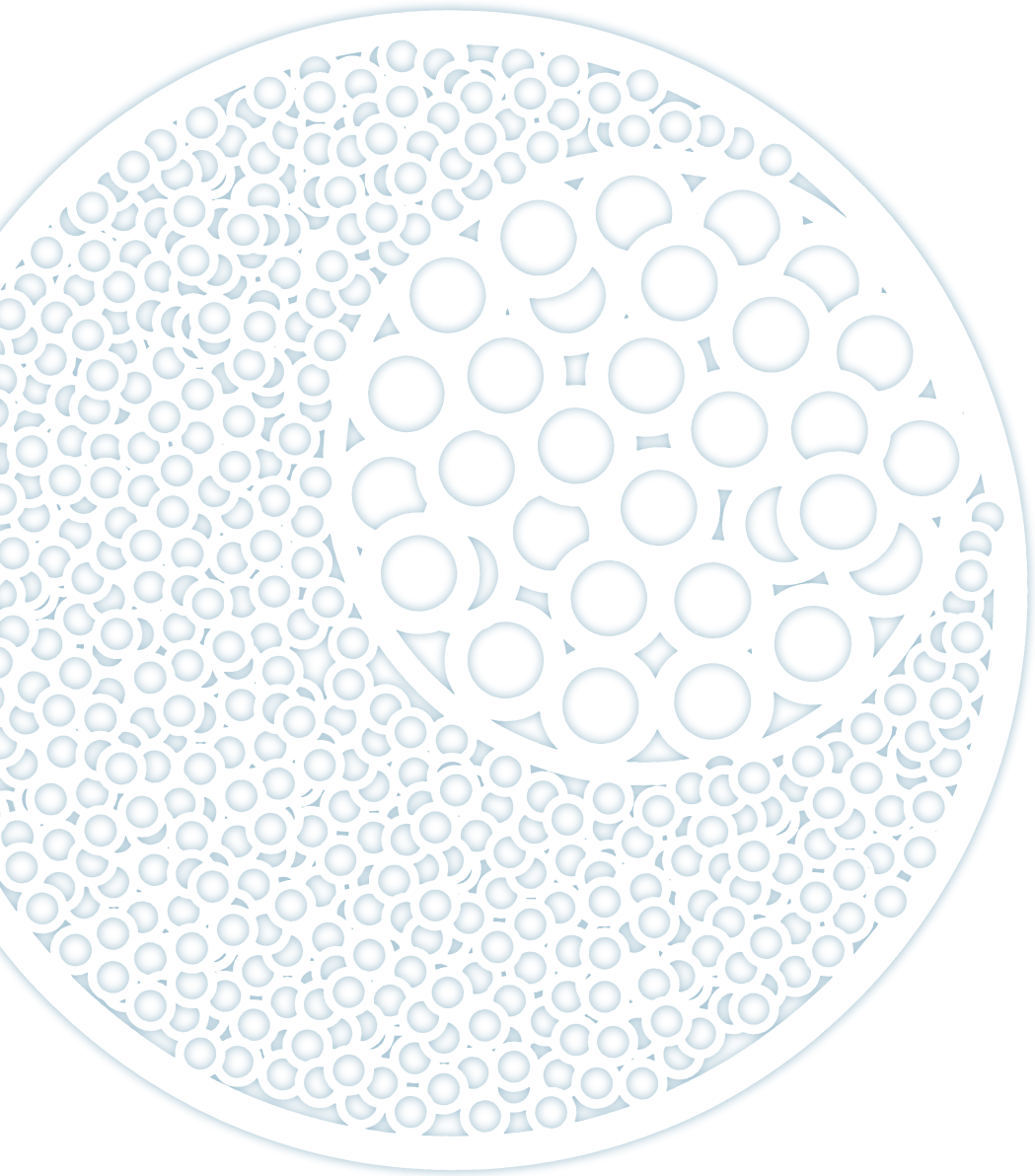
TOSOH

Instruction Manual for TOYOPEARL® and TSKgel® PW-type Resins

Packing and Use Guide



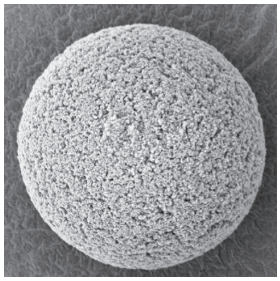
TOSOH BIOSCIENCE

Table of Contents

Introduction

TOYOPEARL chromatographic resins are macroporous polymeric packings for bioprocess chromatography *(Figure 1)*. 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.

Figure 1: TOYOPEARL Base Particle



I. Packing Procedures for TOYOPEARL Resins

1. Preparation for Packing

1.1 General Considerations for Packing

It is best to pack TOYOPEARL resins by the application of pressure from 0.05 to 0.3 MPa across the bed length. Although it is not recommended, TOYOPEARL resins can be packed by simple gravitational settling.

The equipment components required to successfully pack TOYOPEARL resins *(shown in Figure 2)* are: a pump, a pressure gauge, a level, a glass, acrylic or stainless steel column, personal safety equipment, a graduated cylinder and a packing reservoir (optional).

Figure 2: Equipment Required for Packing

1.2 Removal of Fines

Tosoh Bioscience recommends that fines be removed from the gel slurry. Fines 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 (~72% slurry concentration)
Therefore you will need a 20 liter container minimum.
Place the 7-8 liters of re-suspended resin into the 20 liter vessel.

b) Add 12 liters of additional water or packing buffer while stirring to re-suspend the resin in the increased liquid volume. 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 graduated cylinder are listed in *Table 1* below:

Table I: Settling Times for TOYOPEARL Resins in Water

TOYOPEARL Resin Grade	Particle Size	Minutes
Coarse (“C”)	100 µm	15 - 30
GigaCap and Mixed-Mode Medium (“M”)	75 µm	30 - 45
Medium (“M”)	65 µm	30 - 45
Fine (“F”)	45 µm	45 - 60
Superfine (“S”)	35 µm	60 - 90

In larger tanks sedimentation of particles takes longer. *Table 2* lists TOYOPEARL resin settling times in various solutions in 25% and 50% slurry.

Table 2: TOYOPEARL Resin Settling Times in Various Solvents

Solutions	50% slurry	25% slurry
in water	65 µm particles need 3 - 4 hours per meter SD 35 µm particles need 5 - 7 hours per meter SD	65 µm particles need 1.5 - 2.5 hours per meter SD 35 µm particles need 2 - 3.5 hours per meter SD
in 1 mol/L NaCl	65 µm particles need 3 - 5 hours per meter SD 35 µm particles need 12 - 16 hours per meter SD	65 µm particles need 2 - 3 hours per meter SD 35 µm particles need 3 - 7 hours per meter SD
in 1.8 mol/L $(NH_4)_2SO_4$	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)*.

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 *(Figure 4)*. Do not use a magnetic stir bar; it will grind the resin, generating fines.

