cleaning

Toyopearl resins can be cleaned in the column or removed from the column and treated in bulk. The cleaning method and duration of treatment depend on the extent of contamination. At least three bed volumes of cleaning solution are typically employed in column washing procedures.

SEC Resins

In most cases, the resins can be cleaned simply by washing with distilled water to desorb remaining proteins. For more tenaciously bound materials, the following solutions may be required:

Ionically-bound materials

For moderately bound materials, 0.5 - 1 M aqueous salt solutions can be used to clean the resin. For more strongly bound materials, 0.1 - 0.5 M sodium hydroxide or 0.1 - 0.5 M hydrochloric or sulfuric acid is appropriate. Under no circumstances should nitric acid be used to clean Toyopearl resins! Nitric acid can react violently with Toyopearl resins. Because acids sometimes cause protein aggregation, first use an alkaline solution for removing proteins.

Hydrophobically-bound materials

About 10 - 20 % of an alcohol such as ethanol, methanol, or isopropanol can be used to remove hydrophobic materials. Solvents such as acetonitrile and acetone can also be used. It is important to remember that solvents can sometimes cause protein aggregation.

After using any base, acid, or organic solvent, use distilled water as a final rinse.

IEC Resins

For moderate contamination, wash with 0.5 - 1 M sodium chloride, then equilibrate with the starting buffer. For severe contamination, wash with 0.1 - 0.5 M sodium hydroxide, then with 0.1 - 0.5 M sodium chloride, then equilibrate with the starting buffer.

For extremely severe contamination of DEAE and QAE resins, wash with 0.1 - 0.5 M sodium hydroxide, then distilled water, then 0.1 - 0.5 M hydrochloric acid, and then 0.1 - 0.5 M sodium chloride. Equilibrate with the starting buffer.

A high salt mobile phase can be used as a final rinse to assure the correct counter ion is present.

COLUMN OPERATION

HIC Resins

In most cases, the resins can be cleaned simply by washing with distilled water to desorb remaining proteins. For more tenaciously bound materials, the following solutions may be required:

Ionically-bound materials

For moderately bound materials, 0.5 - 1 M aqueous salt solutions can be used to clean the resin. For more strongly bound materials, 0.1 - 0.5 M sodium hydroxide or an appropriate acid such as hydrochloric or sulfuric is appropriate. Under no circumstances should nitric acid be used to clean Toyopearl resins! Because acids sometimes cause protein aggregation, first use an alkaline solution for removing proteins.

Hydrophobically-bound materials

10 - 40% of an alcohol such as ethanol, methanol, or isopropanol can be used to remove hydrophobic materials. Solvents such as acetonitrile and acetone can also be used. It is important to remember that solvents can sometimes cause protein aggregation. Non-ionic detergents may also be used for cleaning.

After using any base, acid, or organic solvent, use distilled water as a final rinse.

AFC Resins

High concentrations of neutral salts, chaotropes, or detergents such as those listed in [Table 9](#) should be used as eluents prior to extensive cleaning efforts. Remaining protein contaminants adsorbed on the resin can be removed by washing with two column volumes of 0.5 M sodium hydroxide followed by distilled water. Sodium hydroxide should be used with AF-Heparin and AF-Protein only in cases of extreme contamination.

3. Storage

SEC, IEC, and HIC

Store the column or used bulk resin in distilled water containing a bacteriostatic agent, such as 20% ethanol, preferably at 4°C to 25°C.

AFC

Store the column or used bulk resin in a neutral solution of 1 M sodium chloride or potassium chloride containing a bacteriostatic agent, such as 20% ethanol, preferably at 4°C to 10°C.

For AF-Formyl 650M, store the column or used bulk resin in a neutral solution of 1 M sodium chloride or potassium chloride in 1% glutaraldehyde, preferably at 4°C to 10°C.

Please note that dye affinity chromatographic resins may release a small amount of dye during storage. Be sure to wash the dye affinity resin before each use to remove any released dye.



