

TOYOPEARL IEC Type

650 Series	TOYOPEARL DEAE-650S, M, C TOYOPEARL CM-650S, M, C TOYOPEARL SP-650S, M, C TOYOPEARL SuperQ-650S, M, C
550 Series	TOYOPEARL QAE-550C TOYOPEARL SP-550C TOYOPEARL MegaCap II SP-550EC

INSTRUCTION MANUAL



TOSOH

TOSOH CORPORATION

Safety Precautions

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

[Notational Conventions]

Notation	Explanation
 WARNING	Indicates a hazard with a medium level of risk which, if not avoided, could result in death or serious injury.
 CAUTION	Indicates a hazard with a low level of risk which, if not avoided, could result in minor or moderate injury.

WARNING

■ **Keep away from fire**

Not taking proper precautions when using flammable solvents could result in 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, or corrosion.
Wear appropriate protective gear when cleaning up a spill.

■ **Wear protective eye gear and gloves**

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

■ **Handle the package with care**

Inappropriate handling may cause rupturing and/or splattering of the product.

■ **Only use this product for its intended use**

This product is intended for the separation and purification of small molecules and proteins. Do not use it for any other purpose.

■ **Make sure compounds are safe**

Check that the target compounds and solutions after separation and purification are safe.

■ **Proper disposal**

Dispose in accordance with local laws and regulations.

NOTE

Keep this manual with the product for future reference.

Precautions: Shipping Solvents

TOYOPEARL IEC Type products are shipped in 20 % aqueous ethanol.

First Aid	Inhalation	<ul style="list-style-type: none">• Move the person to an area with fresh air and rinse the mouth with plenty of water.• Call immediately for medical attention.
	Skin exposure	<ul style="list-style-type: none">• Wash the exposed area with plenty of soap and water.
	Eye exposure	<ul style="list-style-type: none">• Open the eyes as wide as possible and rinse with clean water for at least 15 minutes.• Call immediately for medical attention.
	Ingestion	<ul style="list-style-type: none">• Rinse the mouth with plenty of water.• Call immediately for medical attention.
Handling and Storage	Ventilation	<ul style="list-style-type: none">• Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	<ul style="list-style-type: none">• Container may break if not handled with care.
	Wear appropriate protective equipment	<ul style="list-style-type: none">• Use solvent-resistant gloves and protective eye gear when using this product. Use of a gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	<ul style="list-style-type: none">• If any flammable solvents are used for shipping or storage of this product, keep away from fire or open heat sources.
	Storage temperature	<ul style="list-style-type: none">• Avoid storing this product at very low temperatures (< 0 °C) to prevent product from freezing.
Waste Disposal	Disposal methods	<ul style="list-style-type: none">• Dispose in accordance with local laws and regulations.
	General considerations	<ul style="list-style-type: none">• Please pay attention to all safety precautions with respect to the handling and storage of this product.

Precautions: TOYOPEARL Brand Chromatographic Media

First Aid	Inhalation	<ul style="list-style-type: none"> • Move the person to an area with fresh air and rinse the mouth with plenty of water. • Call immediately for medical attention.
	Skin exposure	<ul style="list-style-type: none"> • Wash the exposed area with plenty of soap and water.
	Eye exposure	<ul style="list-style-type: none"> • Open the eyes as wide as possible and rinse with clean water for at least 15 minutes. • Call immediately for medical attention.
	Ingestion	<ul style="list-style-type: none"> • Rinse the mouth with plenty of water. • Call immediately for medical attention.
Handling and Storage	Ventilation	<ul style="list-style-type: none"> • Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	<ul style="list-style-type: none"> • Container may break if not handled with care.
	Wear appropriate protective equipment	<ul style="list-style-type: none"> • Use solvent-resistant gloves and protective eye gear when using this product. Use of a gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	<ul style="list-style-type: none"> • If any flammable solvents are used for shipping or storage of this product, keep away from fire or open heat sources.
	Fire precautions	<ul style="list-style-type: none"> • Do not expose this chromatographic resin to fire or open heat sources. See below for additional precautions.
Waste Disposal	Disposal methods	<ul style="list-style-type: none"> • Dispose in accordance with local laws and regulations.
	General considerations	<ul style="list-style-type: none"> • Please pay attention to all safety precautions with respect to the handling and storage of this product.
	Disposal precaution	<ul style="list-style-type: none"> • This product can be safely incinerated. • Appropriate nitrogen oxides exhaust emission precautions should be taken specifically for TOYOPEARL DEAE-650S, M, C, TOYOPEARL SuperQ-650S, M, C and TOYOPEARL QAE-550C. • Appropriate sulfur oxides exhaust emission precautions should be taken specifically for TOYOPEARL SP-650S, M, C, TOYOPEARL SP-550C and TOYOPEARL MegaCap II SP-550EC.

