TOSOH

TOYOPEARL® & TSKgel® INSTRUCTION MANUAL

TOSOH BIOSCIENCE

Nomenclature

What's in our names?

Tosoh Bioscience has the most comprehensive selection of process media resins, with a variety of pore and particle size combinations for several modes of chromatography. When it comes to naming our resins, we've got it down to a science (literally). Here's how you can identify the right resin for your purification process:

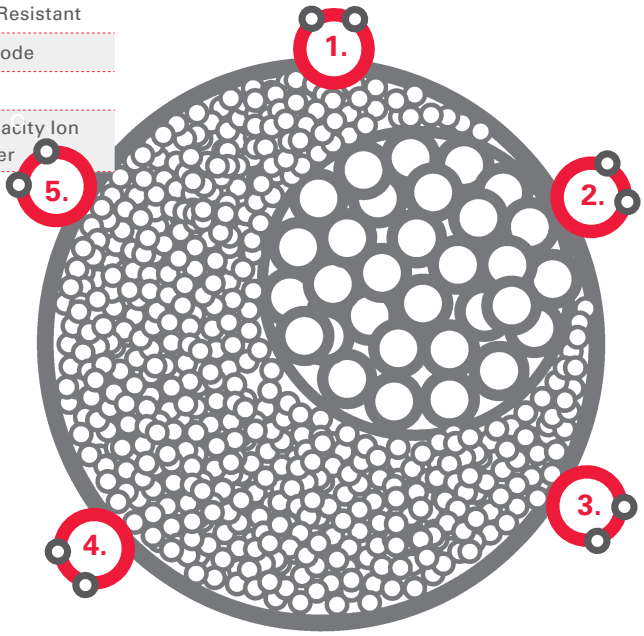
1. Resin type:

Tosoh Bioscience offers 2 base beads for our resin products: TOYOPEARL and TSKgel.
TOYOPEARL and TSKgel products are hydroxylated methacrylic polymer resins and are offered in many different pore sizes and particle diameters. The key differences between the two types are particle size availability, degree of crosslinking, dynamic binding capacity, and operating pressures. Since similarly functionalized TOYOPEARL and TSKgel resins have the same backbone polymer chemistry, the selectivity remains the same as you scale up or down.

5. Additional abbreviations:

Some of our products have additional features or need clarification about what type of product they are.

We use the following abbreviations to highlight these features:	
HC	High Capacity
AR	Alkaline Resistant
MX	Mixed-Mode
AF	Affinity
GigaCap	High Capacity Ion Exchanger



2. Ligand

TOYOPEARL or TSKgel resins are available in the following modes of chromatography functionalized with these ligands:

TOYOPEARL ligands	
Mode	Ligand
HIC	Ether, PPG, Phenyl, Butyl, Hexyl
Anion Exchange	DEAE, QAE, Q, NH_2
Cation Exchange	CM, SP, Sulfate
Antibody Affinity	rProtein A, rProtein L
Affinity	Tresyl, Epoxy, Formyl, Amino, Chelate, Red, Heparin, Carboxy
Mixed-Mode	Tryptophan (Trp)

4. Particle size:

Particle size is typically denoted in the product name as letters or numbers denoting the grade.

Particle size of TOYOPEARL and TSKgel resins (µm)			
Grade	TOYOPEARL	TOYOPEARL GigaCap	TSKgel
EC	200		
C	100 (SEC resins are 75)		
M	65 (MX-Trp is 75)	75	
F	45		
S	35 (SEC resins are 30)	35	
(30)			30
(20)			20

3. Pore size:

TOYOPEARL and TSKgel resin number key		
TOYOPEARL 550 resins	HW-55 base resin	50 nm pore size
TOYOPEARL 600 resins	HW-60 base resin	75 nm pore size
TOYOPEARL 650 resins	HW-65 base resin	100 nm pore size
TOYOPEARL 750 resins	HW-75 base resin	> 100 nm pore size
TSKgel 3PW resin	PW-3000 base resin	25 nm pore size
TSKgel 5PW resin	PW-5000 base resin	100 nm pore size

TOYOPEARL and TSKgel Instruction Manual

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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.

