



# TOYOPEARL INSTRUCTION MANUAL



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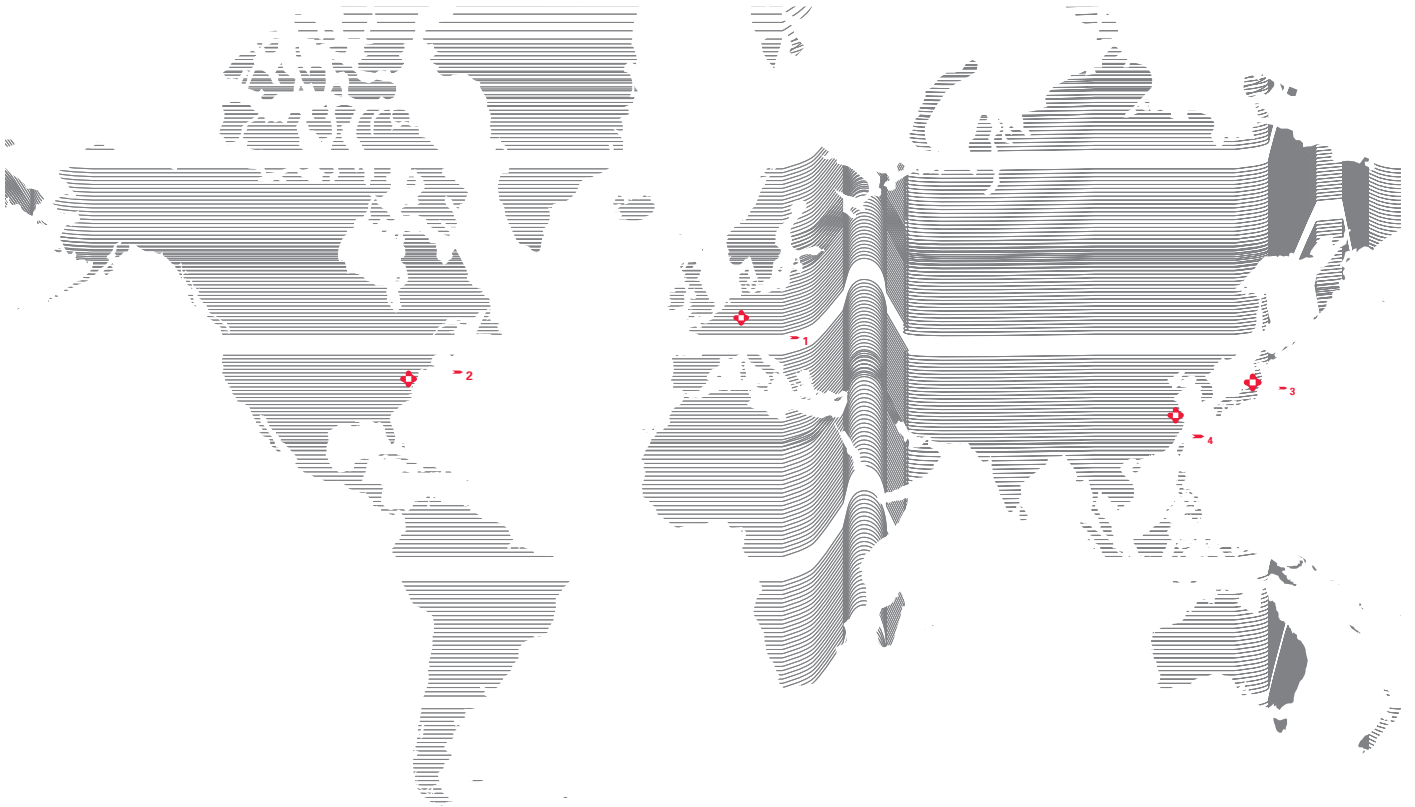
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➤ **TOSOH HISTORY**

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

# Toyopearl® Instruction Manual

## Table of Contents

<b>I. Packing</b>	<b>2</b>
1. Preparation for Packing	2
1.1 General Consideration for Packing	2
1.2 Removal of Fines	2
1.3 Buffer Equilibration	3
1.4 Slurry Preparation	3
1.5 Alternative Slurry Preparation	4
2. Packing Procedure	5
2.1 Constant Velocity / Semi-constant Pressure Method	6
2.2 Alternative Packing Method, Assisted Gravity	8
3. Equilibration and Efficiency Evaluation	9
<b>II. Column Operation</b>	<b>10</b>
1. Chromatographic Separation	10
1.1 Size Exclusion (SEC)	10
1.2 Ion Exchange (IEC)	10
1.3 Hydrophobic Interaction (HIC)	10
1.4 Affinity (AFC)	11
2. Cleaning	11
3. Storage	12
4. Sterilization/Depyrogenation/ Preservative Removal/Column Frits	13
<b>III. Toyopearl Product Overview</b>	<b>14</b>
<b>IV. Process Column Installations</b>	<b>15</b>

# PACKING

## Introduction

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

## I. Packing

### 1. Preparation for Packing

#### 1.1 General Considerations for Packing Toyopearl

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

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

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

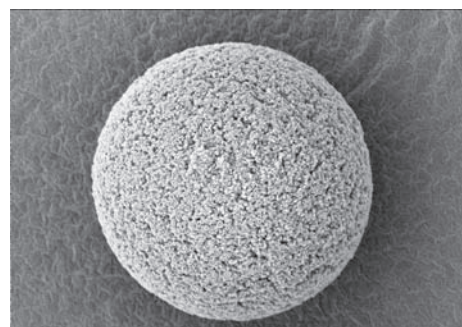
≡ FIGURE 1

Equipment required for packing



≡ FIGURE 2

Toyopearl base particle



#### 1.2 Removal of Fines

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

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

#### Example for Fine Removal:

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

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

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

# PACKING

In larger tanks sedimentation of particles takes longer:

	50 % slurry	25% slurry
<b>in water</b>	65 µm particles need 3 - 4 hours per meter SD	65 µm particles need 1,5 - 2,5 hours per meter SD
	35 µm particles need 5 - 7 hours per meter SD	35 µm particles need 2 - 3,5 hours per meter
<b>in 1 M NaCl</b>	65 µm particles need 3 - 5 hours per meter SD	65 µm particles need 2 - 3 hours per meter SD
	35 µm particles need 12 - 16 hours per meter SD	35 µm particles need 3 - 7 hours per meter SD
<b>in 1.8 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub></b>	65 µm particles need 6 - 9 hours per meter SD	65 µm particles need 4 - 8 hours per meter SD
<b>in 20 % ethanol</b>	65 µm particles need 6 hours per meter SD	

SD = Sedimentation Distance

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

FIGURE 3 + 4



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

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

### 1.3 Buffer Equilibration

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

TABLE 1

### Typical packing buffer

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

### 1.4 Slurry Preparation

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

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

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

# PACKING

## 1.5 Alternative Slurry Preparation

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

**FIGURE 5**  
Settled resin in water



# PACKING

## 2. Packing Procedures

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

➤ TABLE 2

Packing and operating velocities for Toyopearl resins

### LABORATORY SCALE

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

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

### PROCESS SCALE

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

Please call our product specialists for your individual discussion.

# PACKING

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

**TABLE 3**  
Features of packing methods

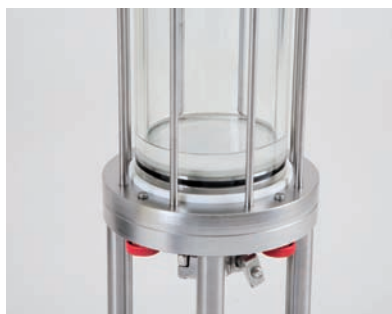
Packing Method	Constant Pressure	Constant Velocity	Assisted Gravity
<b>Packing Velocity</b>	fast	fast	slow
<b>Flow Rate Range</b>	up to high	up to high	limited to low
<b>Pump</b>	constant pressure	constant velocity	peristaltic pump
<b>Pressure Gauge</b>	needed	needed	not needed