□ TOYOPEARL products contain combustible packings based on a methacrylate polymer.

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1. Introduction

TOYOPEARL IEC Type is based TOYOPEARL HW-65 (650 Series, Protein Exclusion Limit 5×10^6) or TOYOPEARL HW-55 (550 Series, Protein Exclusion Limit 7×10^5), which are the porous and spherical polymers.

They have the following features.

- The change of gel volume is negligible in buffer with various pH or salt concentration.
- Applicable to fast flow rate on column chromatography.
- Resistant to microorganisms.
- Applicable to HPLC system.

TOYOPEARL MegaCap II SP-550EC is based TOYOPEARL HW-55, which is the porous and spherical polymer.

- High capacity for peptides and lower molecular weight proteins, such as insulin etc.
- Low pressure drop.

〈Products Line-up〉

650 Series	Weak Anion	TOYOPEARL DEAE-650 S, M, C
	Weak Cation	TOYOPEARL CM-650 S, M, C
	Strong Cation	TOYOPEARL SP-650 S, M, C
	Strong Anion	TOYOPEARL SuperQ-650 S, M, C
550 Series	Strong Anion	TOYOPEARL QAE-550 C
	Strong Cation	TOYOPEARL SP-550 C
	Strong Cation	TOYOPEARL MegaCap II SP-550EC

* Note (Particle Sizes)

S : Superfine	20 μ m - 50 μ m
M : Medium	40 μ m - 90 μ m
C : Coarse	50 μ m - 150 μ m
EC : Extra Coarse	100 μ m - 300 μ m

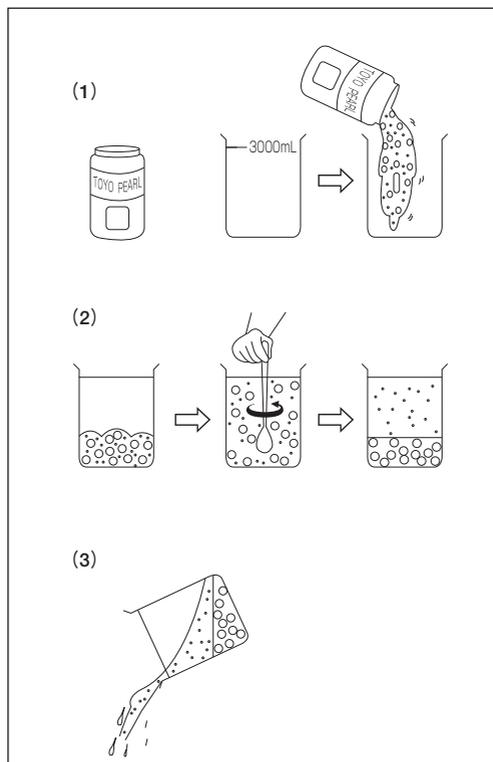
2. Procedure for Chromatography

2-1 Removal of Fines

- (1) As an example, pour the gel of 500 mL in the beaker of 3000 mL. (The capacity has six times of the gel.)
- (2) Add distilled water to a total of 2000 mL (four times of the gel) in the beaker, stir and leave them until the gel settles.

Grade	Settling Time (recommended)
TOYOPEARL DEAE-650C TOYOPEARL CM-650C TOYOPEARL SP-650C TOYOPEARL SuperQ-650C TOYOPEARL QAE-550C TOYOPEARL SP-550C	15 - 30 minutes
TOYOPEARL DEAE-650M TOYOPEARL CM-650M TOYOPEARL SP-650M TOYOPEARL SuperQ-650M	30 - 45 minutes
TOYOPEARL DEAE-650S TOYOPEARL CM-650S TOYOPEARL SP-650S TOYOPEARL SuperQ-650S	60 - 90 minutes
TOYOPEARL MegaCap II SP-550EC	5 - 10 minutes

- (3) Decant and discard the supernatant (containing fines).
- (4) Repeat this process (2) and (3) at least three times.



Removal of Fines

2-2 Cleaning

TOYOPEARL IEC Type is shipped or stored in an aqueous solution containing 20 % ethanol.

The washing of the gel is necessary prior to use.