TOYOPEARL PACKING PROCEDURE

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. TOYOPEARL packing procedure

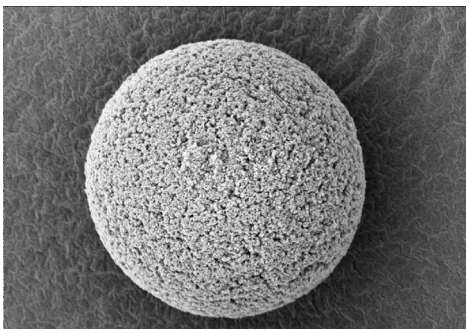
1. Preparation for packing

1.1 General considerations for packing TOYOPEARL
It is best to pack TOYOPEARL resins by the application of pressure from 0.05 to 0.7 MPa 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, glass, 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, especially if you detect any fines. You can detect any potential fines using a simple microscope. 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 resin grade	Particle size	Minutes
Coarse (“C”) grade	100 µm	15 - 30
Medium (“M”) grade	65-75 µm	30 - 45
Fine (“F”) grade	45 µm	45 - 60
Superfine (“S”) grade	35 µm	60 - 90

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

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.

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

TOYOPEARL PACKING PROCEDURE

Figure 3 & 4

Decant

Re-suspend

In larger tanks sedimentation of particles takes longer:

in	50 % slurry	25% slurry
Water	65 µm particles need 3 - 4 h/m SD	65 µm particles need 1,5 - 2,5 h/m SD
	35 µm particles need 5 - 7 h/m SD	35 um particles need 2 - 3,5 h/m SD
1 M NaCl	65 µm particles need 3 - 5 h/m SD	65 µm particles need 2 - 3 h/m SD
	35 µm particles need 12 - 16 h/m SD	35 µm particles need 3 - 7 h/m SD
1.8 M $(NH_4)_2SO_4$	65 µm particles need 6 - 9 h/m SD	65 µm particles need 4 - 8 h/m SD
20 % ethanol	65 µm particles need 6 h/m SD	

SD = Sedimentation Distance

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.

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:

a) Resuspend 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.

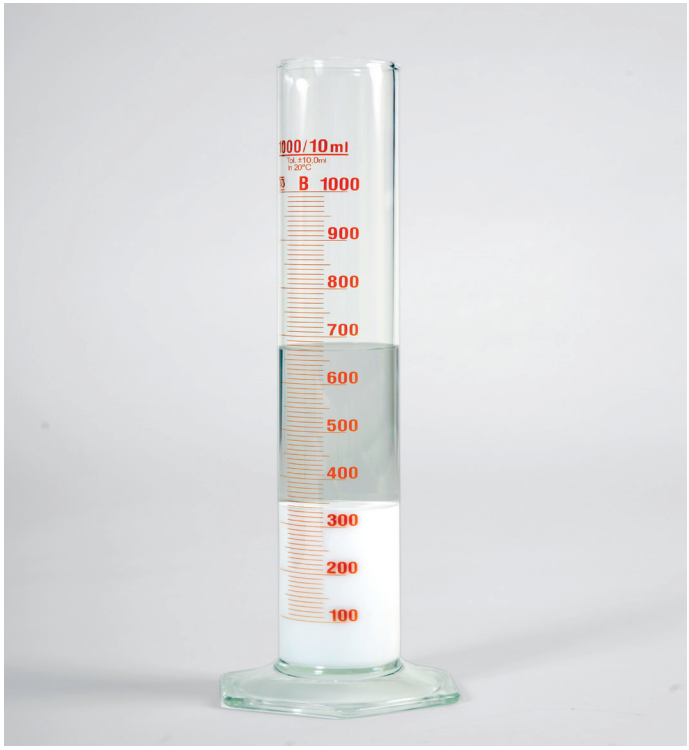
c) Determine the settled resin volume, and adjust the slurry concentration to 30 - 50 % by adding or removing packing buffer.

d) For packing a column of a given volume, use the following amounts of settled resin:

TOYOPEARL resin	Settled resin amount
TOYOPEARL HW resins	use approx. 1.1 x the column volume
QAE-550C and SP-550C MegaCap II SP 550EC, Protein A and Protein L affinity	use approx. 1.25 x the column volume
Other TOYOPEARL resins	use approx. 1.1 - 1.2 x the column volume