## 2.1 Constant Velocity / Semi-Constant Pressure Methods

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

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

**FIGURE 6**  
Bubble free, liquid covered bottom frit



**c)** Resuspend the resin slurry to assure homogeneity.

**FIGURE 7**  
Homogenise slurry



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

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

**FIGURE 8**  
Pouring the resin



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

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

**FIGURE 9**  
Adjusting column



**h)** Two different Packing Methods can be applied:

### Constant Velocity Method

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

# PACKING

## Pressure Method

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

➤ TABLE 4

### Typical packing buffer

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

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

➤ FIGURE 10

### Clear supernatant of sedimenting resin



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

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

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

➤ FIGURE 11

### Flow adapter in place



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

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

➤ FIGURE 12 + 13

### Bubble free (recommendable)



### Air disturbs homogenous settling procedure



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

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

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

# PACKING

## 2.2 Alternative Packing Method, Assisted Gravity

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

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

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

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

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

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

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

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

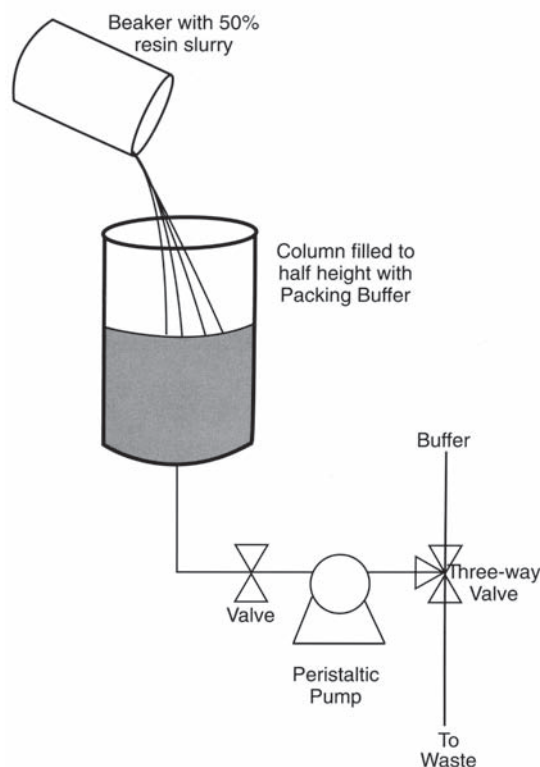
**h)** Carefully place the flow adapter into the column, with the adapter just touching the packing buffer.

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

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

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

**FIGURE 14**  
Assisted cavity packing method



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

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

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

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

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

# PACKING

## 3. Equilibration and Efficiency Evaluation

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

TABLE 5

Typical packing buffer

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

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

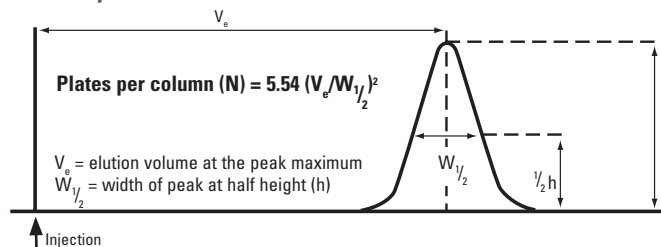
If column diameters > 40 cm are utilized, the number of plates/M can slightly decrease

For further details call our Technical Specialists.

FIGURE 15

How to calculate efficiency & asymmetry Factor

Efficiency



Asymmetry

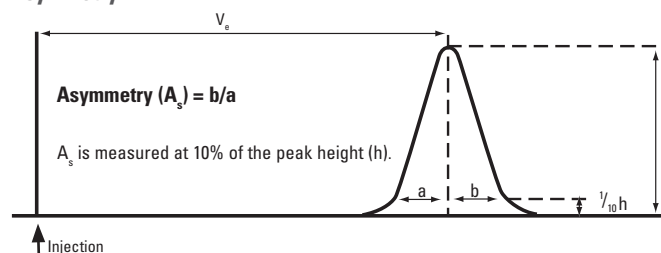


TABLE 6

Troubleshooting performance evaluation

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

\*HETP (Height Equivalence of a Theoretical Plate)

# COLUMN OPERATION

## II. Column Operation

### 1. Chromatographic Separation

#### 1.1 Size Exclusion Chromatography (SEC)

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

≡ TABLE 7

#### Non ideal SEC behavior

Observation	Cause/Solution
<b>Retention time is shorter than expected</b>	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.
<b>Retention time is longer than expected</b>	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 8). The elution is performed by increasing the salt concentration or changing the pH of the eluent.

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

≡ TABLE 8

#### Examples for buffers used in IEC

Resin Type	Buffer	Buffering Range
<b>Cation Exchangers</b>	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
<b>Anion Exchangers</b>	L-Histidine	5.5 - 6.0
	Imidazole	6.6 - 7.1
	Triethanolamine	7.3 - 7.7
	Tris-HCl	7.5 - 8.0
	Diethanolamine	8.4 - 8.8

#### 1.3 Hydrophobic Interaction Chromatography (HIC)

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

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

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

≡ TABLE 9

#### Neutral salts used in HIC

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

\* - based on the Hofmeister series of lyotropic salts

# COLUMN OPERATION

TABLE 10

## Mobile phase additives for HIC

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

### 1.4 Affinity Chromatography (AFC)

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

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

#### Equilibration

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

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

#### Loading and Elution

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

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

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

TABLE 11

## Eluents for exhaustive elution from AF Toyopearl resins

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

## 2. Cleaning

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

### SEC Resins

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

#### Ionically-bound materials

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

#### Hydrophobically-bound materials

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

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

### IEC Resins

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

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

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

# COLUMN OPERATION

## HIC Resins

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

### Ionically-bound materials

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

### Hydrophobically-bound materials

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

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

## AFC Resins

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

## 3. Storage

### SEC, IEC, and HIC

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

### AFC

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

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

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



# COLUMN OPERATION

## 4. Sterilization / Depyrogenation / Preservative Removal / Column Frits

### Sterilization

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

### Depyrogenation

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

### Preservative Removal

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

### Column Frits

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



# TOYOPEARL PRODUCT OVERVIEW

## IEC

### Toyopearl Resin

#### Anion-Exchangers

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

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

#### Cation-Exchangers

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

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

## AFC

### Toyopearl Resin

#### Toyopearl Reactive Resins

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

#### Toyopearl Activated Resins

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

#### Toyopearl Ready to use Resins

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

**M** = 65 µm, **ML** = 65 µm

## HIC

### Toyopearl Resin

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

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

## SEC

### Toyopearl Resin

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

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

# PROCESS COLUMN INSTALLATIONS

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

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

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



# MiniChrom for TOYOPEARL®/TSKgel® Instruction Manual

PRE-PACKED COLUMNS FOR RESIN SCREENING / METHOD DEVELOPMENT

## INTRODUCTION

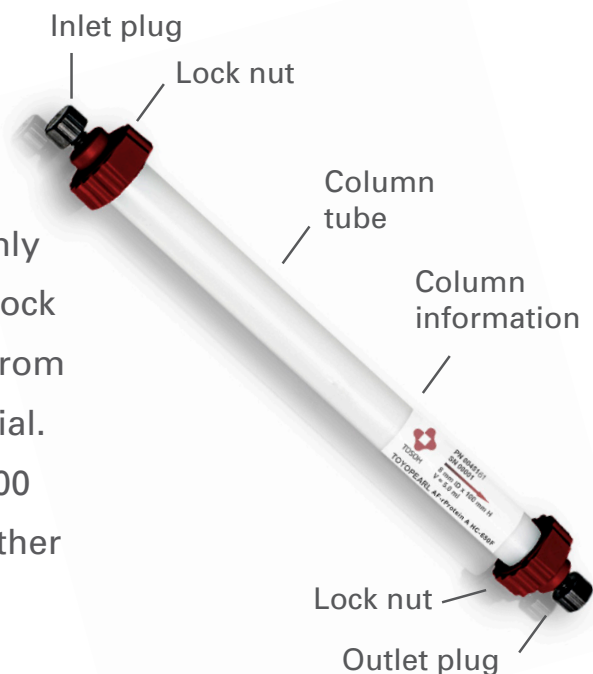
MiniChrom 8-100 columns for TOYOPEARL and TSKgel are small pre-packed columns (8 mm ID x 100 mm) with 5 mL resin volume designed for fast method development or resin screening with TOYOPEARL and TSKgel resins. They guarantee optimal performance and can be connected to common high or medium pressure liquid chromatography systems.

MiniChrom columns are packed with TOYOPEARL or TSKgel chromatography resin. They are reproducibly and individually flow-packed to take account of the varying compressibility of each resin such that the columns provide a more accurate representation of conditions found in full scale columns.

## PRODUCT DESCRIPTION

### MATERIALS

Components of MiniChrom columns are made from bio-compatible polymers, mainly high-density polyethylene (column tube, Lock nut, filter adapter). Filter plates are made from porous polypropylene/polyethylene material. Fig. 1 shows the parts of a MiniChrom 8-100 columns (Upper & lower filter plates, together with filter adapters are internal parts).