Pour the gel slurry on a glass filter and wash with distilled water of three times of the gel volume.

2-3 Preparation of Gel Slurry and Packing

After removing fines from the gel by decantation, wash the gel with packing solvent.

The packing buffer should contain the highest salt concentration that the column will be exposed during normal use, cleaning and storage. Transfer the gel into a beaker and add the packing buffer to make an approximately 30 % - 50 % (V/V) (recommended) slurry.

Packing the column under pressure (0.05 MPa - 0.3 MPa (recommended)) is recommended.

In this case a pump and a reservoir are necessary to pack the column.

Usually the packing flow rate is at least two times faster than that of the operating flow rate.

The gravitational packing method is often applied as conventional one.

In this case the pressure is desired to be as large as possible.

2-4 Equilibration

After packing, the column should be equilibrated with 3 to 5 column volume of buffer.

2-5 Elution

Adsorbed sample can be eluted by increasing of salt concentration up to 1 mol/L or change of pH in buffer.

2-6 Regeneration

The gel can be regenerated by the following procedure.

2-6-1 Batch Method

Pour the gel in a beaker and add the cleaning solvent in it, and stir and leave them until the gel precipitates, then discard the supernatant by decantation.

Repeat this process 2 or 3 times.

 **Caution** : The extremely severe cleaning method that is described below, will use HCl solution. Please note that some proteins will aggregate in acidic conditions

(1) TOYOPEARL DEAE-650S, M, C, TOYOPEARL SuperQ-650S, M, C, TOYOPEARL QAE-550C

*** General cleaning**

Wash the gel with 0.5 mol/L - 1.0 mol/L NaCl solution by the procedure mentioned above, then equilibrate the gel with buffer.

*** Severe cleaning**

Wash the gel with 0.1 mol/L - 0.5 mol/L NaOH followed by washing 0.1 mol/L - 0.5 mol/L NaCl solution. Then equilibrate the gel with buffer.

*** Extremely severe cleaning**

Wash the gel with 0.1 mol/L HCl, then water, then 0.1 mol/L - 0.5 mol/L NaOH,

then 0.1 mol/L - 0.5 mol/L NaCl, followed by washing with buffer.

(2) TOYOPEARL CM-650S, M, C, TOYOPEARL SP-650S, M, C,
TOYOPEARL SP-550C, TOYOPEARL MegaCap II SP-550EC

* General cleaning

At first wash the gel 0.5 mol/L - 1 mol/L NaCl solution by the procedure mentioned above, then equilibrate the gel with buffer.

* Severe cleaning

Wash the gel with 0.1 mol/L - 0.5 mol/L NaOH followed by washing 0.1 mol/L - 0.5 mol/L NaCl solution. Then equilibrate the gel with buffer.

* Extremely severe cleaning

Wash the gel with 0.1 mol/L NaOH, then water, then 0.1 mol/L - 0.5 mol/L HCl, then 0.1 mol/L - 0.5 mol/L NaCl, followed by washing with buffer.

2-6-2 Column Method

The gel in a column can be regenerated easily by flowing the cleaning solvents on the column.

The procedure and the solvents for the cleaning are just same as that of Batch Method.

[Advantages of Column Method]

- * Simple Handling Taking out of the gel from the column and repacking of the gel into the column are not necessary.
- * Good Reproducibility
- * Quick Cleaning By applying a pump the cleaning time becomes shorter than that by Batch Method.
- * Effective Cleaning The gel can be regenerated well with a small amount of solvents compared with Batch Method.

3. Storage

The gel should be stored with 20 % aqueous ethanol at low temperature (preferably 4 °C ~ 35 °C).

4. Remarks

4-1 Removal of Fines

As described in Section 2, remove fines before use. When the fines are not removed completely, there is a possibility that micro-particles may leach from column during chromatography. Leaching of the micro-particles, however, should stop after a short period of time.

4-2 Clogging of Filter

Increasing of pressure-drop or decreasing of flow-rate is typically caused by filter (frit) clogging.

When this happens, remove the chromatographic resin from the column and clean the fitting and screens. Once the hardware is completely clean, repack the chromatographic resin into the column as described above.

4-3 Adsorption of Protein

When protein is not adsorbed well to the column with the initial buffer, sample should be dialyzed or desalted.

4-4 Packing Method

TOSOH recommends packing the resin into the column using a pressure-packing method.

Packing the column using a suction method or by just using gravity settling is not recommended, particularly for columns more than 10 cm in length.



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