Figure 3: Decant

Figure 4: Re-suspend

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

1.3 Buffer Exchange

If packing buffer was used in the de-fining process (Step 1.2), proceed to Slurry Preparation (Steps 1.4 or 1.5).

If water was used in the de-fining process, repeat Step 1.2 b) 3 times with your packing buffer of choice.

The optimal packing buffer for each resin will vary from application to 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 3*.

Table 3: Typical Packing Buffers

TOYOPEARL Resin	Packing Buffer
SEC	
HW-40, HW-50, HW-55, HW-65 and HW-75	0.1 mol/L Na_2SO_4, $NaNO_3$, or NaCl in 50 mmol/L phosphate or Tris buffer
IEC	
DEAE-type, QAE, Q-type, CM-type, SP-type, S-type, NH_2-type , Sulfate-type, MegaCap II-SP-type, all GigaCap-type resins	1 mol/L NaCl in 50 mmol/L phosphate, Tris, or acetate buffer
HIC	
Ether-650, SuperButyl-550, Phenyl-type, Butyl-type, Hexyl-650, PPG-600	2 mol/L Na_2SO_4, $(NH_4)_2SO_4$ or NaCl in 50 mmol/L phosphate buffer
Protein A and L	
AF-rProtein A-650, AF-rProtein A HC-650, AF-rProtein L-650F	1 mol/L NaCl in 100 mmol/L phosphate or $NaHCO_3$ buffer
AFC	
AF-Tresyl-650 and AF-Epoxy-650	0.5 mol/L NaCl in 0.1 mol/L $NaHCO_3$ or phosphate buffer
AF-Formyl-650, AF-Amino-650 and AF-Carboxy-650	1 mol/L NaCl in 100 mmol/L phosphate or $NaHCO_3$ buffer
AF-Chelate-650, AF-Blue HC-650 and AF-Red-650	0.5 mol/L NaCl or 0.2 mol/L glycine in 20 mmol/L phosphate or Tris buffer
MMC	
MX-Trp-650	1 mol/L NaCl in 50 mmol/L phosphate, Tris, or acetate buffer

NOTE: Avoid the use of multivalent salts when using TOYOPEARL NH_2-750F

Call customer service: +49 6155 7043730,
technical service: +49 6155 7043736

1.4 Slurry Preparation

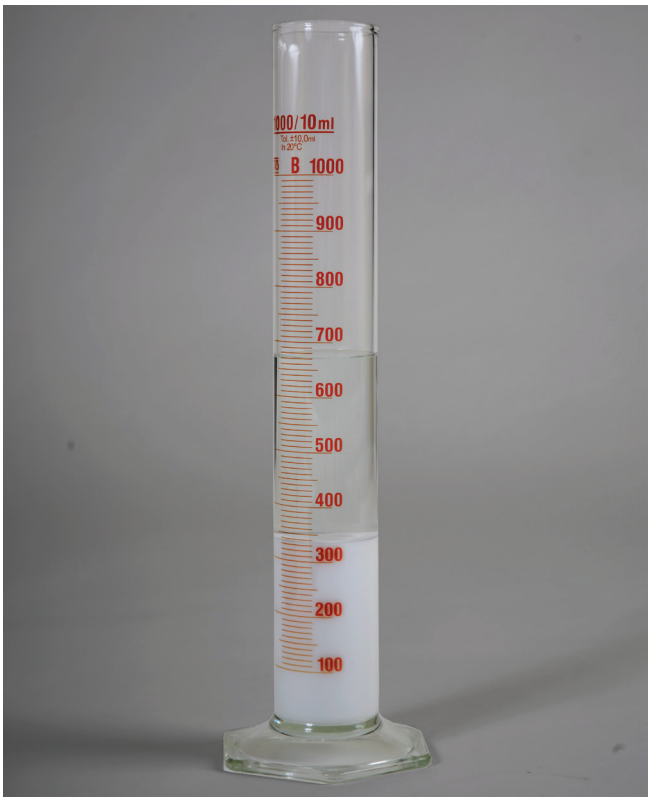
After de-fining and buffer exchange of the resin, the slurry concentration can be adjusted for packing the column. The slurry concentration is calculated as the volume of settled gel divided by the total volume of the slurry. The slurry concentration is adjusted as follows:

a) Re-suspend the resin slurry in the de-fining vessel and transfer the homogeneous slurry to a graduated cylinder.

b) Allow the slurry to settle overnight (>12 hours) for best results *(Figure 5)*.

c) Determine the settled resin volume and adjust the slurry concentration to 30-50% by adding or removing packing buffer. For TOYOPEARL AF-rProtein A HC-650F resin, a 40% slurry is recommended.

Figure 5: Settled Resin in Water



d) For packing a column of a given volume, use the amounts of settled resin as listed in *Table 4* (assuming a packing pressure between 0.24 and 0.3 MPa):

Table 4: Approximate Resin Quantity Needed

TOYOPEARL Resin	Settled Resin Amount
HW-40, HW-50, HW-55	use approximately 1.1 × the column volume
HW-65, and HW-75F NH_2-750F type, Sulfate-650F, Ether-650, Butyl-type, Hexyl-650, PPG-600, DEAE-type, Q-type, CM-650, SP-type, GigaCap-type, non-protein A affinity and mixed-mode	use approximately 1.2 × the column volume
Phenyl-type*, QAE-550C, SP-550C, protein A and protein L affinity	use approximately 1.25 × the column volume

*For TOYOPEARL Phenyl-600M resin, please consult with the Technical Service group to obtain the latest information regarding the packing of this particular resin.

1.5 Alternative Slurry Preparation

a) Re-suspend the resin slurry in the de-fining vessel and transfer the homogeneous slurry to a Büchner funnel or equivalent.

b) Filter the slurry under suction until the slurry becomes a wetcake (all excess liquid has been removed).

c) Weigh out the appropriate amount of resin wetcake (1 g of wetcake = 1 mL of gravity settled gel) referencing *Table 4* above.

d) Transfer the wetcake to a beaker and add enough packing buffer to make a slurry concentration of 30-50%.

2. Packing Procedures

Do not pack TOYOPEARL and TOYOPEARL GigaCap® resins like traditional soft gels. For best results, TOYOPEARL and TOYOPEARL GigaCap resins should be packed at higher flow rates and pressures.