**TABLE 1**

PROPERTIES OF Minichrom COLUMNS 8-100 FOR TOYOPEARL/TSKgel

Inner dimension	8 mm ID x 100 mm bed height
Cross section	0.5 cm <sup>2</sup>
Volume	5 mL
Outer dimensions	10 mm ID x 164 mm L including plugs
Flow rate	1.3 mL/min /150 cm/h
Max. flow rate	13 mL/min /1500 cm/h (2.5 mL/min / 300 cm/h for TOYOPEARL AF-rProtein A HC-650F)
Connectors	Standard HPLC (10-32 for 1/16 inch capillaries)
Max. operating pressure	0.3 MPa for TOYOPEARL, 2 Mpa for TSKgel
Shipment buffer	Unless otherwise stated, 20% ethanol or a mixture of 20% ethanol and 0.150 mol/L sodium chloride (IEC & HIC resins)
Chemical stability <sup>1</sup>	Columns are tolerant to aqueous buffers and salt solutions, 1 M alkaline solutions, 1 M non-oxidizing mineral acids, 8 M urea, 6 M guanidine hydrochloride, non-halogenated organic solvents and detergents. They are not compatible with strong oxidants and halogenated solvents.

<sup>1</sup> The chemical stability refers to the column hardware parts only. The individual packed chromatography media may have different stability. To avoid damage to the chromatography media, please refer to the relevant resin data.

## CONNECTABILITY

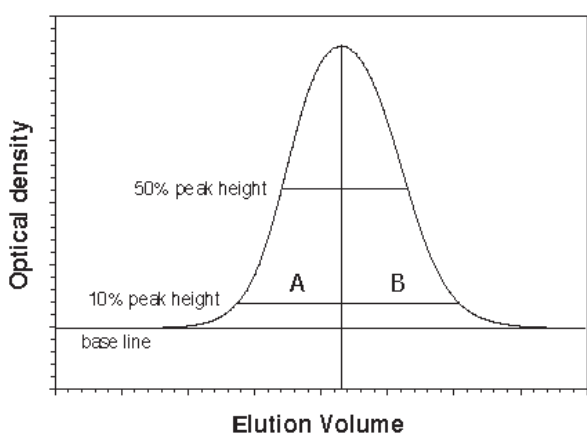
MiniChrom columns are delivered ready to use, packed with TOYOPEARL or TSKgel chromatography media for bioseparation. Columns may be connected directly to most liquid chromatography systems or workstations.

MiniChrom columns may be reused as long as the resin remains in acceptable condition, which will depend largely upon the care taken, the cleaning performed and the tolerance of the resin to user conditions. The columns are not designed to be repacked. The most important properties of MiniChrom 8-100 columns are summarized in Table 1.

## PERFORMANCE TESTING

The chromatographic performance of MiniChrom columns should be checked initially and at regular intervals by determination of plate number and asymmetry factor.

The evaluation of the elution profile of an acetone or other suitable sample should be carried out as follows, with parameters as shown in the schematic drawing: The theoretical plate number ( $N$ , as number per meter) and the asymmetry factor ( $A_s$ ) are calculated according to equations (1) and (2):



$$N = 5.54 \times \left( \frac{V_e}{W_{1/2}} \right)^2 \times \frac{100}{BH} \quad (1)$$

$$A_s = \frac{B}{A} \quad (2)$$

With  $V_e$  = elution volume,  $W_{1/2}$  = width at 50% peak height, BH = bed height in cm, B and A = left and right section of a line, drawn parallel to the base line at 10% peak height drawn vertically from peak maximum to base line.

The functional test for columns packed with chromatography media incompatible with acetone, e.g. Protein A resins, should be performed with an alternative low molecular weight sample.

## INSTRUCTIONS FOR USE

Install the column following the recommended flow direction as indicated by the arrow on the tag. For best results and increased column life, samples and buffers should be degassed and filtered through a 0.45  $\mu\text{m}$  filter.

A sudden pressure change may impair the performance of the column. Therefore, slowly step-up the mobile phase flow rate until it reaches the desired value.

Before the first sample injection equilibrate the column with at least 10 CV (column volumes) of the respective loading buffer. For washing and equilibration a flow rate of 5 mL/min (2.5 mL/min for TOYOPEARL AF-Protein A HC-650F) can be applied.

Buffer composition and gradient design is dependent on the resin type. Please refer to the TOYOPEARL Instruction Manual and/or the respective Product Datasheet of the resin for more information about recommended eluents, storage and cleaning solutions.

Check out our website [www.tosohbioscience.de](http://www.tosohbioscience.de) to download resin datasheets and the latest version of the TOYOPEARL instruction manual.



TOSOH

# **INSTRUCTION MANUAL**

Toyoscreen™ Series



Packed Columns for TOYOPEARL® Screening

**TOSOH CORPORATION**

## Safety Precautions

To help protect your property from potential damage and ensure personal safety, please read this manual thoroughly before using the product.

### Notational Conventions

Notation	Explanation
 <b>WARNING</b>	Alerts the user to the potential for serious injury or death.
 <b>CAUTION</b>	Alerts the user to the potential for damage to hardware or bodily harm.

### **WARNING**

#### ■ **Keep away from fire**

Take proper precautions when using flammable solvents. There is the potential for fire, explosion, or poisoning.

### **CAUTION**

#### ■ **Use only in well ventilated areas**

In case of insufficient ventilation, flammable and toxic solvents can cause fire, explosion, or poisoning.

#### ■ **Do not spill solvents**

Spillage and leakage can cause fire, electric shock, poisoning, injury, and corrosion.

When cleaning up a spill, wear appropriate protective gear.

#### ■ **Wear eye protection and protective gloves**

Organic solvents and acids should not come in direct contact with the skin.

#### ■ **Handle package with care**

Inappropriate handling may cause rupturing and splattering.

#### ■ **Only use this product as intended**

This product is for separation and purification, do not use for any other purpose.

#### ■ **Confirm compounds are safe**

Check that obtained compounds and solutions after separation and purification are safe.

#### ■ **Proper disposal**

Dispose of in accordance with local laws and regulations.

### **NOTE**

Keep this manual for future reference.

## **CONTENTS**

- 1. Introduction**
- 2. Column Dimensions and Basic Properties of Packing Material**
- 3. Column Components**
- 4. Column Attachment**
- 5. Connecting the ToyoScreen Column to the Instrument**
- 6. Mobile Phase**
- 7. Analytical Conditions**
- 8. Storage**

## 1. Introduction

The ToyoScreen™ Series consist of small pre-packed screening columns packed with TOYOPEARL®, a packing material for semi-preparative and preparative liquid chromatography. These columns are suitable for evaluating different TOYOPEARL resins or for developing the purification conditions of biological target molecules such as proteins or nucleic acids.

## 2. Column Dimensions and Basic Properties of Packing Material

### 2-1 Column Dimensions

The ToyoScreen Series is available in two column volumes; 1mL and 5mL formats. The two different column sizes can be used in the following way:

Column Volume	(inside diameter) × (length)	Purpose
1mL column	6.4mm × 3cm	Selection of TOYOPEARL Preliminary evaluation of purity and recovery Purification of small amounts of sample
5mL column	14.6mm × 3cm	Selection of TOYOPEARL Preliminary evaluation of purity and recovery Detailed evaluation of purification conditions Purification of small amounts of sample * Sample dynamic capacities should be evaluated using a minimum column length of 7.5cm.

### 2-1 Basic Properties of Packing Materials

TOYOPEARL used to pack in the ToyoScreen Series have the following basic properties:

IEC type	Particle size (µm)	Ion exchange capacity (eq/L-gel)	Static protein adsorption capacity (g/L-gel)
DEAE-650M	40-90	0.08-0.12	25-35 <sup>1)</sup>
SuperQ-650M	40-90	0.20-0.30	105-155 <sup>1)</sup>
QAE-550C	50-150	0.28-0.38	60-80 <sup>1)</sup>
CM-650M	40-90	0.08-0.12	30-50 <sup>2)</sup>
SP-650M	40-90	0.13-0.17	40-60 <sup>2)</sup>
SP-550C	50-150	0.14-0.18	80-120 <sup>2)</sup>
HIC type			
Ether-650M	40-90		10-30 <sup>2)</sup>
Phenyl-650M	40-90		30-50 <sup>2)</sup>
Butyl-650M	40-90		30-50 <sup>2)</sup>
Hexyl-650C	50-150		30-50 <sup>2)</sup>
PPG-600M	40-90		20-35 <sup>3)</sup>
SuperButyl-550C	50-150		52-70 <sup>2)</sup>

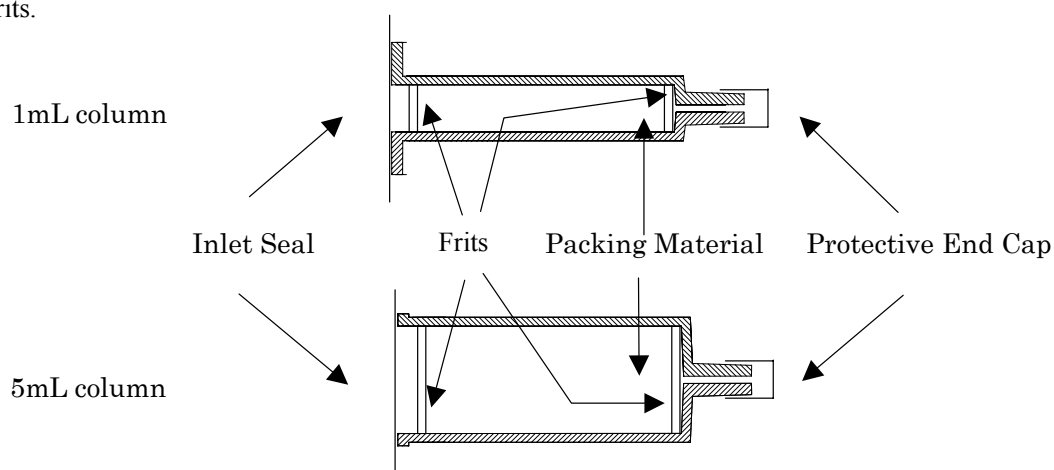
AFC type			
AF-Chelate-650M	40-90	0.025-0.045	
AF-Blue HC-650M	40-90		18 or more <sup>4)</sup>
AF-Red-650M	40-90		2.5-4.5 <sup>4)</sup>
AF-Heparin HC-650M *	40-90		5 or more <sup>5)</sup>

\*AF-Heparin HC-650M is not available in US market.