Figure 5

Settled resin in water



TOYOPEARL PACKING PROCEDURE

Table 1

Typical packing buffer

SEC	
HW-40, HW-50, HW-55, HW-65 and HW-75	0.1 M Na_2SO_4, $NaNO_3$, or NaCl in 50 mM phosphate or Tris buffer
IEC	
GigaCap-type, DEAE-type, Q-type, CM-type, SP-type, MegaCap II-SP NH_2-type	1 M NaCl in 50 mM phosphate, Tris, or acetate buffer 1M NaCl in 100mM acetate pH 5 (low pH recommended!)
MIXED MODE	
MX-Trp	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_2SO_4, $(NH_4)_2SO_4$ or NaCl in 50 mM phosphate buffer
Antibody AFC	
AF-rProtein A-650F, AF-rProtein A HC-650F, AF-rProtein L-650F	1 M NaCl in 100 mM phosphate or $NaHCO_3$ buffer
AFC	
AF-Tresyl, AF-Epoxy-650	0.5 M NaCl in 0.1 M $NaHCO_3$ or phosphate buffer
AF-Formyl-650, AF-Amino-650, AF-Carboxy-650	1 M NaCl in 100 mM phosphate or $NaHCO_3$ buffer
AF-Chelate-650, AF-Red-650	0.5 M NaCl or 0.2 M glycine in 20 mM phosphate or Tris buffer

NOTE: Avoid the use of multivalent salts when working with TOYOPEARL NH_2-750F. Please consult the dedicated Instruction Manual for TOYOPEARL NH_2-750F for more information.

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) using the above table.

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

TOYOPEARL PACKING PROCEDURE

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

	TOYOPEARL resin type	Column size (cm ID x cm L)	Grade	Packing velocity (cm/hr)	Operating velocity (cm/hr)
SEC	HW-40	2.2 x 60	S (30 µm) F (45 µm) C (75 µm)	30 - 40 60 - 80 120 - 160	10 - 25 25 - 50 50 - 100
	HW-50/HW-55	2.2 x 60	S (30 µm) F (45 µm)	25 - 35 50 - 70	10 - 20 25 - 35
	HW-65	2.2 x 60	S (30 µm) F (45 µm)	20 - 75 40 - 150	10 - 15 15 - 30
	HW-75	2.2 x 60	F (45 µm)	40 - 150	15 - 30
IEC*	DEAE-650, SuperQ-650 CM-650, SP-650 GigaCap S, CM, Q, DEAE	2.2 x 20	S (35 µm) M (65/75 µm) C (100 µm)	400 - 600 800 - 1,000 800 - 1,200	45 - 65 80 - 130 80 - 600
	Sulfate-650, NH_2-750**	2.2 x 20	F (45 µm)	400 - 600	45 - 65
	SP-550 QAE-550	2.2 x 20	C (100 µm)	700 - 1,000	80 - 240
	MegaCap II SP-550		EC (100-300 µm)	800 - 1,200	80 - 500
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) M (65 µm) C (100 µm)	400 - 600 800 - 1,000 800 - 1,200 700 - 1,000	45 - 65 80 - 130 80 - 500 80 - 240
Antibody AFC	AF-rProtein A-650, AF-rProtein A HC-650***, AF-rProtein L-650	2.2 x 10	F (45 µm)	400 - 600	45 - 65
Mixed Mode	MX-Trp-650	2.2 x 20	M (75 µm)	800 - 1,000	80 - 130
AFC	AF-Amino-650, AF-Tresyl-650, AF-Carboxy-650, AF-Red-650 AF-Formyl-650, AF-Chelate-650 AF-Epoxy-650	2.2 x 20	M (65 µm)	800 - 1,000	30 - 130

** Not all resins are available in all particle sizes.*

*** See the dedicated Instruction Manual for TOYOPEARL NH_2-750F for more information.*

**** See application note A15P64A for packing procedures of TOYOPEARL AF-rProtein A 650F for three common process columns.*

PROCESS SCALE

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

PLEASE CALL OUR TECHNICAL SUPPORT SPECIALISTS AT TECHSUPPORT.TBG@TOSOH.COM OR TEL: +49 (0)6155 7043736 FOR INDIVIDUAL SUPPORT.