Table 5: Packing and Operating Velocities for TOYOPEARL Resins

LABORATORY SCALE

Mode	TOYOPEARL Resin Type	Column Size (cm ID x cm L)	Resin Grade	Packing Velocity (cm/hr)	Max Operating Pressure (Pressure drop)
SEC	HW-40	2.2 × 60	S (30 µm) F (45 µm) C (100 µm)	30 - 40 60 - 80 120 - 160	0.3 MPa 0.3 MPa 0.3 MPa
	HW-50	2.2 × 60	S (30 µm) F (45 µm)	25 - 35 50 - 70	0.3 MPa 0.3 MPa
	HW-55	2.2 × 60	S (30 µm) F (45 µm)	25 - 35 50 - 70	0.3 MPa 0.3 MPa
	HW-65	2.2 × 60	S (30 µm) F (45 µm)	20 - 75 40 - 150	0.3 MPa 0.3 MPa
	HW-75	2.2 × 60	F (45 µm)	40 - 150	0.3 MPa
IEC*	NH_2-750	2.2 x 20	F (45 µm)	400-600	0.3 MPa
	Sulfate-650F	2.2 x 20	F (45 µm)	400-600	0.3 MPa
	DEAE-650, SuperQ-650, CM-650 CM-650, SP-650, Q-600C AR	2.2 × 20	S (30 µm) M (65 µm) C (100 µm)	400 - 600 800 - 1000 800 - 1200	0.3 MPa 0.3 MPa 0.3 MPa
	GigaCap S-650, GigaCap Q-650 GigaCap CM-650, GigaCap DEAE-650	2.2 × 20	S (35 µm) M (75 µm)	400 - 600 800 - 1000	0.3 MPa 0.3 MPa
	SP-550, QAE-550	2.2 × 20	C (100 µm)	700 - 1000	0.3 MPa
	MegaCap II SP-550	2.2 × 20	EC (200 µm)	800 - 1200	0.3 MPa
HIC*	Ether-650, Phenyl-650, Butyl-650, Hexyl-650	2.2 × 20	S (30 µm) M (65 µm) C (100 µm)	400 - 600 800 - 1000 800 - 1200	0.3 MPa 0.3 MPa 0.3 MPa
	PPG-600, Phenyl-600, Butyl-600	2.2 × 20	M (65 µm)	800 - 1000	0.3 MPa
	SuperButyl-550	2.2 × 20	C (100 µm)	800 - 1200	0.3 MPa
Protein A	AF-rProtein A-650	2.2 × 20	F (45 µm)	400 - 600	0.3 MPa
	AF-rProtein A HC-650	2.2 × 20	F (45 µm)	400 - 600	0.3 MPa
Protein L	AF-rProtein L-650F	2.2 × 20	F (45 µm)	400 - 600	0.2 MPa
Affinity	AF-Amino-650, AF-Tresyl-650 AF-Carboxy-650, AF-Formyl-650 AF-Epoxy-650, AF-Chelate-650 AF-Blue HC-650, AF-Red-650	2.2 × 10	M (65 µm)	800 - 1000	0.3 MPa
MMC	MX-Trp-650	2.2 × 20	M (75 µm)	800 - 1000	0.3 MPa

* Not all resins are available in all particle sizes.

PROCESS SCALE

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

Please call our Technical Specialists to discuss your unique packing process.

Call customer service: +49 6155 7043730,
technical service: +49 6155 7043736

Table 6 lists the features of packing methods valid for packing under flow. If you have other equipment, such as Dynamic Axial Compression or self-packing columns, or pack columns greater than 5 liters, please call our Technical Specialists. We have experience with many different column designs and brands.

Table 6: Features of Packing Methods

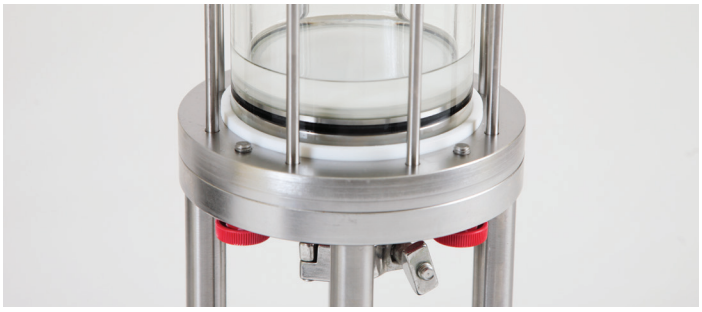
	Packing Method		
	Semi-Constant Pressure	**Constant Velocity**	**Assisted Gravity**
Packing velocity	fast	fast	slow
Flow rate range	up to high	up to high	limited to low
Pump	constant pressure	constant velocity	peristaltic pump
Pressure gauge	needed	needed	not needed

2.1 Constant Velocity / Semi-Constant Pressure Methods

a) If necessary, place a 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 *(Figure 6)*. Plug the outlet of the column while leaving 1-2 cm of buffer in the bottom of the column.

Figure 6: Bubble Free, Liquid Covered Bottom Frit



c) Re-suspend the resin slurry to assure homogeneity *(Figure 7)*.

Figure 7: Homogenize Slurry

d) Carefully pour the resin slurry down the inside wall of the column. Prevent air from being trapped in the resin slurry *(Figure 8)*.

Figure 8: Pouring the Resin

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

f) Immediately place the flow adapter of the column onto the resin slurry *(Figure 9)*. There should be no trapped air between the flow adapter and the buffer.

Figure 9: Adjusting Column

g) Open the column outlet, and start the pump. Slowly increase the flow of packing buffer through the column. Record the system pressure for future reference.

If packing with an FPLC system, record the system pressure prior to beginning packing operations. Subtracting the system pressure from the total pressure will yield the column ΔP.

h) At this point one of two methods should be followed:

Constant Velocity Method

Slowly increase to the final flow rate as mentioned in Step *g*. Increasing the flow rate gradually prevents hydraulic shock to the forming bed and avoids uneven packing of the column bed. The flow rate can be increased 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 7*.

Table 7: Examples of Flow Rate Increments

Column Size (ID × L)	TOYOPEARL Resin	Target Flow Rate (mL/min)	Increment (mL/min)	Hold Time (min)
2.2 cm × 60 cm	HW-55S	2	0.5	0.5
9 cm × 30 cm	QAE-550C	300	50	2
25 cm × 30 cm	DEAE-650M	2000	400	3

Semi-Constant Pressure Method

Slowly increase the flow rate until the target pressure (max 0.3 MPa) is obtained. Increasing the flow rate gradually prevents hydraulic shock to the forming bed, and therefore avoids uneven packing of the column. The packing pressure must 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 approximately 0.3 MPa across the bed length.

For TOYOPEARL AF-rProtein A HC-650F resin and TOYOPEARL AF-rProtein L-650F resin, open the column outlet and begin flow packing at 100 cm/hr linear velocity. Increase the linear velocity by 100 cm/hr every minute until the column ΔP reaches approximately 0.3 MPa.

i) When the packed bed is stable, and with approximately 2-3 cm of buffer above the bed, shut off the pump and close the column outlet.

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 *(Figure 10)*. Avoid introduction of air into the column *(Figure 11a-b)*.

Figure 10: Clear Supernatant of Partially Packed Bed

Figure 11a: Bubble Free (recommended)

Figure 11b: Air Disturbs Homogeneous Settling Procedure

l) Secure the flow adapter, restart the pump as described in Step *g*, and open the column outlet.

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

n) Carefully loosen the flow adapter seal and lower the adapter to within 0.5 to 1.0 cm of the resin bed. Do not disturb the resin bed when moving the flow adapter *(Figure 12)*.