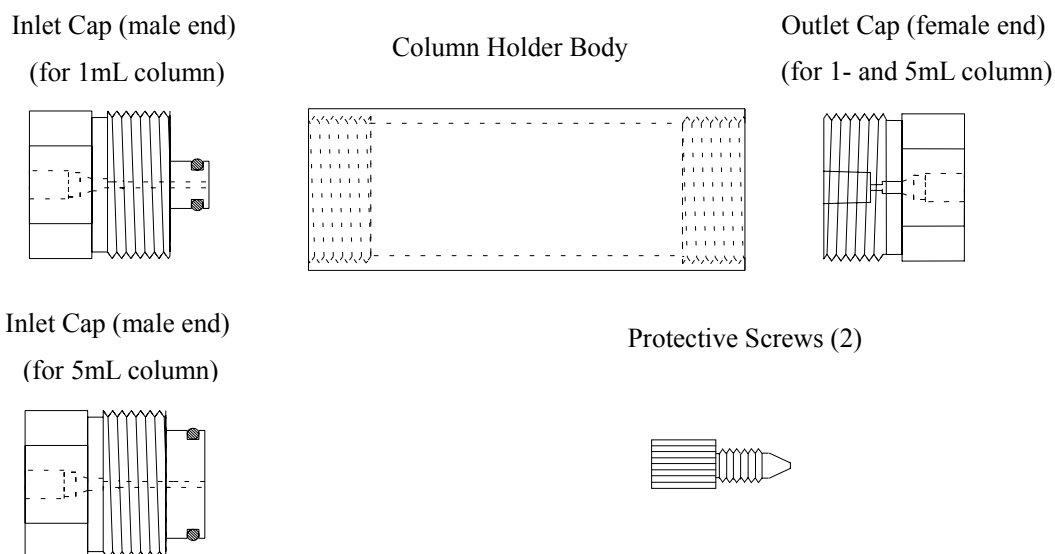
Measured with 1) Bovine serum albumin, 2) Lysozyme, 3)  $\gamma$ -Globulin, 4) Human serum albumin, and 5) Antithrombin-III (Tosoh original method.)

### 3. Column Components

The column housing is essentially a syringe barrel that is filled with the packing material sandwiched between two frits.



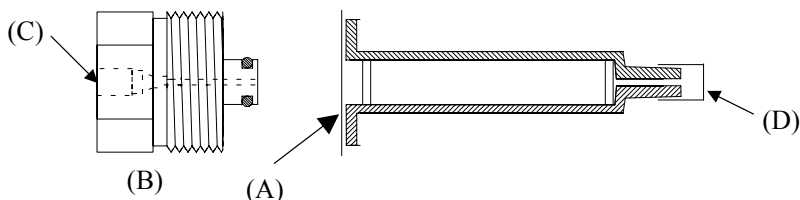
ToyoScreen must be used with the dedicated holder (ToyoScreen Holder: Part No.21400), which is sold separately. The holder consists of the following components:



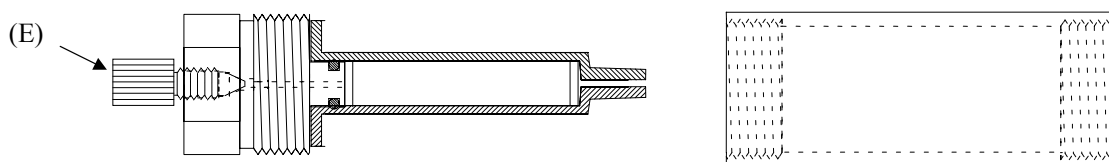
#### 4. Column Attachment

Install the column into the holder according to the procedure described below. Care should be taken to avoid applying pressure to the frit while placing the column inside the holder. Doing so may lower column performance. The final frit position is not determined until the column is properly placed into the holder.

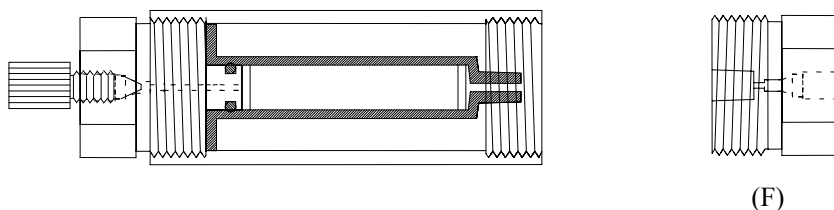
1) Carefully peel the foil inlet seal (A) from the column making sure all seal material is removed from the column inlet. Place the Inlet Cap (B) on top of the column and slowly push the male end of the Inlet Cap into the column housing. When attaching the column, leave the flow hole of the Inlet Cap open (C). Do not remove Protective End Cap (D). (If you have removed the Protective End Cap by mistake, block the column outlet using your finger or by some other means to avoid air from entering the column.) Please note that the device may leak if the male end of the holder is attached with too much force.



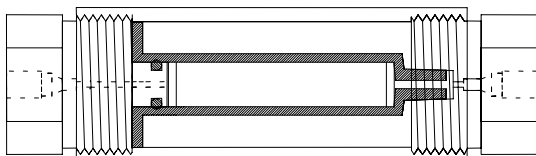
2) Attach the Protective Screw (E) to the Inlet Cap as shown below. Slowly remove the column Protective End Cap (D) and hand-tighten the column into the Column Holder Body. **DO NOT USE A WRENCH TO TIGHTEN THE HOLDER.**



3) Hand-tighten the Outlet Cap (F) into the Column Holder Body. **DO NOT USE A WRENCH TO TIGHTEN THE HOLDER.**



4) Remove the Protective Screw (E) and connect the column to the liquid chromatograph according to step 5 below.



## 5. Connecting the ToyoScreen Column to the Instrument

### 5-1 Fittings

The column should be attached to the injector and detector using 1/16" tubing. Narrow internal diameter PEEK<sup>®</sup> or plastic tubing is recommended to prevent sample dilution. Metal fittings may damage the column, therefore PEEK fittings should be used to attach tubing to the ToyoScreen column. These fittings are sold separately (Part No.16566).

When using a FPLC<sup>®</sup> system, a T-F union should be used (sold separately, Part No.20028).

### 5-2 Flow Direction

Mobile phase should only pass through the column in the flow direction indicated on the label.

### 5-3 Preventing Air from Entering the Column

When attaching or detaching the column from the instrument, take great care to prevent air from entering the column. Flush out all air bubbles from the tubing using mobile phase before attaching the column to the instrument. Air bubbles in the column may result in uneven flow (channeling) and lower performance.

### 5-4 Connection to Instrument

Prior to connecting the column, connect two pieces of tubing (one piece to connect the column to the injector, the other connecting the column to the detector) with a 1/16" union, and pump mobile phase through the tubing in order to expel the air from the column inlet tubing.

The next step is to remove air, if present, from the column. Connect the outlet end of the column to the injector or pump and slowly pump mobile phase through the column to expel any remaining air bubbles. It is important to prevent a sudden surge of mobile phase or pressure as this may lower the performance of the column. Therefore, slowly step-up the mobile phase flow rate until it reaches the desired value (we recommend 1mL/min for the 1mL columns and 5mL/min for 5mL columns). After confirming that there are no bubbles escaping from the inlet (male) end of the column, stop the flow and disconnect the column. Arrange the column in the direction of the flow arrow and reconnect the top of the column to the injector/pump. Please note that if a detector is connected, the column backpressure may increase significantly due to resistance of tubing inside the detector. In this case, replace the tubing or lower the flow rate.

### 5-5 Prior to Analysis

After the column is properly installed between the injector and detector, avoid sudden pressurization by slowly stepping-up the flow rate of the mobile phase until the desired flow rate is obtained (see section 7; for flow rate guidelines).