TOYOPEARL PACKING PROCEDURE

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

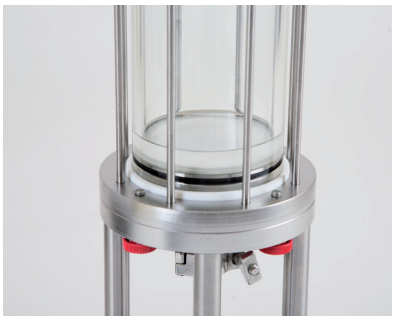
Constant velocity / Semi-constant pressure methods

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.

TOYOPEARL PACKING PROCEDURE

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 flow rate increments

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

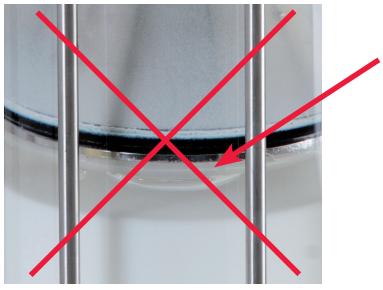
m) The bed will compress further. When compression is complete and pressure is stable, stop the pump and close the column outlet.

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 (desired)

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)

TOYOPEARL PACKING PROCEDURE

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 14. 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 in an optimal hardware setup.

Table 5

Typical plate numbers in ideal systems

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	3,500	4,000	2,000
	5.5	6,000	3,300	4,000	-
	10.8	6,000	2,500	4,000	-
	21.0	4,000	2,200	2,600	2,000
	31.0	-	2,000	2,000	1,000
	40.0	-	1,800	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
Anti-body AFC	2.2	-	3,500	-	-
	5.5	-	3,300	-	-
	10.8	-	2,500	-	-
	21.0	-	2,200	-	-
	31.0	-	2,000	-	-
	40.0	-	1,800	-	-
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 assymetry factors, please repeat the packing procedure.

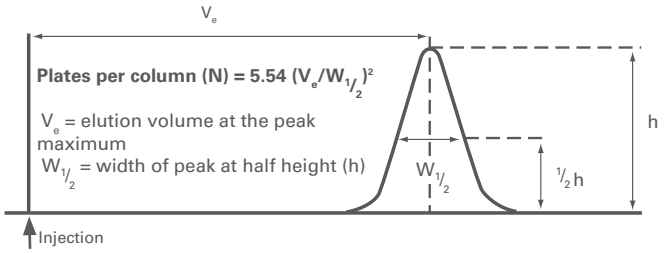
If column diameters > 40 cm are utilized, the number of plates/M can slightly decrease.

For further information contact our technical specialists at techsupport.tbg@tosoh.com or +49 (0)6155 7043736 .

Figure 14

How to calculate efficiency & asymmetry Factor

Efficiency



Asymmetry

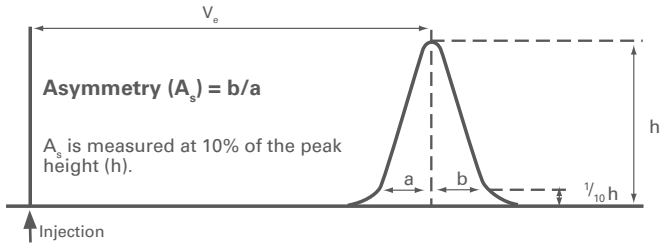


TABLE 6

Troubleshooting performance evaluation

A_S < 0.8	A_S > 1.4
Column overpacked. 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 plate number)	Low HETP* (high plate numbers)
Injection port or detector too far from column. Injection volume too large. Column not packed efficiently.	Probe molecule retained on column due to interaction with functional group or backbone.

*HETP (Height equivalence of a theoretical plate)

TSKgel PACKING PROCEDURE

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 8), 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 7.
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 7

Settling times for TSKgel PW-type resins

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

Table 8

Packing and operational velocities for TSKgel PW-type resins

Resin type	Column size (cm ID × L)	Particle size	Packing velocity (cm/hr)	Operating velocity (cm/hr)
TSKgel 3PW TSKgel 5PW	5.5 × 20	20 µm	100 - 400	50 - 300
	10.8 × 20	20 µm	100 - 400	50 - 300
	15.8 × 30	30 µm	100 - 500	50 - 400
	21 × 30	30 µm	100 - 500	50 - 400
	30 × 20 - 30 (DAC*)	20 - 30 µm	(N/A)	50 - 400

*DAC = Dynamic axial compression at applied piston pressures > 0.3 MPa.