Figure 12: Flow Adapter in Place

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) Lower the adapter 1-2 mm into the bed and lock the adaptor into place.

q) The column is now ready for efficiency evaluation. Examples of TOYOPEARL AF-rProtein A HC-650F performance testing results are shown beginning on page 15.

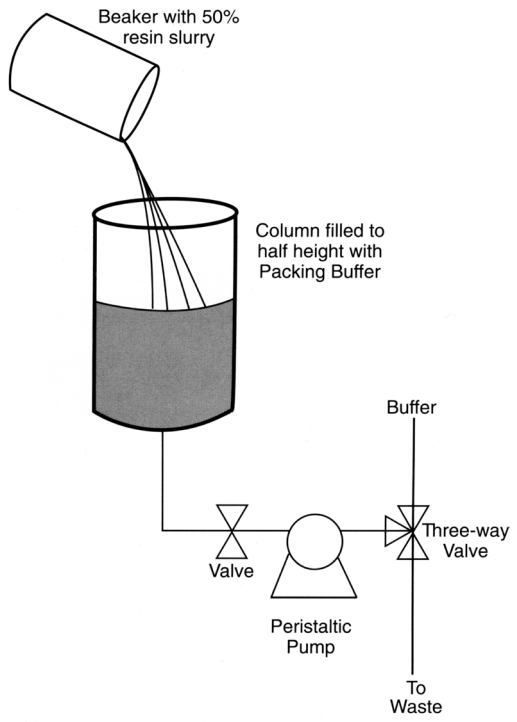
2.2 Alternative Packing Method, Assisted Gravity

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.

Figure 14: Assisted Gravity Packing Method



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 wash 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 so that flow will enter through the top of the column. Eliminate any air in the tubing.

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

k) Slowly increase the pump speed until the desired flow rate is obtained. Increasing the flow rate gradually prevents hydraulic shock to the forming bed, and therefore avoids uneven packing of the column. The flow rate can be increased 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 7)*.

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**) and **m**) until there is no further bed compression from the flow adapter (< 0.5 cm).

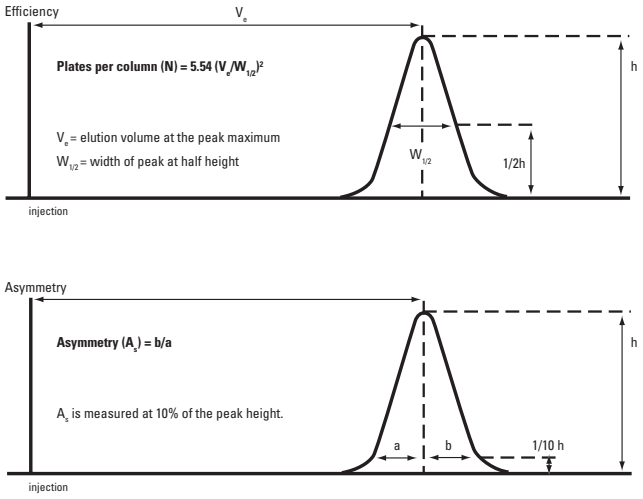
o) As the final step, lower the adapter 1-5 mm into the bed.

p) The column is now ready for an efficiency evaluation.

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 total 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 8*, and asymmetries between 0.8-1.4 when tested.

Figure 15: How to Calculate Efficiency & Asymmetry Factor



If there is a large deviation from expected plate count and asymmetry factors, please repeat the packing procedure. If column diameters > 40 cm are utilized, the number of plates/M will decrease proportionally.

Table 8: Typical Plate Counts

Mode	Column ID (cm)	S Grade (plates/M)	F Grade (plates/M)	M Grade (plates/M)	C Grade (plates/M)
SEC	2.2	5000	3500	-	3000
	5.5	5000	3300	-	-
	10.8	5000	2500	-	-
	21	4000	2200	-	1500
	31	-	2000	-	1200
	40	-	1800	-	1000
IEC	2.2	6000	3500	4000	2000
	5.5	6000	3300	4000	-
	10.8	6000	2500	4000	-
	21	4000	2200	2600	2000
	31	-	2000	2000	1000
	40	-	1800	1500	750
HIC	2.2	6000	-	4000	2000
	5.5	6000	-	4000	-
	10.8	6000	-	4000	-
	21	4000	-	2600	2000
	31	-	-	2000	1000
	40	-	-	1500	750
Protein A	2.2	-	3500	-	-
	5.5	-	3300	-	-
	10.8	-	2500	-	-
	21	-	2200	-	-
	31	-	2000	-	-
	40	-	1800	-	-
Protein L	2.2	-	3500	-	-
	5.5	-	3300	-	-
	10.8	-	2500	-	-
	21	-	2200	-	-
	31	-	2000	-	-
	40	-	1800	-	-
AFC	2.2	-	-	4000	-
	5.5	-	-	4000	-
	10.8	-	-	4000	-
	21	-	-	2600	-
	31	-	-	2000	-
	40	-	-	1500	-
MMC	2.2	-	-	4000	-
	5.5	-	-	4000	-
	10.8	-	-	4000	-
	21	-	-	2600	-
	31	-	-	2000	-
	40	-	-	1500	-

The TOYOPEARL GigaCap series of ion exchange resins require special running conditions to properly perform the efficiency evaluations when using a conductivity probe. The equilibrium buffer for performing the efficiency test must contain at least 250-300 mmol/L of salt. If the salt is not added, the performance test will result in peak fronting (most likely due to ion exclusion effects).

If column diameters > 40 cm are utilized, the number of plates/M will decrease slightly. If there is a large deviation from expected plate count and asymmetry factors, please repeat the packing procedure. Some of the potential reasons for poor column packing are listed in *Table 9*.

For further details call our Technical Specialists.

Table 9 : Troubleshooting Performance Evaluation

$A_S < 0.8$	$A_S > 1.4$
Column overpacked. Packing at too high a 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 is the height equivalent to the theoretical plate.

Call customer service: +49 6155 7043730,
technical service: +49 6155 7043736

II. Packing Procedures for TSKgel PW-type Resins

In general the packing procedure for TSKgel PW-type resins is the same as for TOYOPEARL resins noted in Section I. Because the TSKgel PW-type resins are more highly crosslinked to withstand the higher pressures generated by their smaller particle sizes *(See Table 10)*, some guidelines for the packing procedure shown in Section I should be modified for the TSKgel PW-type resins:

1. TSKgel PW-type resins are best packed at 0.3 - 1.0 MPa and should not be packed at pressures higher than 2 MPa.

2. Removal of fines is as described in Section 1.2 with settling times as shown in *Table 11*.

3. Slurry preparation is as described in Section 1.4 using a slurry range between 20-75%. Best results are obtained at 40-50% slurry concentration.