### 5-6 After Analysis

After operation, do not detach the column from the instrument until the pump stops and the flow of the mobile phase has stopped. If the column is detached before the flow has halted, the column will be subjected to a sudden

pressure drop that may deteriorate its performance.

## 6. Mobile Phase

### 6-1 Mobile Phase Viscosity

When using a high viscosity mobile phase, such as mixtures of alcohol with aqueous buffer, use a lower flow rate because the backpressure increases with viscosity of the mobile phase. High backpressure may deteriorate the column.

### 6-2 Impurities in Mobile Phase

In order to prevent ghost peaks, we recommend the use of analytical grade solvents.

### 6-3 Dissolution of Sample

Select a solvent that completely dissolves the sample. If there is precipitate in the injected sample, the inlet tubing and/or inlet frit may become plugged resulting in lower performance.

### 6-4 Degassing

If the mobile phase is not adequately degassed, bubbles may enter the column and decrease column performance. To prevent this problem, it is recommended to degas the mobile phase thoroughly. If only a few bubbles are present, column performance may be recovered by running a well-degassed mobile phase (such as distilled water) through the column.

## 7. Analytical Conditions

### 7-1 Flow Rate

The operational flow rate is selected by considering the resolution, measurement time, and column life. As flow rate increases, analysis times decrease but a column void can develop more readily. Recommended flow rates are as follows:

Column volume	Recommended flow rate	Maximum flow rate
1mL column	0.2 to 1 mL/min	1.5 mL/min
5mL column	1 to 5 mL/min	7.5 mL/min

These flow rates are based on the viscosity of distilled water at 25°C. Use proportionally lower flow rates when using a more viscous mobile phase.

### 7-2 Gradient Profile

Stepwise or continuous gradient elution method is effective for protein separation.

In the case of continuous gradient elution, gradient profile significantly influences separation efficiency. In

general, shallower gradients result in higher resolution at the expense of longer run time. Conversely, steeper gradients result in shorter run times and lower resolution.

Typical gradient volume is 10-30 column volumes (IEC; 0→0.5mol/L sodium chloride, HIC; 1.0→0mol/L ammonium sulfate).

### 7-3 Sample Loading

In term of separation, excess sample loading results in lower resolution.

Less than about 0.5mg protein loading (\*) for each 1mL of column volume is recommended for optimum resolution.

(\*The value is an approximation, because loading capacity is dependent on sample properties, analytical conditions, etc.)

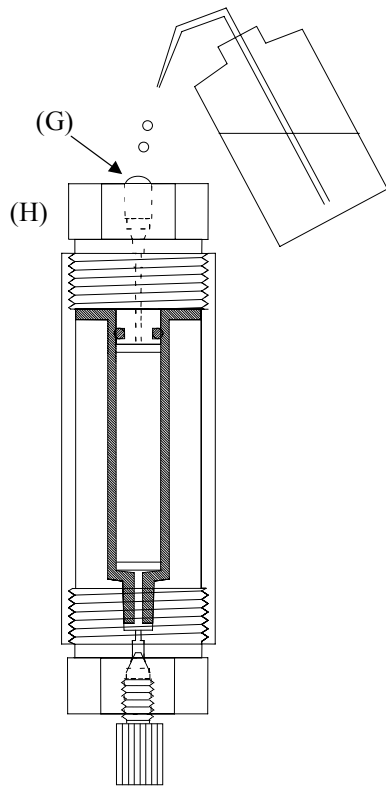
## 8. Storage

We recommend that, after use, the column is washed with water and stored in 20% aqueous ethanol (IEC & AFC type) or 1.8mol/L ammonium sulfate (HIC type). The Protective Screws should be hand tightened and the column should be stored at the temperature indicated on the label and protected from exposure to direct sunlight.

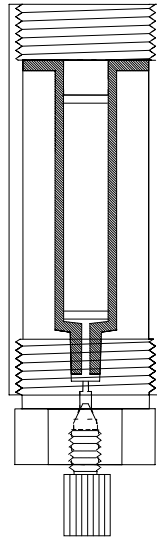
When removing the column from the holder, the column can be sealed with the dedicated cap in order to prevent the column from drying out. The procedure is described below. (Please refer to the illustration on next page.)

- 1) Remove only the Protective Screw located at the top of the column, while leaving the bottom Protective Screw in place. Fill the body of the Inlet Cap with water (G) and then remove the Inlet Cap (H) from the holder.
- 2) Loosen the Outlet Cap of the holder, and detach the column from the Column Holder Body.
- 3) Fill the column top with water. Confirm that liquid drops exit from the bottom of the column through gravity and that no air bubbles are present in the column outlet (I).
- 4) Fill Protective End Cap (J) with water using a washing bottle and attach the cap to the column outlet side.
- 5) Attach the dedicated Seal Cap (K) to top of the column.

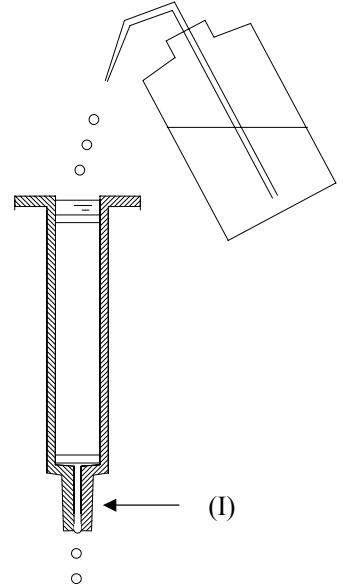
1)



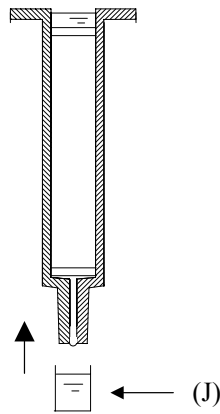
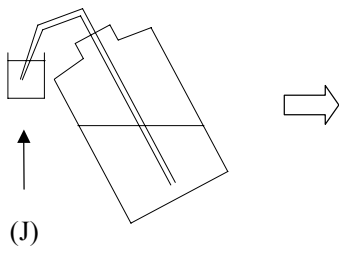
2)



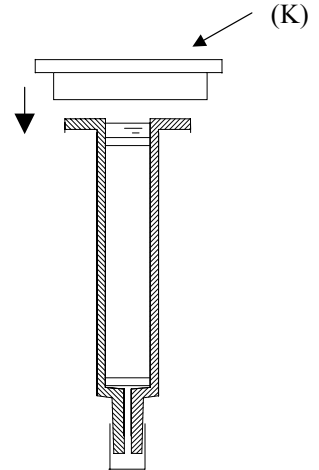
3)



4)



5)





TOSOH

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Website: [www.tosoh.com](http://www.tosoh.com)

**HPLC database:** [www2.tosoh.co.jp/hlc/hlcdb.nsf/StartE?OpenForm](http://www2.tosoh.co.jp/hlc/hlcdb.nsf/StartE?OpenForm)

TOYOPEARL is a registered trademark of Tosoh Corporation

ToyoScreen is a trademark of Tosoh Corporation

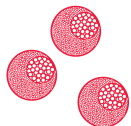
PEEK is a registered trademark of Randal Styka (DBA COMPUTRONICS)

FPLC is a registered trademark of Amersham Bioscience

# ToyoScreen<sup>®</sup> RoboColumn<sup>®</sup> INSTRUCTION MANUAL

## TABLE OF CONTENTS

1.	Introduction	2
2.	Characteristics	2
3.	Handling & Operation	3
	a. Equipment	3
	b. Sample Preparation	3
	c. Experimental setup	5
	d. Te-Chrom <sup>™</sup> Wizard	5
4.	Protocols	6
	a. Detailed Protocol	6
5.	Troubleshooting	11
6.	Ordering information	12



# ToyoScreen RoboColumn INSTRUCTION MANUAL

## 1. INTRODUCTION

ToyoScreen RoboColumns are miniaturized chromatography columns pre-packed with TOYOPEARL biopurification chromatography resins covering a wide range of chromatographic modes, such as ion exchange (IEX), hydrophobic interaction chromatography (HIC), multimodal chromatography (MMC), and affinity chromatography (AFC). Bed containment between two filter frits and professional packing with consideration to individual material compression requirements ensure high efficiency and peak symmetry similar to that of preparative and process separation columns.

ToyoScreen RoboColumns are compatible with 96-well formats and designed for fully automated chromatographic separation with robotic liquid handling workstations such as the Freedom EVO® from Tecan. The column units are packed with TOYOPEARL® media by Atoll GmbH and are identical to the MediaScout® RoboColumns® supplied by Atoll GmbH. One package contains eight pre-packed ToyoScreen RoboColumn units with two removable silicon cover seals for proper storage. A 96-well array plate is available to arrange up to 96 RoboColumn units according to application requirements.

