



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
 WARNING	Alerts the user to the potential for serious injury or death.
 CAUTION	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.

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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 ¹⁾
SuperQ-650M	40-90	0.20-0.30	105-155 ¹⁾
QAE-550C	50-150	0.28-0.38	60-80 ¹⁾
CM-650M	40-90	0.08-0.12	30-50 ²⁾
SP-650M	40-90	0.13-0.17	40-60 ²⁾
SP-550C	50-150	0.14-0.18	80-120 ²⁾
HIC type			
Ether-650M	40-90		10-30 ²⁾
Phenyl-650M	40-90		30-50 ²⁾
Butyl-650M	40-90		30-50 ²⁾
Hexyl-650C	50-150		30-50 ²⁾
PPG-600M	40-90		20-35 ³⁾
SuperButyl-550C	50-150		52-70 ²⁾

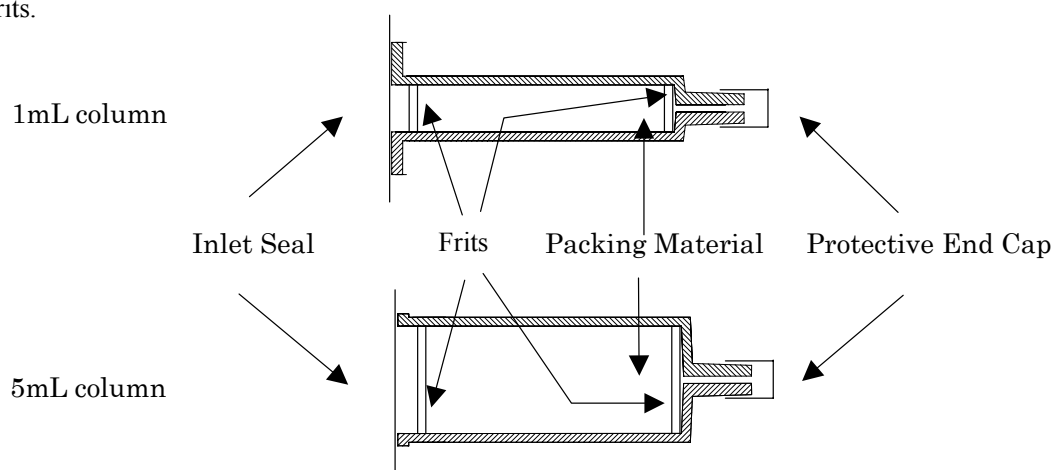
AFC type			
AF-Chelate-650M	40-90	0.025-0.045	
AF-Blue HC-650M	40-90		18 or more ⁴⁾
AF-Red-650M	40-90		2.5-4.5 ⁴⁾
AF-Heparin HC-650M *	40-90		5 or more ⁵⁾

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

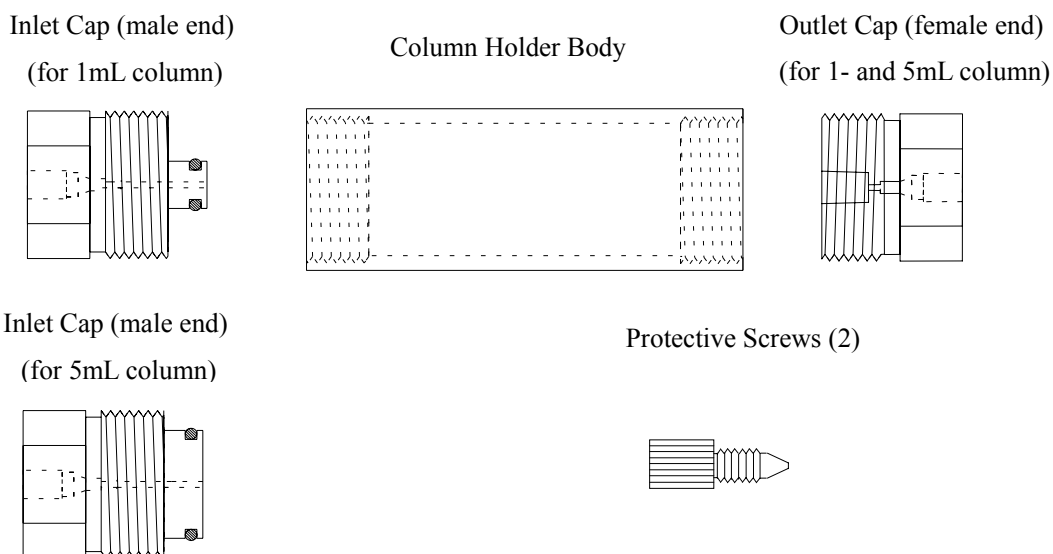
Measured with 1) Bovine serum albumin, 2) Lysozyme, 3) γ -Globulin, 4) Human serum albumin, and 5) Antithrombin-III (Tosoh original method.)

3. Column Components

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



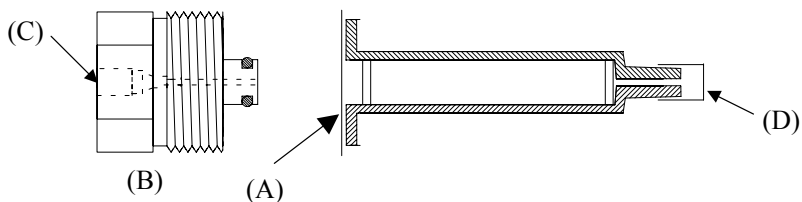
ToyoScreen must be used with the dedicated holder (ToyoScreen Holder: Part No.21400), which is sold separately. The holder consists of the following components:



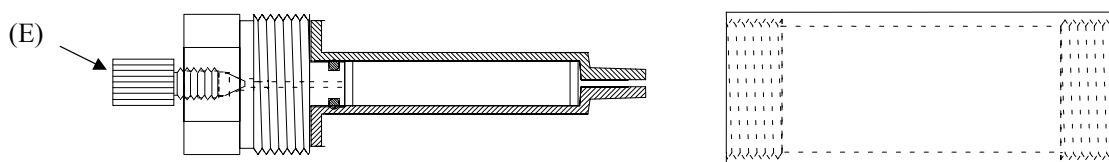
4. Column Attachment

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.