4. Use 1.2× the final column volume needed, in settled resin, to pack the column.

Table 10: Packing and Operational Velocities for TSKgel PW-type Resins

Resin Type	Column Size (cm ID × L)	Particle Size	Packing Velocity Flow Rate (cm/hr)	Operating Velocity (cm/hr)	Operating Velocity (mL/min)
TSKgel 3PW TSKgel 5PW	5.5 × 20	20 µm	100 - 400	50 - 300	19.8 - 119
	10.8 × 20	20 µm	100 - 400	50 - 300	76.3 - 458
	15.8 × 30	30 µm	100 - 500	50 - 400	163 - 1307
	21 × 30	30 µm	100 - 500	50 - 400	289 - 2309
	30 × 20 - 30 (DAC*)	20 - 30 µm	(N/A)	50 - 400	589 - 4712

*DAC = Dynamic Axial Compression at applied piston pressures > 0.3 MPa.

Table 11: Settling Times for TSKgel PW-type Resins:

TSKgel PW-type Resin Particle Size	Settling time
30 µm	90 - 120 minutes
20 µm	>120 minutes

III. 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 I, Section 1.3)*. Size exclusion separations on TOYOPEARL HW columns are performed under isocratic conditions using buffered salt solutions of moderate ionic strength. Sample volumes injected onto the column 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 12* for suggested mobile phase changes.

Table 12: Non Ideal SEC Behavior

Observation	Cause/Solution
Retention time is shorter than expected	Sample can be partially or totally excluded from column, confirm MW of sample and use a resin with higher exclusion limit if necessary Anionic molecules can be repulsed by ionic exclusion, increase the ionic strength of the mobile phase
Retention time is longer than expected	Cationic molecules can be retarded by ionic attraction, increase the ionic strength of the mobile phase. 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.

1.2 Ion Exchange Chromatography (IEC)

Equilibrate the column with 5 to 10 column volumes of an appropriate starting buffer solution *(Table 13)*. The elution is performed by increasing the salt concentration or changing the pH of the eluent.

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

Table 13: Examples of Buffers used in IEC

Resin Type	Buffer	Buffering Range
Cation exchange	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 exchange	L-Histidine	5.5 - 6.0
	imidazole	6.6 - 7.1
	triethanolamine	7.3 - 7.7
	Tris-Cl	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 mol/L to 3 mol/L) salt such as one listed in *Table 14*. 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.

Table 14: 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. May be more difficult to dispose.
Sodium sulfate	Solubility is low (1.5 mol/L 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

Call customer service: +49 6155 7043730,
technical service: +49 6155 7043736

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 15* for suggestions on what organic solvents, detergents, or chaotropes to use.

Table 15: Mobile Phase Additives for HIC

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

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, adjust the pH of the buffer closer to the isoelectric point of the protein, or select a resin with a more hydrophobic ligand.

1.4 Affinity Chromatography (AFC)

Included among the TOYOPEARL affinity resins are both group specific ligand resins (AF-Chelate-650, AF-Red-650, and AF-Blue HC-650) 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 AF-Formyl-650, AF-Carboxy-650, AF-Amino-650, AF-Epoxy-650, and AF-Tresyl-650.

Equilibration
AF Red-650, AF Blue HC-650, and AF-Chelate-650, 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 any released dye. Wash a column containing new resin with 1 mol/L sodium chloride or 1 mol/L potassium chloride. Use 2 mol/L potassium chloride or 4 mol/L urea for washing used resin. Equilibrate a column containing old or new resin with an appropriate starting buffer such as 20 mmol/L 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 mol/L sodium chloride or 3 mol/L potassium chloride. Proteins not eluted with these eluents can be eluted with solutions listed in *Table 16*.

Table 16: Eluents for Exhaustive Elution from AF TOYOPEARL Resins

Preferred choice	2 mol/L NaCl or 3 mol/L KCl
Alternate choice	1% Triton X-100 / 1 mol/L NaSCN / 75% ethylene glycol / 4 mol/L urea or 0.1 mol/L NaOH / 4.2 mol/L $(NH_4)_2SO_4$

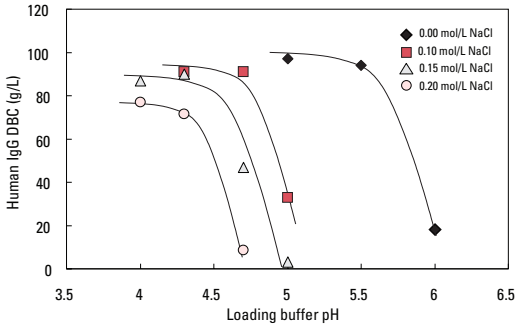
In specific elution, an enzyme is eluted with a solution containing its substrate or coenzyme. A substrate or coenzyme concentration below 10 mmol/L usually is sufficient for elution.

1.5 Mixed-Mode Chromatography (MMC)

TOYOPEARL MX-Trp-650M resin is a mixed-mode resin that exhibits both high dynamic binding capacities and tolerance to feedstocks with high concentrations of salt. This resin also has excellent binding and elution kinetics.

Equilibrate the packed column with an appropriate buffer solution containing a conductivity similar to the conductivity of the feedstock. The pH of the feedstock should be lower (pH < 5.0) for samples having approximately 0.1 mol/L of salt or more. For feedstocks containing < 0.1 mol/L of salt, the pH can be increased up to pH 6.0 *(See figure 16)*.

Figure 16: Effect of Buffer and Salt on DBC



Resin: TOYOPEARL MX-Trp-650M
Column: 6 mm ID × 4 cm
Mobile phase: Buffer A: 0.05 mol/L acetate buffer (pH 4.0 - 6.0) + 0 - 0.2 mol/L NaCl
Buffer B: 0.1 mol/L Tris-HCl buffer (pH 8.5) + 0.3 mol/L NaCl
Flow rate: 1.0 mL/min (212 cm/hr)
Detection: UV @ 280 nm
Sample: human polyclonal IgG (1 mg/mL)

Dynamic binding capacity (DBC) calculated from 10 % height of breakthrough curve

To elute the proteins, increase the salt concentration (e.g. 0.3 mol/L) and raise the pH above 7.0. Always take into consideration the conditions and how they might influence the stability of the target molecule.

2. Cleaning

TOYOPEARL and TSKgel PW-type 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 TOYOPEARL SEC 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 mol/L aqueous salt solutions can be used to clean the resin. For more strongly bound materials, 0.1-0.5 mol/L sodium hydroxide or 0.1-0.5 mol/L 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 or damage column hardware. 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 mol/L sodium chloride, then equilibrate with the starting buffer. For severe contamination, wash with 0.1-0.5 mol/L sodium hydroxide, then with 0.1-0.5 mol/L sodium chloride, and finally equilibrate with the starting buffer.

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

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

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 more strongly bound materials, 0.1-0.5 mol/L sodium hydroxide or an appropriate acid such as hydrochloric or sulfuric is appropriate. Under no circumstances should nitric acid be used to clean TOYOPEARL and TSKgel PW-type 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 or damage column hardware. Non-ionic detergents may also be used for cleaning.