## APPLICATIONS

ToyoScreen RoboColumn technology is used in a broad spectrum of applications, such as parallel screening of chromatographic media and optimization of chromatographic conditions, scale-down experiments, sample preparation prior to subsequent analysis like MS, ELISA or CGE/SDS-Page as well as for the concentration of low titer compounds. It can also be applied for high throughput small scale purifications of a high number of samples.

## FORMATS

ToyoScreen RoboColumns are available in two formats with 200 µL and 600 µL resin volume, respectively. All chromatographic media used in the ToyoScreen RoboColumns are also available in larger pre-packed ToyoScreen columns of 1 mL or 5 mL volume and as bulk resins for use at all scales.

## 2. CHARACTERISTICS

ToyoScreen RoboColumns are ready-to-use chromatography columns pre-packed with TOYOPEARL chromatography media. ToyoScreen RoboColumns are available in 200 µL and 600 µL volume. Table 1 presents the column geometries and characteristics of ToyoScreen RoboColumns.

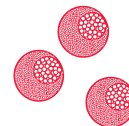
➤ **TABLE 1**

### ToyoScreen RoboColumn CHARACTERISTICS

Column volume	200 µL	600 µL
Bed height	10.0 mm	30.0 mm
Column inner diameter	5 mm	
Column material	Polypropylene	
Chemical stability	All commonly used aqueous buffers, pH 1 – 14, organic solvents <sup>1</sup>	
Avoid	Halogenated organic solvents Hexane	
Storage solution	AIEX and CIEX: 20% ethanol + 150 mmol/L NaCl HIC, SEC, AFC and MMC: 20 % ethanol	
Recommended storage temperature	IEX, HIC & MMC Media: +4°C to +30°C AFC Media: +4°C to +8°C	
Working temperature	+4°C to +30°C	
Max. working pressure	Up to 8.0 bar	
Flow velocity	16 – 1000 cm/h	

<sup>1</sup> Note: The chemical stability of the packed chromatography media also has to be taken into consideration. For more information please refer to the specification of the respective TOYOPEARL resin at [www.tosohbioscience.com](http://www.tosohbioscience.com).

# ToyoScreen RoboColumn INSTRUCTION MANUAL



## 3. HANDLING AND OPERATION

### A) EQUIPMENT


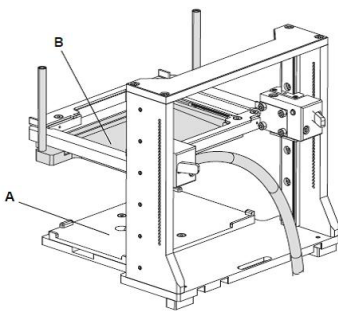
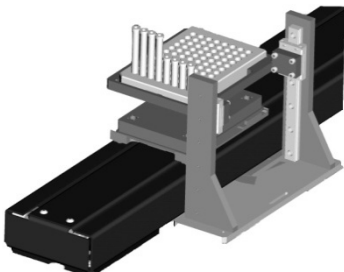
ToyoScreen RoboColumns are designed for robotic handling. Table 2 is a guide for the equipment required for robotic handling of RoboColumns using Tecan's Freedom EVO workstation.

### B.) SAMPLE PREPARATION

We recommend loading a clarified sample on RoboColumns, since unclarified sample may cause clogging of the top and bottom filter frits.

**TABLE 2**

RECOMMENDED EQUIPMENT FOR AUTOMATED HANDLING OF ToyoScreen RoboColumns

EQUIPMENT	DETAILS	ADVICE
<p>96-WELL ARRAY PLATE</p> 	<p>Arrange up to 96 individual RoboColumns onto the 96- well array plate according to application requirements.</p>	<ul style="list-style-type: none"> <li>➤ Do not mix short (200 µL) and long (600 µL) RoboColumns.</li> <li>➤ Make sure that RoboColumns are well clicked into the 96- well array plate before using them.</li> </ul>
<p>Te-Chrom™ MODULE<sup>1)</sup></p> 	<p>Use the Te-Chrom module (A) to integrate the RoboColumn array onto the robotic worktable. The Te-Chrom Module is height adjustable and contains a waste option (B)</p>	<ul style="list-style-type: none"> <li>➤ Use the Te-Chrom module in combination with the plate stacker Te-Stack™ for the optimized handling and storage of collection plates.</li> <li>➤ Use the waste container to drain off e.g. conditioning or regeneration solvents.</li> <li>➤ Make sure that the array is in an appropriate distance to the collection plate before starting the experiment.</li> </ul>
<p>Te-Chrom SHUTTLE</p> 	<p>Use the Te-Chrom Shuttle to move a collection plate under the array to collect the fractions.</p>	<ul style="list-style-type: none"> <li>➤ Use the robotic manipulator (RoMa) arm to transport the collection plate from a pre-defined transfer position to any destination on the robotic worktable (e.g. plate reader, hotels or auto sampler) for further analysis such as UV measurement, ELISA, HPLC, MS, etc.</li> </ul>

(Courtesy of Atoll GmbH)

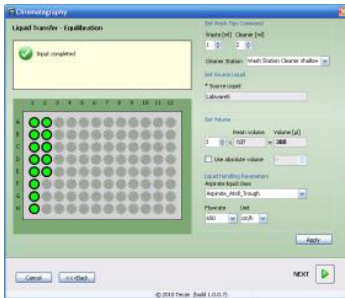
(Courtesy of Tecan)

(Courtesy of Tecan)



# ToyoScreen RoboColumn INSTRUCTION MANUAL

## Te-Chrom WIZARD<sup>2)</sup>



Use the Te-Chrom Wizard to configure the chromatographic process and to set all process relevant parameters, such as volume, flow rate, etc.

- Assign labels (e.g. resin name and column types) by using the plate layout configurator.
- Use the neutralization option to adjust the pH of fractions after acidic elution of mAbs.

Note: Confirm suitability for your specific application with Tecan.

(Courtesy of Tecan)

## PLATE READER

Use an internal or external plate reader for data collection.

- Determine the precise volume of your fractions for subsequent data plotting.

## REAGENT AND SAMPLE RESERVOIRS

A broad spectrum of troughs, tubes and plates can be positioned onto the robotic worktable by using the appropriate Tecan carrier.

- Use an 8-, 48- or 96- deep well reservoir for buffer solution/preparation.

## COLLECTION PLATE

Use any type of multi-well plate which matches the ANSI/SDS standard (UV- or non UV-readable).

- Use a 96-well half area micro-plate to collect fractions in a range from 25  $\mu$ L to 175  $\mu$ L.
- Use standard 96-well micro-plates to collect fractions in a range from 75  $\mu$ L to 340  $\mu$ L. Use 96-deep well reservoirs to collect fraction up to 2 mL.

<sup>1)</sup> For more detailed information about the Te-Chrom Module, see Te-Chrom Operating Manual from Tecan

<sup>2)</sup> For more detailed information about the Te-Chrom Wizard, see Te-Chrom Wizard software Manual from Tecan.

# ToyoScreen RoboColumn INSTRUCTION MANUAL



## C. EXPERIMENTAL SETUP

ToyoScreen RoboColumns are suitable for a broad variety of applications such as parallel screening and optimization of chromatographic conditions in downstream process development, sample preparation prior to subsequent analysis as well as the concentration of low titer compounds in drug discovery.

When using the high-throughput process development approach for RoboColumns, it is suggested to use Design of Experiments (DoE) for the experimental setup. This enables screening of many different chromatographic parameters, such as resin type, pH, conductivity/ionic strength or salt type simultaneously.