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 9 for suggested mobile phase changes.

Table 9

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 10). 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.

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 11. 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 10

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

* not recommended for resin with NH_2 ligand.

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 12 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 11

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 12

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 Mixed Mode (MXC)

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.

1.5 Antibody Affinity Chromatography (Antibody AFC)

equilibration

Antibody affinity resins (Protein A and Protein L) should be equilibrated with 3 - 5 column volumes of the appropriate starting buffer. Suitable load/wash buffers are 20-100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 6.5 - 7.5 or 100 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.2 - 7.5. Washing at reduced pH (e.g. pH 6) might further improve host cell protein reduction.

Loading and elution

Elution from AF-rProtein A and AF-rProtein L type resins is typically performed at low pH. Suitable elution buffer systems are 50-100 mmol/L acetate, citrate or glycine-HCl. The pH shift required for mAb elution depends on a particular mAb and ranges from pH 3.0 to 4.5.

1.6. Affinity Chromatography (AFC)

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

Please contact our technical specialists at techsupport.tbg@tosoh.com or +49 (0)6155 7043736 for information concerning coupling chemistries for the attachment of ligands to TOYOPEARL Formyl, Carboxy, Amino, Epoxy, and Tresyl.

Equilibration

AF Red 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.

Non-specific 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 13.

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 13

Eluents for exhaustive elution from TOYOPEARL AFC resins

1st Choice:	2 M KCl or 3 M NaCl
2nd Choice:	1 % Triton X-100 / 1 M NaSCN / 75% ethylene glycol / 4 M urea or 0.1 M NaOH / 4.2 M $(NH_4)_2SO_4$

2. Cleaning (CIP)

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 and/or 20-30 min contact time of cleaning solution are typically employed in column washing procedures.

2.1 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:

COLUMN OPERATION

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.

2.2 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, 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. 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. Please note that acidic solutions with pH < 3 are not recommended as cleaning solvent for Sulfate-650F. For NH_2-750F, please consult the dedicated Instruction Manual for specific conditions.

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

2.3 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.

2.4 MXC resin
For moderate contamination, wash with 0.5-1.0 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, 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.

2.5 Antibody AFC resins
For cleaning and sanitization the use of 0.1 to 0.3 M NaOH is recommended. Depending on the origin and subclass of the antibody, contact time (< 15 min), concentration, and frequency of CIP cycles the conditions should be optimized. 0.5 M NaOH can be used in case of extreme contamination

2.6 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.

3. Storage

3.1 SEC, IEC, and HIC resins
Store the column or used bulk resin in distilled water containing a bacteriostatic agent, such as 20% ethanol, preferably at 4°C to 35°C.

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

3.3 Antibody AFC resins
Store the column or used bulk resin in distilled water containing a bacteriostatic agent, such as 20% ethanol, preferably at 2°C to 8°C.

COLUMN OPERATION

3.4 AFC resins
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 2°C to 8°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 2°C to 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.

4. Sterilization / Depyrogenation / Preservative removal / Column frits

Sterilization
Most TOYOPEARL and TSKgel PW type 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.

For further information contact our technical specialists at techsupport.tbg@tosoh.com or +49 (0)6155 7043736.

Depyrogenation
Most 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 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 most TOYOPEARL resins contain 20% ethanol. The resin preparation procedures outlined in this document will reduce the ethanol level in the packed column effluent.

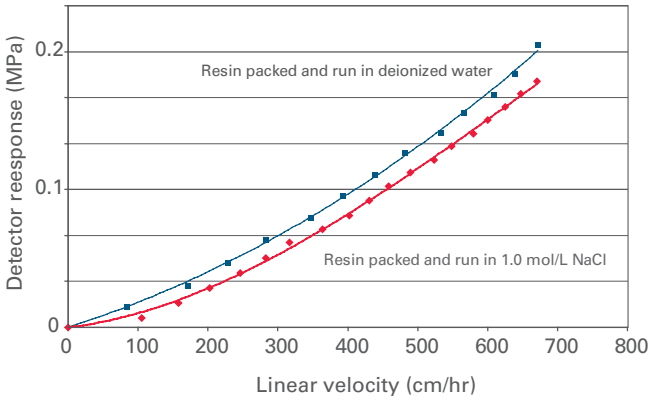
Column frits
Pressure-related problems are often caused by clogged column frits. Remove the frits and clean thoroughly as recommended by the column manufacturer. If the problem persists, replace the frits.