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

Protein A and L Resins
A solution of 0.1 mol/L NaOH at 15 minutes contact time, may be used as an effective cleaning solution for the resins. (A solution of 0.2 mol/L NaOH may also be used as a cleaning solution for the Protein A resin only.) 0.5 mol/L NaOH should only be used as a cleaning solution in cases of extreme contamination. Contact time should be kept to 15 minutes or less as prolonged exposure to elevated concentrations of NaOH is detrimental to the ligand.

AFC Resins
High concentrations of neutral salts, chaotropes, or detergents such as those listed in *Table 16* 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 mol/L sodium hydroxide followed by distilled water.

MMC Resin
For moderate contamination, wash with 0.5-1.0 mol/L sodium chloride, then equilibrate with the starting buffer. For severe contamination, wash with 0.1-0.5 mol/L sodium hydroxide, then with 0.1-0.5 mol/L sodium chloride, and finally equilibrate with the starting buffer.

Strongly bound hydrophobic impurities may require 10-40% of an alcohol such as ethanol, methanol, or isopropanol. It is important to remember that solvents can sometimes cause protein aggregation or damage column hardware.

Call customer service: +49 6155 7043730,
technical service: +49 6155 7043736

3. Storage

SEC and IEC Resins
Store the column or used bulk resin in distilled water containing a bacteriostatic agent, such as 20% ethanol, preferably at 4-35 °C. As an alternative, SEC and IEC resins can be stored in a solution of 2% benzyl alcohol. For information on TOYOPEARL resins and storage in benzyl alcohol and NaOH solutions, please contact the Tosoh Bioscience Technical Service department.

HIC Resins
Store the column or used bulk resin in distilled water containing a bacteriostatic agent, such as 20% ethanol, preferably at 4-35 °C. It is not recommended to store HIC resins in 2% benzyl alcohol. For information on storage in NaOH, please contact the Tosoh Bioscience Technical Service department.

Protein A and L Resins
Store the column or used bulk resin in distilled water containing a bacteriostatic agent, such as 20% ethanol, preferably at 2-8 °C. The TOYOPEARL AF-rProtein A-650F resin and TOYOPEARL AF-rProtein L-650F resin can be, as an alternative to 20% EtOH, stored in a solution of 2% benzyl alcohol. For information on TOYOPEARL protein A resins and storage in benzyl alcohol, please contact the Tosoh Bioscience Technical Service department.

AFC Resins
Store the column or used bulk resin in a neutral solution of 1 mol/L sodium chloride or potassium chloride containing a bacteriostatic agent, such as 20% ethanol, preferably at 2-8 °C. For AF-Formyl 650M, store the column or used bulk resin in a neutral solution of 1mol/L sodium chloride or potassium chloride in 1% gluteraldehyde, preferably at 2-8 °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.

MMC Resin
Store the column or used bulk resin in distilled water containing a bacteriostatic agent, such as 20% ethanol, preferably at 4-35 °C. Store the bulk resin in a dark container such as that provided during shipment. Make sure that a packed column is kept out of direct light during storage as the resin may slightly discolor over time. This discoloration does not influence the performance of the resin in subsequent operations.

4. Sterilization / Depyrogenation / Preservative Removal / Column Frits

Sterilization
TOYOPEARL and TSKgel PW-type resins can be sterilized by autoclaving at 121 °C for 20 minutes 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. Please consult with a Technical Specialist if considering the use of sodium hypochlorite.

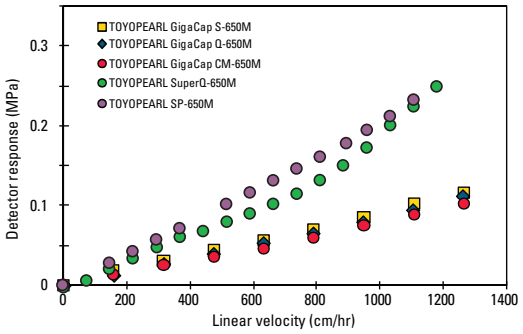
Depyrogenation
TOYOPEARL and TSKgel PW-type resins are recommended for use from pH 2 to 12. However, short exposures (< 12 hours) to higher pH (0.5 mol/L NaOH) are acceptable for depyrogenation. Typically endotoxin levels are reduced by at least 4 logs following a 4 hour treatment with 0.5 mol/L NaOH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

Preservative Removal
Shipping solvents for TOYOPEARL and TSKgel PW-type resins contain 20% ethanol (with the 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.

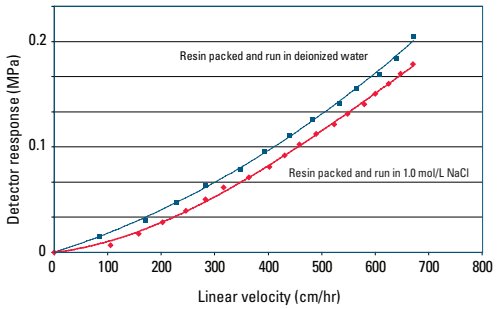
5. Pressure-flow Curves and Performance Testing Results for Selected TOYOPEARL and TOYOPEARL GigaCap Resins

Figure 17: Pressure-flow Curve Comparison of TOYOPEARL Resins



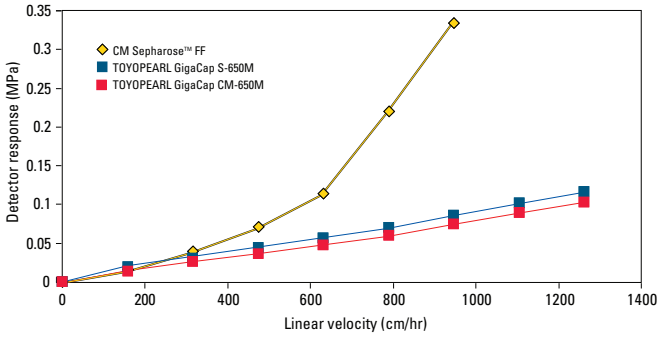
Resins:	TOYOPEARL GigaCap S-650M TOYOPEARL GigaCap Q-650M TOYOPEARL GigaCap CM-650M TOYOPEARL SuperQ-650M TOYOPEARL SP-650M
Column size:	22 mm ID × 20 cm
Mobile phase:	distilled water
Temperature:	25 °C

Figure 18: Pressure Flow Data for TOYOPEARL GigaCap S-650M



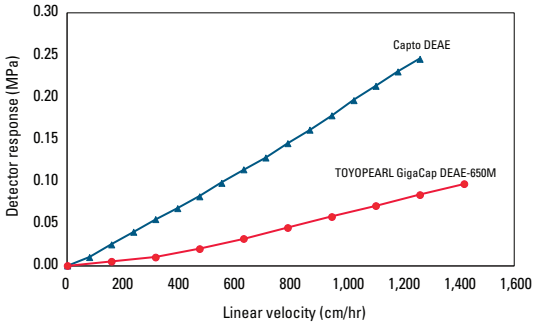
TOYOPEARL GigaCap S-650M was packed into a 36 cm ID x 25 cm bed height Eastern Rivers BioStream column to measure the pressure-flow characteristics. The resin had similar profiles when packed and run in both water and 1.0 mol/L NaCl.