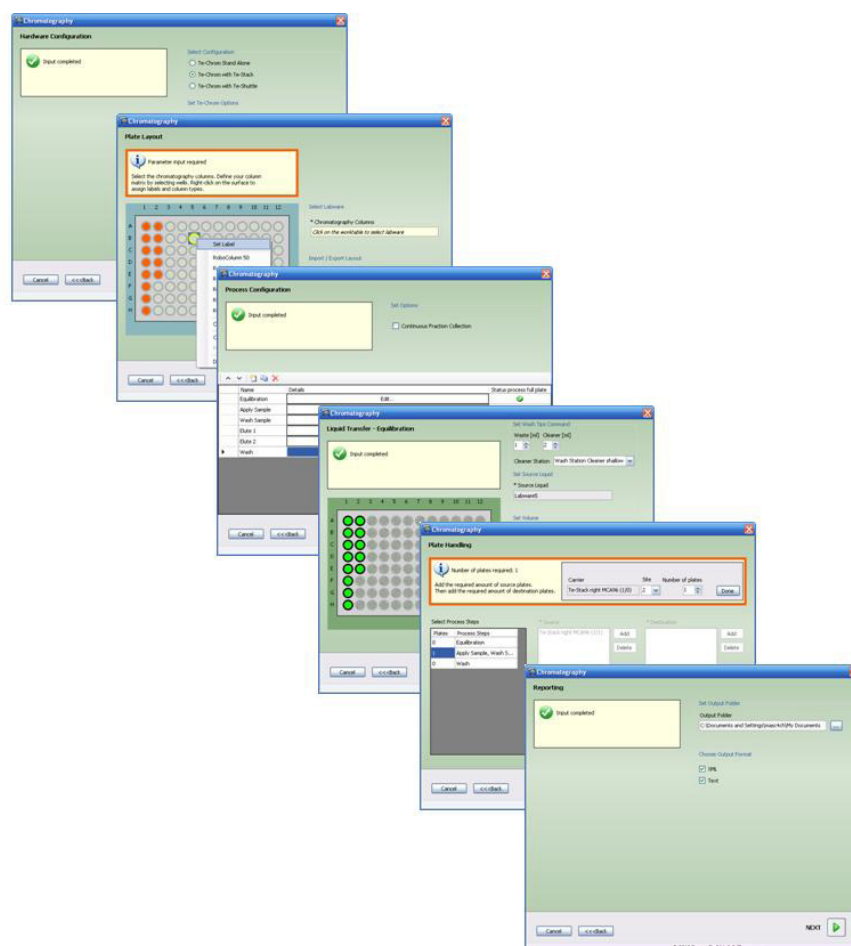
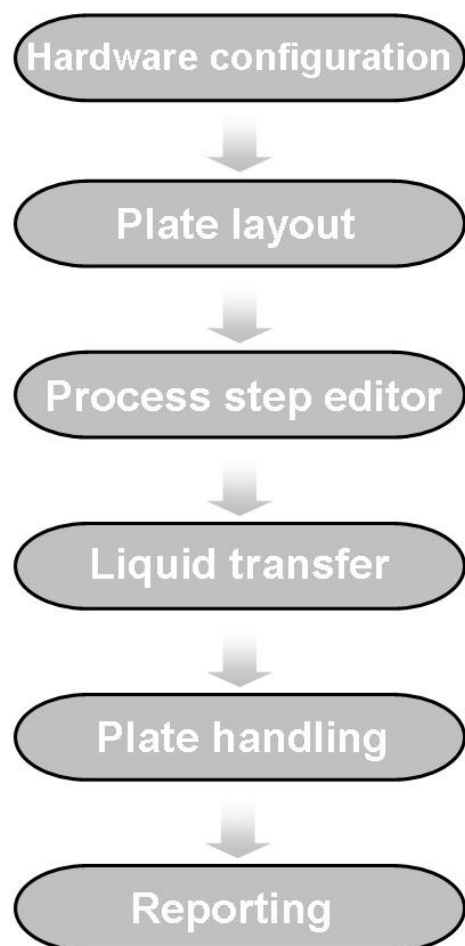
For experimental set-up and configuration of the RoboColumn array plate, the Te-Chrom Wizard is recommended.

## D. Te-Chrom WIZARD

The Te-Chrom Wizard is a dialog-based graphic user interface offered by Tecan and based on the Freedom EVOware® software. The Te-Chrom Wizard was developed for the use of RoboColumns and provides the possibility to configure hardware, plate layout, and the chromatographic process and to set some chromatographic parameters, such as flow-rate or volume without direct script writing. See the illustration below for an overview of some of the Te-Chrom Wizard functions.

➤ **FIGURE 18**

### Te-Chrom WIZARD FEATURES





# ToyoScreen RoboColumn INSTRUCTION MANUAL

## 4. PROTOCOLS

The protocol is designed as a general guideline for working with TOYOPEARL RoboColumns. Adjustment may be required depending on type of application, sample, resin type and RoboColumn dimension.

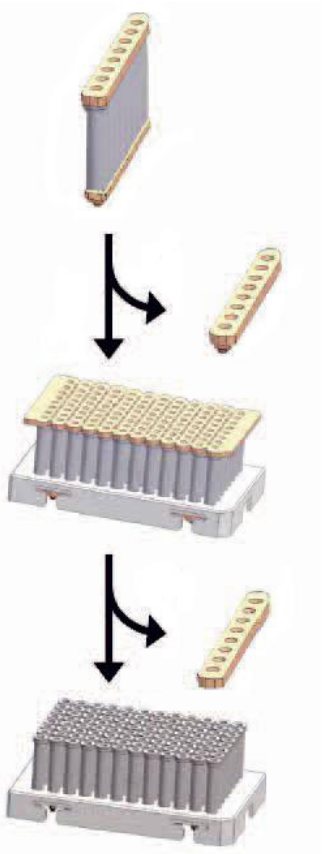
In order to operate RoboColumns the Freedom EVO workstation should be equipped with Te-Chrom Module and Te-Chrom Shuttle.

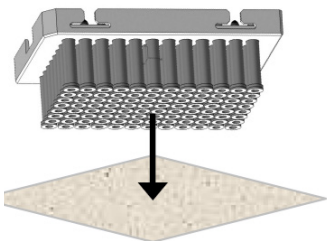
The user should have a general knowledge of chromatography and should be familiar with the use of robotic workstations.

### A) DETAILED PROTOCOL

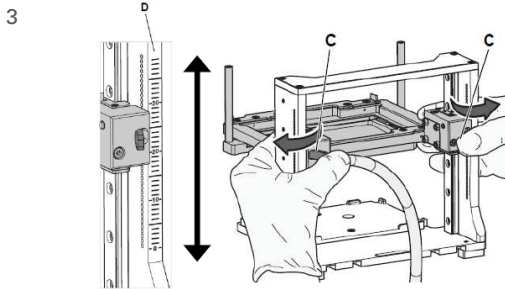
Follow the actions described below in order to perform a complete experiment.

STEP	ILLUSTRATION	ACTION
1		<p><b>REMOVE COVER SEALS</b></p> <ul style="list-style-type: none"> <li>➤ Gently peel off the bottom seal from the row of eight RoboColumn units.</li> <li>➤ Assemble the Robocolumn units onto the 96-well array plate. Up to 12 rows of eight RoboColumn units can be assembled. Do not mix different formats (200 µl and 600 µl) on the same array plate.</li> <li>➤ Gently peel off the top seal on each row of eight RoboColumns.</li> </ul>
2		<p><b>REMOVE EXCESS STORAGE SOLUTION</b></p> <ul style="list-style-type: none"> <li>➤ Remove remaining storage solution in the top part of the columns by blotting the RoboColumn® array upside down on a soft paper tissue.</li> </ul>



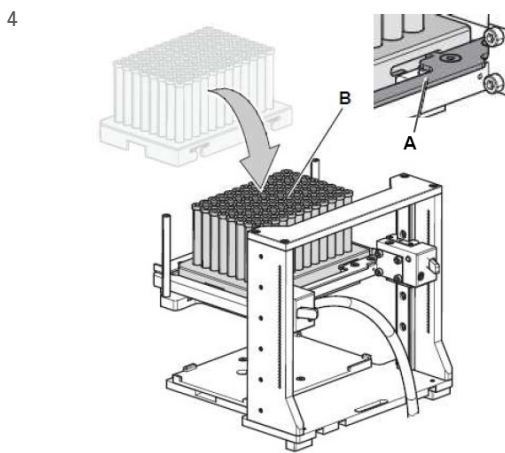



# ToyoScreen RoboColumn INSTRUCTION MANUAL



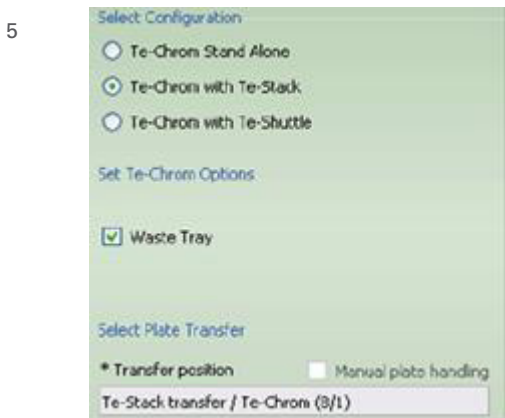
### Te-Chrom - ADJUST THE OPERATING HEIGHT

- Press and hold the fastener (C) to set the Te-Chrom to the required height of the Te-Chrom module scale (D).
- *Make sure that the RoboColumn array is as close to the collection plate as possible.*



### Te-Chrom - LOADING THE 96-WELL ARRAY EQUIPPED WITH RoboColumns

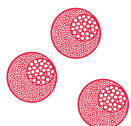
- Move the locking slider (A) to the left mechanical stop.
- Load the RoboColumn array (B).
- Move the locking slider (A) to the right mechanical stop to lock the RoboColumn array.
- *Make sure that all RoboColumns are well fixed into the 96-well array plate before starting the experiment.*



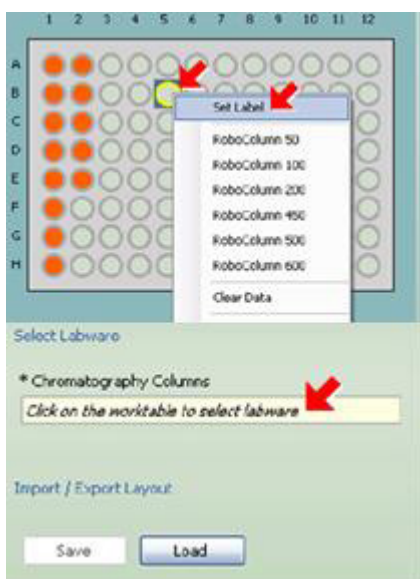
### Te-Chrom WIZARD – EXPERIMENTAL SETUP

#### A) Hardware configuration

- Select the appropriate configuration of the Te-Chrom
- If the Te-Chrom is equipped with a waste tray, check the 'Waste Tray' box.
- Define a transfer position for the collection plate or check the 'manual plate handling' box to select manual plate handling.