COLUMN OPERATION

5. Pressure-flow curves and performance testing results for selected TOYOPEARL resins

Figure 15

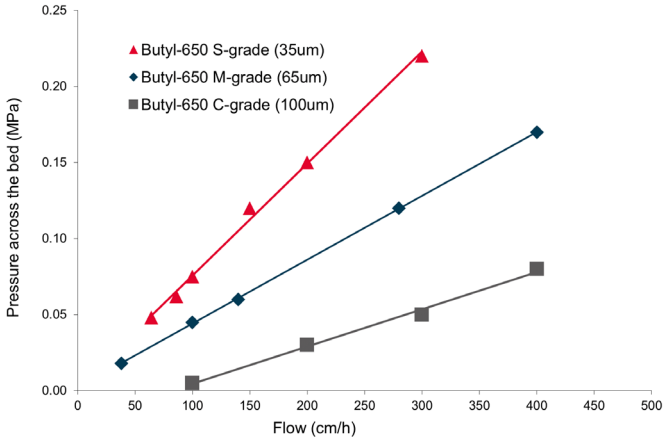
Pressure flow data for TOYOPEARL GigaCap S-650M



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 16

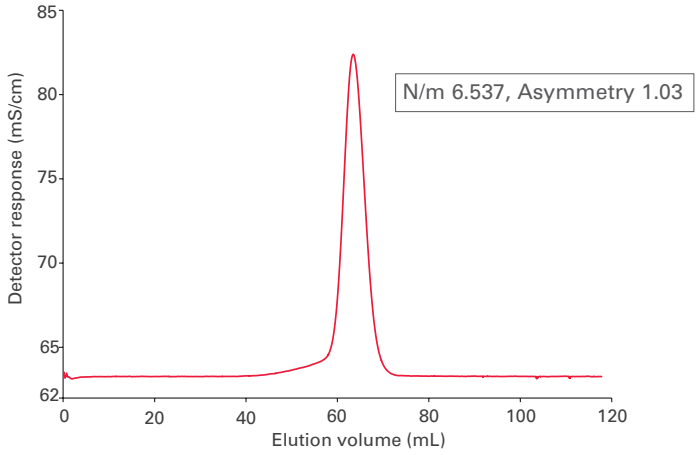
Pressure-flow curve for TOYOPEARL Butyl-650 resins of various particle sizes



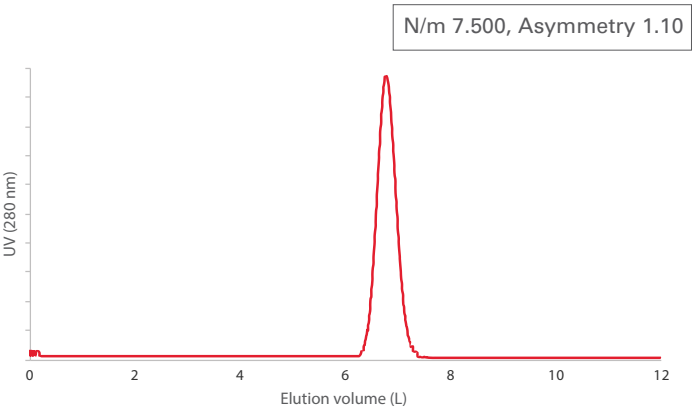
Resins:	A. TOYOPEARL Butyl-650S
	B. TOYOPEARL Butyl-650M
	C. TOYOPEARL Butyl-650C
Column size:	40 mm ID × 18 cm
Mobile phase:	2.0 mol/L $(NH_4)_2SO_4$
Flow rate:	as indicated in figure

Figure 17

HETP and asymmetry evaluations of packed TOYOPEARL



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



Resin:	TOYOPEARL Sulfate-650F
Column size:	20 cm ID x 21.2 cm (6.65 L)
Mobile phase:	water
Flow rate:	100 cm/hr (31.4 L/h)
Detection:	UV@280 nm
Temperature:	ambient
Sample:	5 % acetone
Sample load:	0.5 % CV