COLUMN OPERATION

4. Sterilization / Depyrogenation / Preservative Removal / Column Frits

Sterilization

Toyopearl resins can be sterilized by autoclaving at 121°C for 20 min. without altering their properties. Alternatively, columns already packed may be exposed to 200 ppm sodium hypochlorite for periods up to 12 hours without loss of function.

Depyrogenation

Toyopearl resins are recommended for use from pH 2 to 12. However, short exposures (< 12 hours) to higher pH (0.5 N NaOH) are acceptable for depyrogenation. Typically endotoxin levels are reduced by at least 4 logs following a 4-hour treatment with 0.5 N NaOH followed by a wash with 3 column volumes of endotoxin-free equilibration buffer.

Preservative Removal

Shipping solvents for Toyopearl resins contain 20% ethanol (with exception of some affinity products). The resin preparation procedures outlined in this document will reduce the ethanol level in the packed column effluent.

Column Frits

Pressure-related problems are often caused by clogged column frits. Remove the frits and clean thoroughly as recommended by the column manufacturer. If the problem persists, replace the frits.



TOYOPEARL PRODUCT OVERVIEW

IEC

Toyopearl Resin

Anion-Exchangers

| Toyopearl Resin | Pore Size |
|--------------------------|-----------|
| SuperQ-650 (S, M, C), | 400 Å |
| QAE-550C, | 500 Å |
| DEAE-650 (S, M, C), | 1.000 Å |
| GigaCap Q-650M (M=75 µm) | 1.000 Å |
| Q-600C AR | 750 Å |

S = 35 µm, **M** = 65 µm, **C** = 100 µm

Cation-Exchangers

| Toyopearl Resin | Pore Size |
|------------------------------|-----------|
| CM-650 (S, M, C), | 1.000 Å |
| SP-650 (S, M, C), | 1.000 Å |
| SP-550C, | 500 Å |
| MegaCap II SP-550EC, | 500 Å |
| GigaCap S-650M, (50-100 µm) | 1.000 Å |
| GigaCap CM-650M, (50-100 µm) | 1.000 Å |

S = 35 µm, **M** = 65 µm, **C** = 100 µm, **EC** = 200 µm

AFC

Toyopearl Resin

Toyopearl Reactive Resins

| Toyopearl Resin | Pore Size |
|------------------|-----------|
| AF-Amino-650M, | 1.000 Å |
| AF-Carboxy-650M, | 1.000 Å |
| AF-Formyl-650M, | 1.000 Å |

Toyopearl Activated Resins

| Toyopearl Resin | Pore Size |
|-----------------|-----------|
| AF-Epoxy-650M, | 1.000 Å |
| AF-Tresyl-650M, | 1.000 Å |

Toyopearl Ready to use Resins

| Toyopearl Resin | Pore Size |
|--------------------|-----------|
| AF-BlueHC-650M, | 1.000 Å |
| AF-Chelate-650M, | 1.000 Å |
| AF-HeparinHC-650M, | 1.000 Å |
| AF-Red-650ML, | 1.000 Å |

M = 65 µm, **ML** = 65 µm

HIC

Toyopearl Resin

| Toyopearl Resin | Pore Size |
|-----------------------|-----------|
| Ether-650 (S, M), | 1.000 Å |
| PPG-600M , | 750 Å |
| Phenyl-600M, | 750 Å |
| Phenyl-650 (S, M, C), | 1.000 Å |
| Butyl-650 (S, M, C), | 1.000 Å |
| Butyl-600M, | 750 Å |
| SuperButyl-550C, | 500 Å |
| Hexyl-650C, | 1.000 Å |

S = 35 µm, **M** = 65 µm, **C** = 100 µm

SEC

Toyopearl Resin

| Toyopearl Resin | Pore Size |
|------------------|-----------|
| HW-40 (S, F, C), | 50 Å |
| HW-50 (S, F), | 125 Å |
| HW-55 (S, F), | 500 Å |
| HW-65 (S, F, C), | 1.000 Å |
| HW-75 (F), | > 1.000 Å |