Figure 19: TOYOPEARL GigaCap CM-650M Pressure-flow Properties



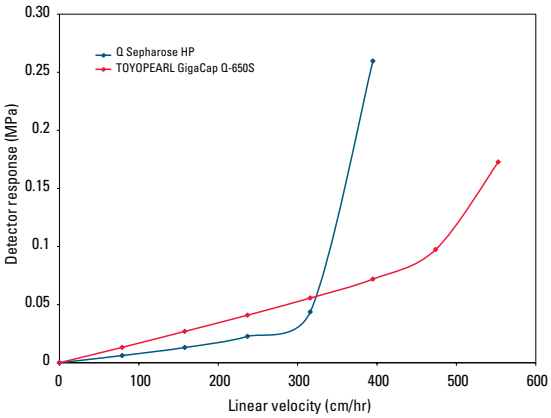
Resin:	TOYOPEARL GigaCap CM-650M
Column size:	22 mm ID × 20 cm
Mobile phase:	H_2O
Detection:	pressure (MPa)

Figure 20: TOYOPEARL GigaCap DEAE-650M Pressure-flow Curves



Resin:	TOYOPEARL GigaCap DEAE-650M
Column size:	22 mm ID × 20 cm
Mobile phase:	0.1 mol/L NaCl
Detection:	pressure (MPa)

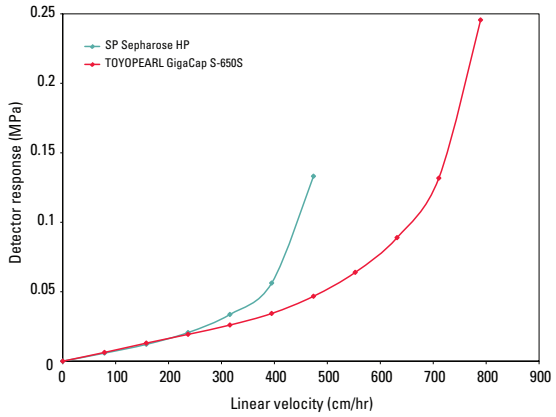
Figure 21: Comparison of TOYOPEARL GigaCap Q-650S and Q Sepharose HP Pressure-flow Curves



Resin:	TOYOPEARL GigaCap Q-650S Q Sepharose HP
Column size:	22 mm ID × 20 cm
Mobile phase:	0.1 mol/L NaCl
Detection:	pressure (MPa)

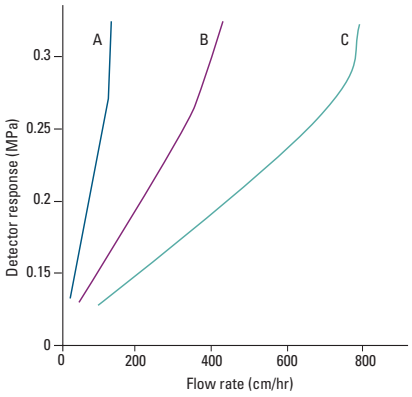
Call customer service: +49 6155 7043730,
technical service: +49 6155 7043736

Figure 22: Comparison of TOYOPEARL GigaCap S-650S and SP Sepharose HP Pressure-flow Curves



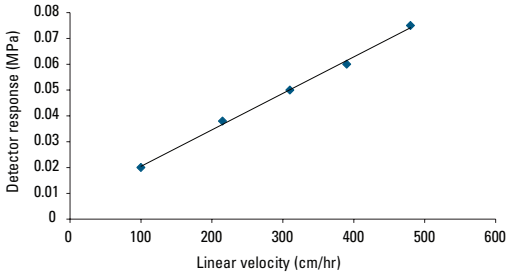
Resin:	TOYOPEARL GigaCap S-650S SP Sepharose HP
Column size:	22 mm ID × 20 cm
Mobile phase:	0.1 mol/L NaCl
Detection:	pressure (MPa)

Figure 23: Pressure-flow Curve for TOYOPEARL Phenyl-650 Resins of Various Particle Sizes



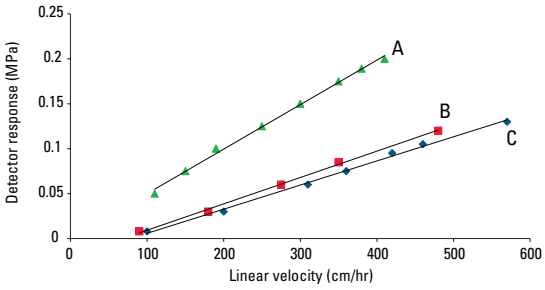
Resins:	A. TOYOPEARL Phenyl-650S B. TOYOPEARL Phenyl-650M C. TOYOPEARL Phenyl-650C
Column size:	25 mm ID × 25 cm
Mobile phase:	2.0 mol/L $(NH_4)_2SO_4$
Flow rate:	as indicated in figure

Figure 24A: Linear Velocity and Pressure Curve of Packed TOYOPEARL AF-rProtein A-650F Column



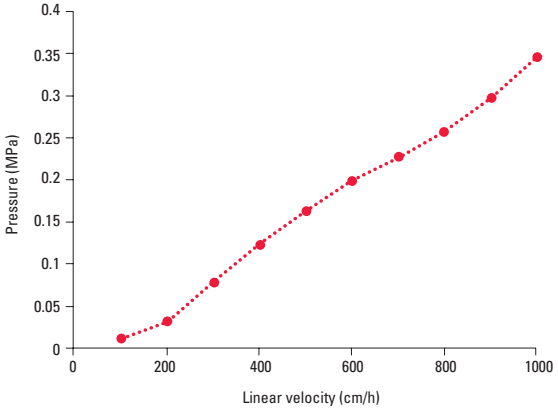
Resin:	TOYOPEARL AF-rProtein A-650F
Column and size:	Resolute, 40 cm ID × 8.4 cm
Mobile phase:	H_2O
Linear velocity:	various
Detection:	pressure (MPa)

Figure 24B: Comparison of Linear Velocity and Pressure Curves



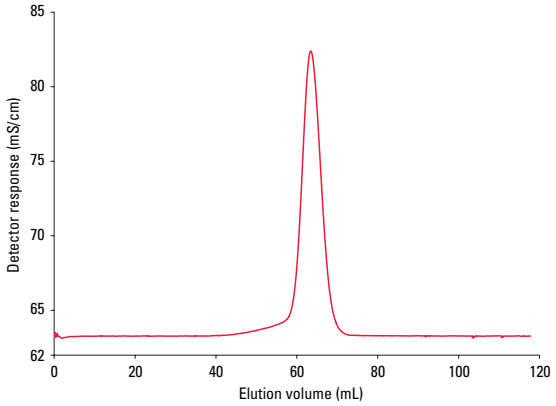
Resins:	A. TOYOPEARL AF-rProtein A-650F - 45 µm (20 cm ID × 32 cm) B. TOYOPEARL AF-rProtein A-650F - 45 µm (20 cm ID × 18 cm) C. TOYOPEARL Butyl-650M - 65 µm (20 cm ID × 21 cm)
Column:	QuikScale 20 cm ID
Mobile phase:	Water
Linear velocity:	Various
Detection:	Pressure (MPa)