TOYOPEARL PRODUCT OVERVIEW

IEC

TOYOPEARL resin	Pore size (nm)
Anion-Exchangers	
SuperQ-650 (S, M, C)	100
QAE-550C	50
DEAE-650 (S, M, C)	100
GigaCap Q-650M (S, M)	100
GigaCap DEAE (50-100 µm)	100
NH_2-750F	> 100

S = 35 µm, **M** = 65/75 µm, **C** = 100 µm

Cation-Exchangers	
CM-650 (S, M, C)	100
SP-650 (S, M, C)	100
SP-550C	50
MegaCap II SP-550EC	50
GigaCap S-650M, (S, M)	100
GigaCap CM-650M, (50-100 µm)	100

S = 35 µm, **M** = 65/75 µm, **C** = 100 µm, **EC**= 200 µm

MIXED MODE

TOYOPEARL resin	Pore size (nm)
MX-Trp-650M	100

M = 75 µm

HIC

TOYOPEARL resin	Pore size (nm)
Ether-650 (S, M)	100
PPG-600M	75
Phenyl-600M	75
Phenyl-650 (S, M, C)	100
Butyl-650 (S, M, C)	100
Butyl-600M	75
SuperButyl-550C	50
Hexyl-650C	100

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

* Pore size of base matrix. Resulting effective pore size might be smaller

AFC

TOYOPEARL resin	Pore size (nm)
TOYOPEARL Antibody Affinity resins	
AF-rProtein A-650F	100
AF-rProtein A HC-650F	100
AF-rProtein L-650F	100
TOYOPEARL reactive resins	
AF-Amino-650M	100
AF-Carboxy-650M	100
AF-Formyl-650M	100
TOYOPEARL activated resins	
AF-Epoxy-650M	100
AF-Tresyl-650M	100
TOYOPEARL ready to use resins	
AF-Chelate-650M	100
AF-HeparinHC-650M	100
AF-Red-650ML	100

M= 65 µm, **ML** =65 µm

SEC

TOYOPEARL resin	Pore size (nm)
HW-40 (S, F, C)	5
HW-50 (S, F)	12.5
HW-55 (S, F)	50
HW-65 (S, F, C)	100
HW-75 (F)	> 100

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

HYDROXYAPATITE

Please consult the dedicated Instruction Manual for Ca^{++}Pure-HA.

PROCESS COLUMN INSTALLATIONS

				Column performances	
Column manufacturer	**Column type**	**Bed dimensions (ID x L in cm)**	**Asym-metry**	**Plate count [N/m]**	**Resin type**
BioRad	InPlace™/ Geltec™	20 - 45 x 15 - 25	0.8 - 1.4	3,000 - 4,000 (60 cm/h-salt)	HIC - 65 µm
		130 x 24	1.1 - 1.2	3,500 - 3,900 (300 cm/h-salt)	IEC - 65 µm
Cytiva/GE Healthcare Lifesciences	AxiChrom™	30 x 20	1.2	3,500	Sulfate-650F - 45 µm
		60 x 20	1.1	8,000 (100 cm/h-salt)	HIC - 65 µm
		60 x 20	1.2	3.000	GigaCap S-650M - 75 µm
		20 - 60 x 15	1.2	4.000 - 7.000	AF-rProtein A-650F - 45 µm
	BPG™	20 x 11	1.3	7.200 (100 cm/h-salt)	AF-rProtein A-650F - 45 µm
		20 - 30 x 11 - 25	0.9 - 1.3	4,000 - 11,000 (40cm/h-salt)	IEC/HIC - 65 µm
		30 x 21	1.5 - 1.7	6.000 - 7.000 (100 cm/h-salt)	NH_2-750F - 45 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	1.3 - 1 4	14,000 - 20,000 (20cm/h-acetone)	IEC - 20 µm
		20 x 15 - 25	0.8 - 1.6	3,000 - 6,000 (100cm/h-acetone)	IEC/HIC - 35/65 µm
Merck	Superformance ®	20 - 30 x 15 - 30	1.0 - 1.3	2,500 - 3,500 (100cm/h-acetone)	IEC - 65 µm
		20 x 30	1.2	7,000 (250cm/h-acetone)	IEC - 20 µm
Merck Milli-pore	IsoPak® / Access ®	44 x 25	1.2 - 1.5	6,000 - 9,000 (acetone-60 cm/h)	IEC - 35 µm
		44 x 13 - 30	1.1 - 1.4	3,000 - 8,000 (130 - 20 cm/h)	IEC/HIC - 65 µm
		100 - 160 x 15 - 25	1.2 - 1.4	4,000 - 6,000 (salt-60 cm/h)	IEC/HIC - 65 µm
		140 x 25	1.4 - 1.7	5,000 - 7,000 (salt-60cm/h)	IEC - 35 µm
		160 x 13 - 15	1.0	600 - 900 (acetone-100 cm/h)	IEC - 100 µm
		200 x 30	1.2 - 1.4	4,000 - 5,500 (100cm/h-salt)	HIC - 65 µm
	QuikScale ®	20 - 30 x 13 - 20	1.2 - 1.6	4,000 - 9,000 (acetone-100 cm/h)	HIC - 35 µm
		14 - 30 x 13 - 33	1.3 - 1.6	2,500 - 5,000 (acetone-100 cm/h)	IEC - 65 µm
		63 x 15	1.2	~7,000	AF-rProtein A-650F - 45 µm
		63 x 17	1.2 - 1.4	2,500 - 4,000 (acetone-130 cm/h)	IEC - 65 µm
		20 x 11 - 20	1.1 - 1.7	4000 - 6000 (acetone-100 cm/h)	AF-rProtein A-650F - 45 µm
	Moduline®	140 x 20 - 25	0.8	5,000 - 6,000 (salt-30 cm/h)	IEC - 65 µm