S = 30 µm, **F** = 45 µm, **C** = 75 µm

PROCESS COLUMN INSTALLATIONS

| Column Manufacturer | Column Type | various bed dimensions (ID x L in cm) | Column Performances Af | plate count [N/m] | Resin Type |
|------------------------------|--------------------|---|--|---|---|
| BioRad | InPlace/Geltec | 20 - 45 x 15 - 25 130 x 24 | 0,8-1,4 1,1-1,2 | 3.000-4.000 (60 cm/h-salt) 3.500-3.900 (300 cm/h-salt) | HIC - 65 µm IEC - 65 µm |
| GE Healthcare Lifesciences | AxiChrom | 60 x 20 | 1,1 | 8.000 (100 cm/h-salt) | HIC - 65 µm |
| | BPG | 20 - 30 x 11 - 25 | 0,9-1,3 | 4.000-11.000 (40cm/h-salt) | IEC/HIC - 65 µm |
| | Chromaflow | 40 - 80 x 15 - 24 | 1,1-1,4 | 3.000-5.000 (100cm/h-salt) | IEC - 65 µm |
| | Index | 20 - 35 x 28 - 32 20 x 15 - 25 | 1,3-1,4 0,8-1,6 | 14.-20.000 (20cm/h-acetone) 3.000-6.000 (100cm/h-acetone) | IEC - 20 µm IEC/HIC - 35/65 µm |
| Merck | Superformance | 20 - 30 x 15 - 30 20 x 30 | 1,0-1,3 1,2 | 2.500-3.500 (100cm/h-acetone) 7.000 (250cm/h-acetone) | IEC - 65 µm IEC - 20 µm |
| Millipore | IsoPak / Access | 44 x 25 44 x 13 - 30 100 - 160 x 15 - 25 140 x 25 160 x 13 - 15 200 x 30 | 1,2-1,5 1,1-1,4 1,2-1,4 1,4-1,7 1,0 1,2-1,4 | 6.000-9.000 (acetone-60 cm/h) 3.000-8.000 (130-20 cm/h) 4.000-6.000 (salt-60 cm/h) 5.000-7.000 (salt-60cm/h) 600-900 (acetone-100 cm/h) 4.000-5.500 (100cm/h-salt) | IEC - 35 µm IEC/HIC - 65 µm IEC/HIC - 65 µm IEC - 35 µm IEC - 100 µm HIC - 65 µm |
| | QuikScale | 20 - 30 x 13 - 20 14 - 30 x 13 - 33 63 x 17 | 1,2-1,6 1,3-1,6 1,2-1,4 | 4.000-10.000 (acetone-100 cm/h) 2.500-5.000 (acetone-100 cm/h) 2.500-4.000 (acetone-130 cm/h) | HIC - 35 µm IEC - 65 µm IEC - 65 µm |
| | Moduline | 140 x 20 - 25 | 0,8 | 5.000-6.000 (salt-30cm/h) | IEC - 65 µm |
| Pall/Euroflow | Resolute™ | 40 - 80 x 12 - 32 40 - 80 x 14 - 32 40 - 100 x 21 - 28 100 - 140 x 20 - 25 | 1,1 0,8-1,2 1,0-1,2 1,0-1,3 | 16.000-19.000 (salt-60cm/h) 3.000-7.000 (salt-30cm/h) 1.000-3.000 (salt-100cm/h) 3.000-7.000 (salt-80cm/h) | HIC - 35 µm HIC/IEC - 65 µm IEC - 100/200 µm HIC - 65 µm |
| Peak Biotech/ DAN Process | LPLC-DAC | 30 x 19 - 21 30 x 20 30 x 20 | 1,3-1,4 1,2-1,8 1,2 | 13.000-17.000 (salt-100cm/h) 6.000-8.000 (salt-100cm/h) 4.000 (salt-80cm/h) | HIC/IEC - 20 µm HIC/IEC - 35 µm IEC - 65 µm |
| Proxcys | CRIO - radial flow | 5 - 20 liter BV, 6 - 11,6 cm L | 1,0-1,2 | 3.000-7.000 (salt-100cm/h) | IEC - 65 µm |

These examples show real values for any packing condition given. It need not to be the achievable optimum.