Figure 25: Pressure flow curve for TOYOPEARL AF-rProtein L-650F Resin



Resin:	TOYOPEARL AF-rProtein L-650F
Column size:	4.4 cm ID × 28 cm
Mobile phase:	Water
Linear velocity:	Various
Detection:	Pressure (MPa)

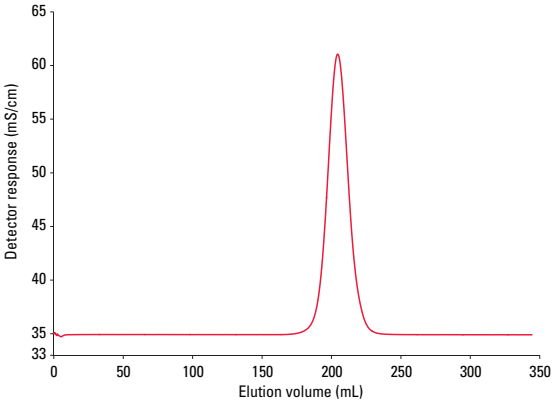
Figure 26A: HETP and Asymmetry Evaluations of Packed TOYOPEARL AF-rProtein A HC-650F Columns



Resin:	TOYOPEARL AF-rProtein A HC-650F
Column size:	2.6 cm ID × 14.8 cm (78.5 mL)
Mobile phase:	0.4 mol/L NaCl
Flow rate:	60 cm/hr (5.3 mL/min)
Detection:	conductivity (mS/cm)
Temperature:	ambient
Sample:	3.0 mol/L NaCl
Sample load:	1.0% CV
Instrument:	AKTA Avant

N/m	6537
Asymmetry	1.03

Figure 26B: HETP and Asymmetry Evaluations of Packed TOYOPEARL AF-rProtein A HC-650F Columns



Resin:	TOYOPEARL AF-rProtein A HC-650F
Column size:	4.4 cm ID x 15 cm (228 mL)
Mobile phase:	0.4 mol/L NaCl
Flow rate:	60 cm/hr (15.2 mL/min)
Detection:	conductivity (mS/cm)
Temperature:	ambient
Sample:	3.0 mol/L NaCl
Sample load:	1.0% CV
Instrument:	AKTA Avant

N/m	5932
Asymmetry	1.10

Call customer service: +49 6155 7043730,
technical service: +49 6155 7043736

6. Examples of Process Column Installations

Column Manufacturer	Column Type	Various Bed Dimensions (ID × L in cm)	Column Performance		Resin Type
			As	Plate Count (N/m)	
Bio-Rad®	InPlace™/GelTec™	20-45 × 15-25 130 × 24	0.8-1.4 1.1-1.2	3,000-4,000 (salt-60 cm/h) 3,500-3,900 (salt-300 cm/h)	HIC-65 µm IEC-65 µm
GE Healthcare Lifesciences	AxiChrom™	60 × 20	1.1	8,000 (salt-100 cm/h)	HIC-65 µm
	BPG™	20-30 × 11-25	0.9-1.3	4,000-11,000 (salt-40 cm/h)	IEC/HIC-65 µm
	Chromaflow™	40-80 × 15-24	1.1-1.4	3,000-5,000 (salt-100 cm/h)	IEC-65 µm
	INdEX™	20-35 × 28-32 20 × 15-25	1.3-1.4 0.8-1.6	14,-20,000 (acetone-20 cm/h) 3,000-6,000 (acetone-100 cm/h)	IEC-20 µm IEC/HIC-35/65 µm
Merck	Superformance®	20-30 × 15-30 20 × 30	1.0-1.3 1.2	2,500-3,500 (acetone-100 cm/h) 7,000 (acetone-250 cm/h)	IEC-65 µm IEC-20 µm
Millipore	IsoPak® / Access®	44 × 25	1.2-1.5	6,000-9,000 (acetone-60 cm/h)	IEC-35 µm
		44 × 13-30	1.1-1.4	3,000-8,000 (130-20 cm/h)	IEC/HIC-65 µm
		100-160 × 15-25	1.2-1.4	4,000-6,000 (salt-60 cm/h)	IEC/HIC-65 µm
		140 × 25	1.4-1.7	5,000-7,000 (salt-60 cm/h)	IEC-35 µm
		160 × 13-15	1.0	600-900 (acetone-100 cm/h)	IEC-100 µm
		200 × 30	1.2-1.4	4,000-5,500 (100 cm/h-salt)	HIC-65 µm
	QuikScale®	20-30 × 13-20	1.2-1.6	4,000-10,000 (acetone-100 cm/h)	HIC-35 µm
		14-30 × 13-33	1.3-1.6	2,500-5,000 (acetone-100 cm/h)	IEC-65 µm
		63 × 17	1.2-1.4	2,500-4,000 (acetone-130 cm/h)	IEC-65 µm
	Moduline®	140 × 20-25	0.8	5,000-6,000 (salt-30 cm/h)	IEC-65 µm
Pall/Euroflow	Resolute™	40-80 × 12-32	1.1	16,000-19,000 (salt-60 cm/h)	HIC-35 µm
		40-80 × 14-32	0.8-1.2	3,000-7,000 (salt-30 cm/h)	HIC/IEC-65 µm
		40-100 × 21-28	1.0-1.2	1,000-3,000 (salt-100 cm/h)	IEC-100/200 µm
		100-140 × 20-25	1.0-1.3	3,000-7,000 (salt-80 cm/h)	HIC-65 µm
Peak Biotech/ DAN Process	LPLC-DAC	30 × 19-21	1.3-1.4	13,000-17,000 (salt-100 cm/h)	HIC/IEC-20 µm
		30 × 20	1.2-1.8	6,000-8,000 (salt-100 cm/h)	HIC/IEC-35 µm
		30 × 20	1.2	4,000 (salt-80 cm/h)	IEC-65 µm
Proxcys	CRIO - radial flow	5 - 20 liter BV, -11.6 cm L	1.0-1.2	3,000-7,000 (salt-100 cm/h)	IEC-65 µm

These examples show real values for user defined packing conditions. These are not necessarily the optimum values.

Tosoh Bioscience has more than 20 years of experience in packing production columns using various manufacturers. Please call the Tosoh Bioscience Technical Service department with your concerns and questions.

TOSOH BIOSCIENCE

Tosoh Bioscience GmbH
Im Leuschnerpark 4
64347 Griesheim, Germany

Tel: +49 6155-7043700
info.tbg@tosoh.com

IM02_1022 - IM0088A-L