# ToyoScreen RoboColumn INSTRUCTION MANUAL



## B) PLATE LAYOUT

- ➔ Click load to import an existing plate layout. To create a new layout, follow the actions below:
- ➔ Select the RoboColumn labware.
- ➔ Define the required RoboColumn matrix.
- ➔ Select the RoboColumn dimension.
- ➔ Set a desired label e.g. resin name.
- ➔ To import or export the plate layout, click the save button.



## C) PROCESS CONFIGURATION

- ➔ Define the chromatographic workflow by setting a desired number of process steps.
- ➔ Use the 'Continuous Fraction Collection' option to collect fractions over multiple plates, without leaving gaps between plates pipetted in different process steps. This minimizes the consumption of collection plates.



## D) CONFIGURE YOUR PROCESS STEPS

- ➔ Edit a 'process step name' e.g. Equilibration:
- ➔ Check the 'Process Full Plate' option according to the desired procedure:
- ➔ When checked: The full RoboColumn array will be processed.
- ➔ When not checked: The RoboColumn array will be processed column per column.
- ➔ Set parameters for tip washing.



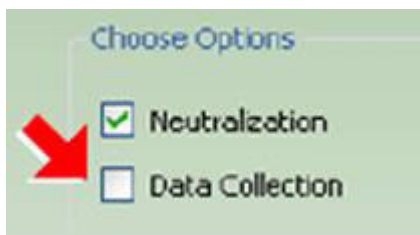
# ToyoScreen RoboColumn INSTRUCTION MANUAL



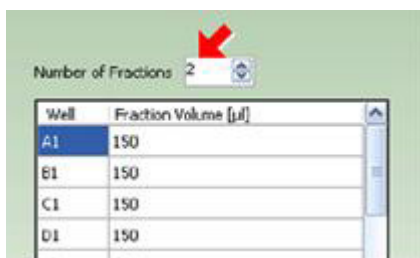
- Set individual chromatographic parameters:
- Select the source of liquid.
- Set the dispensing volume or check the 'Use absolute volume' box to enter an absolute volume.
- Set the required liquid class for aspiration.
- Set the required linear velocity [cm/h] or flow rate [μL/s].



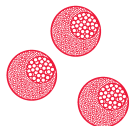
- Check the waste option accordingly:
- When checked: The waste tray will be engaged by the RoMa / LiHa.
- When not checked: The flow-through will be collected in a desired collection plate.



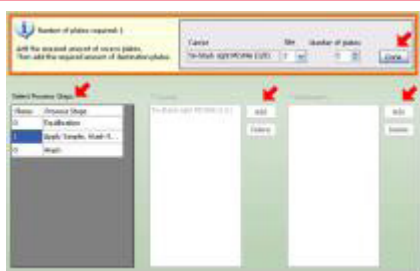
- Check the 'Neutralization' option to perform a pH adjustment e.g. after acidic elution of mAbs.
- Check the Data Collection option to evaluate the collection plate in a required reader device by e.g. UV measurement.



- Select the number of fractions according to the given dispensing volume for the chromatography step if the waste option is not checked.
- Repeat these steps in order to configure all chromatographic process steps.

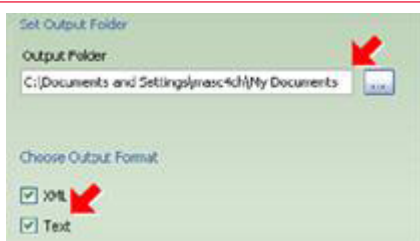


# ToyoScreen RoboColumn INSTRUCTION MANUAL



## E) PLATE HANDLING

- Add the required amount of source plates.
- Add the required amount of destination plates.



## F) REPORTING

- Set output folder.
- Select output format

After the experimental set-up the Te-Chrom wizard will generate a chromatographic method in Freedom EVOware software automatically.

6



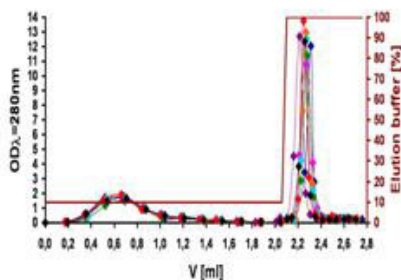
## RUN CHROMATOGRAPHIC METHOD

7

## DATA EVALUATION

- Determine the precise volume of the collected fractions by using an appropriate plate reader.
- Determine the protein concentration by using UV measurement.

8



## DATA PLOTTING

- Plot the UV data against the fraction volume to create a pseudo-chromatogram.
- *Note: Make sure that blank values of the used solvents are subtracted from the UV data before plotting.*

9

## REGENERATION AND STORAGE

- For appropriate regeneration of RoboColumns please refer to the recommendation for the corresponding TOYOPEARL resin.
- For appropriate storage of RoboColumns please refer to Table 1

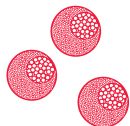
# ToyoScreen RoboColumn

## INSTRUCTION MANUAL



### 5. TROUBLESHOOTING

ERROR	POSSIBLE CAUSE	ACTION
RoboColumns are clogged.	The sample is too viscous.	Increase dilution of the cell paste before lysis, or dilute after the lysis.
	There is too much cell debris in the sample.	Centrifuge and/or filtrate the sample if unclarified sample has been used.
	The column has been regenerated and used too many cycles.	Reduce the number of cycles.
RoboColumns are leaking.	Columns are clogged.	See above.
	The o-ring sealing is damaged.	Check the taught X- and Y coordinates for RoboColumn labware for precision.
	The insertion depth of the robotic needle is incorrect.	Check the insertion depth (16 mm from the top of the column) of the robotic needle.
RoboColumns are lifted up from the 96-well array plate during the pull out of the robotic needles.	RoboColumns are not well fixed into the 96-well array plate.	Make sure that all RoboColumns are well fixed into the 96-well array plate before starting the experiment.
Low protein recovery in the elution fraction(s).	The residence time for sample loading is too low.	Decrease the flow-rate (increase residence time) for sample loading.



# ToyoScreen RoboColumn

## ORDERING INFORMATION

### ► ORDERING INFORMATION

P/N	ToyoScreen RoboColumn	RESIN
45001	ToyoScreen RoboColumn	GigaCap S-650M, 200 µL
45002	ToyoScreen RoboColumn	GigaCap S-650M, 600 µL
45003	ToyoScreen RoboColumn	GigaCap Q-650M, 200 µL
45004	ToyoScreen RoboColumn	GigaCap Q-650M, 600 µL
45005	ToyoScreen RoboColumn	GigaCap CM-650M, 200 µL
45006	ToyoScreen RoboColumn	GigaCap CM-650M, 600 µL
45011	ToyoScreen RoboColumn	Q-600C AR, 200 µL
45012	ToyoScreen RoboColumn	Q-600C AR, 600 µL
45031	ToyoScreen RoboColumn	Phenyl-600M, 200 µL
45032	ToyoScreen RoboColumn	Phenyl-600M, 600 µL
45033	ToyoScreen RoboColumn	Butyl-600M, 200 µL
45034	ToyoScreen RoboColumn	Butyl-600M, 600 µL
45035	ToyoScreen RoboColumn	PPG-600M, 200 µL
45036	ToyoScreen RoboColumn	PPG-600M, 600 µL
45037	ToyoScreen RoboColumn	Phenyl-650M, 200 µL
45038	ToyoScreen RoboColumn	Phenyl-650M, 600 µL
45051	ToyoScreen RoboColumn	MX-Trp-650M, 200 µL
45052	ToyoScreen RoboColumn	MX-Trp-650M, 600 µL
45061	ToyoScreen RoboColumn	AF-rProtein A-650F, 200 µL
45062	ToyoScreen RoboColumn	AF-rProtein A-650F, 600 µL
45099	Array Plate	

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Freedom EVO® and Freedom EVOware® are registered trademarks of Tecan Group Ltd.

Te-Chrom™ and Te-Stack™ are trademarks of Tecan Group Ltd.