We have more than 10 years of experience in packing production columns of various manufacturers. Please call our specialist for your individual discussion. In addition we assist you on-site.

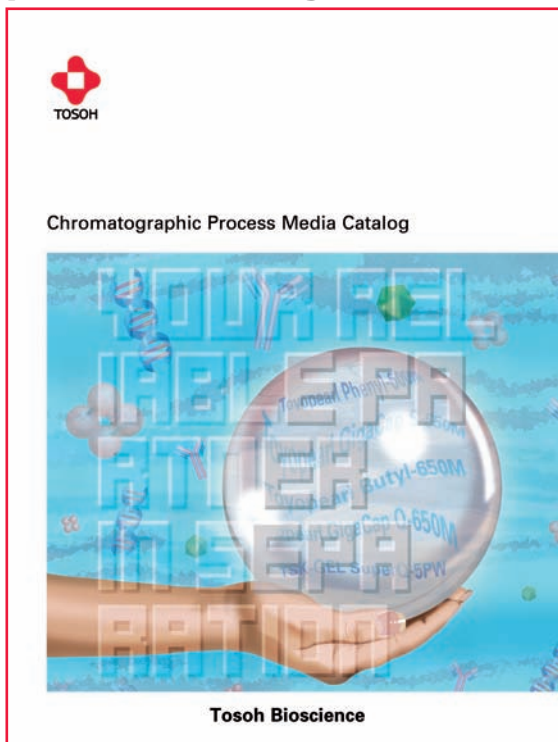


TOSOH

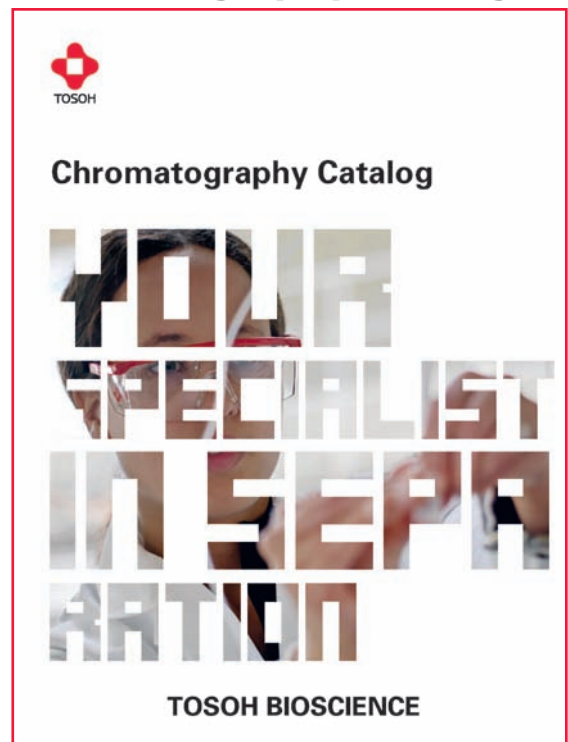
YOUR NOTES

To get an overview about the whole range of our:

bulk media for biopurification, please request our **process catalog**



columns and small bulk media, please request our **chromatography catalog**



For a deeper insight into applications and all questions related to the practical use of TSK-GEL and Toyopearl check our website www.tosohbioscience.com and the related **catalogues** or **instruction manuals**.

Our **technical experts** are happy to discuss your specific separation needs via

hotline: +49 (0) 1805 012299

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