				Column performances	
Column manufacturer	**Column type**	**Bed dimensions (ID x L in cm)**	**Asym-metry**	**Plate count [N/m]**	**Resin type**
Sartorius/Pall	Resolute™ Linear	20 x 20	0.9 - 1.3	4.000 - 7.000 (acetone-100 cm/h)	Sulfate-650F - 45 µm
		40 x 15 / 40 x 25	1.1 - 1.2	7.000 - 10.000 (salt-100 cm/h)	NH_2-750F - 45 µm
		40 - 80 x 12 - 32	1.1	16,000 - 19,000 (salt-60 cm/h)	HIC - 35 µm
		40 - 80 x 14 - 32	0.8 - 1.2	3,000 - 7,000 (salt-30 cm/h)	HIC/IEC - 65 µm
		40 - 100 x 21 - 28	1.0 - 1.2	1,000 - 3,000 (salt-100 cm/h)	IEC - 100/200 µm
		100 - 140 x 20 - 25	1.0 - 1.3	3,000 - 7,000 (salt-80 cm/h)	HIC - 65 µm
	Resolute™ Manual	20 x 19	1.0 - 1.4	4.000 - 7.500 (acetone-100 cm/h)	Sulfate-650F - 45 µm
		20 x 19	0.8 - 1.0	4.000 - 6.000 (salt-100 cm/h)	NH_2-750F - 45 µm
		20 x 22	1.1	4.800 - 5.600 (acetone-100 cm/h)	GigaCap Q-650S - 35 µm
Peak Biotech/ DAN Process	LPLC-DAC	30 x 19 - 21	1.3 - 1.4	13,000 - 17,000 (salt-100 cm/h)	HIC/IEC - 20 µm
		30 x 20	1.2 - 1.8	6,000 - 8,000 (salt-100 cm/h)	HIC/IEC - 35 µm
		30 x 20	1.2	4,000 (salt-80 cm/h)	IEC - 65 µm
Proxcys	CRIO - radial flow	5 liter, 6 cm L	1.1	3.500 (acetone - 100 cm/h)	AF-rProtein A HC-650F - 45 µm
		5 - 100 liter BV, 6 - 12 cm L	1.0 - 1.2	3.000 - 7.000 (salt-100 cm/h)	HIC/IEC - 65 µm

These examples show real values for any packing condition given. The achievable optimum might be different.
We have more than 30 years of experience in packing production columns of various manufacturers.
Please call our specialist for an individual discussion, to schedule a packing training. Our chromatography experts can also assist you on site. Contact us at techsupport.tbg@tosoh.com or +49 (0)6155 7043736.

TOSOH BIOSCIENCE

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