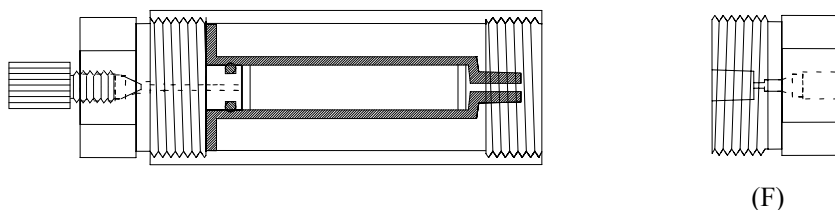
- 1) Carefully peel the foil inlet seal (A) from the column making sure all seal material is removed from the column inlet. Place the Inlet Cap (B) on top of the column and slowly push the male end of the Inlet Cap into the column housing. When attaching the column, leave the flow hole of the Inlet Cap 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.



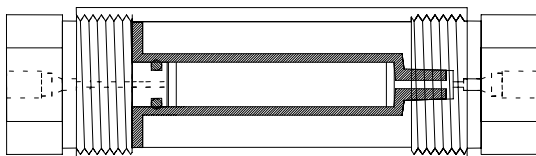
- 2) Attach the Protective Screw (E) to the Inlet Cap as shown below. Slowly remove the column Protective End Cap (D) and hand-tighten the column into the Column Holder Body. **DO NOT USE A WRENCH TO TIGHTEN THE HOLDER.**



- 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[®] 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[®] 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.)

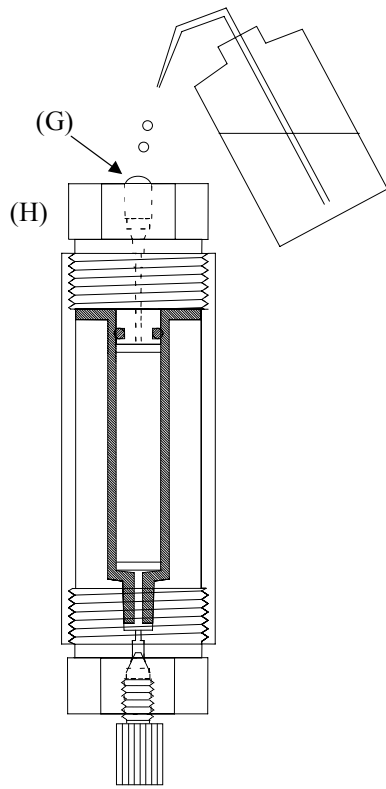
8. Storage

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.

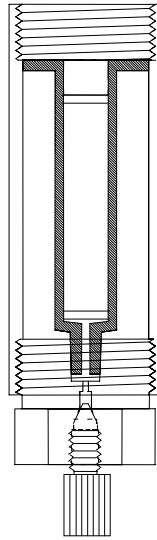
When removing the column from the holder, the column can be sealed with the dedicated cap in order to prevent the column from drying out. The procedure is described below. (Please refer to the illustration on next page.)

- 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.

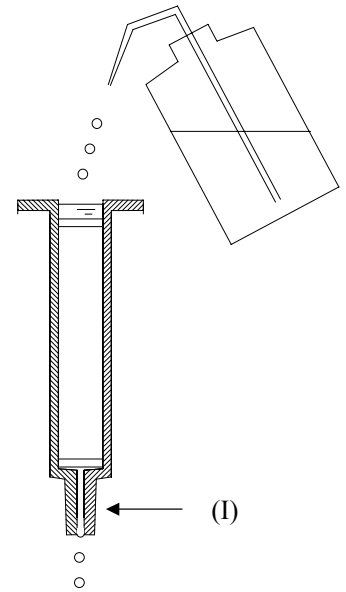
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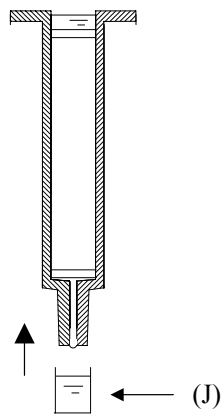
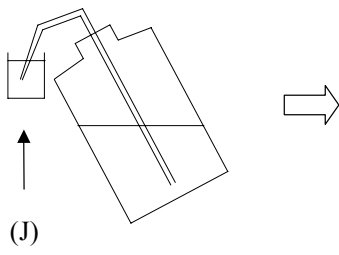
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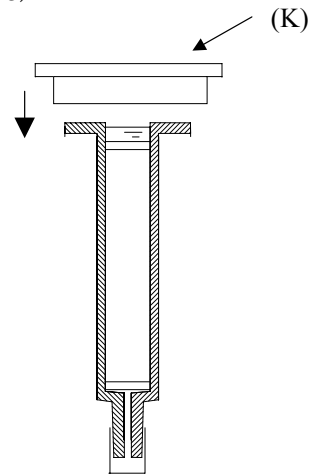
3)



4)



5)





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