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TOYOPEARL®
Ion Exchanger

650 Series TOYOPEARL GigaCap® S-650S, M
TOYOPEARL GigaCap® Q-650S, M
TOYOPEARL GigaCap® CM-650M
TOYOPEARL GigaCap® DEAE-650M

FOR IN VITRO USE ONLY / FOR PACKED-BED USE ONLY

INSTRUCTION MANUAL

TOSOH

TOSOH CORPORATION

Safety Precautions

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

[Notation Conventions]

Notation	Explanation
⚠WARNING	Indicates a potentially hazardous situation which could result in death or serious injury.
⚠CAUTION	Indicates a potentially hazardous situation which could result in 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, and corrosion.
Wear appropriate protective gear when cleaning up a spill.

■ **Wear protective eye gear and gloves**

Organic solvents and acids should not come in 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 as intended**

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

■ **Make sure compounds are safe**

Check that obtained compounds in the feedstock 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 Ion Exchangers are shipped in 20 % aqueous ethanol.

First Aid	Inhalation	・Move the person to an area with fresh air. Immediately rinse the mouth with plenty of water. ・Call for medical attention immediately.
	Skin exposure	・Wash exposed area with plenty of soap and water.
	Eye exposure	・Open eyes as wide as possible and rinse with clean water for at least 15 minutes. ・Call for medical attention immediately.
	Ingestion	・Wash the mouth with plenty of water and immediately call for medical attention.
Handling and Storage	Ventilation	・Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	・Container may break if not handled with care.
	Wear appropriate protective equipment	・Use solvent-resistant gloves and protective eye gear when using this product. Use of gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	・If any flammable solvents are used for shipping or storage, keep away from fire and open heat.
	Storage temperature	・Avoid storing this product at very low temperatures (＜0 °C) to prevent product from freezing.
Waste Disposal	Disposal methods	・Follow local guidelines for disposal. This product can be incinerated safely.
	General considerations	・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	・Move the person to an area with fresh air. Rinse the mouth with plenty of water immediately. ・Call for medical attention immediately.
	Skin exposure	・Wash exposed area with plenty of soap and water.
	Eye exposure	・Open eyes as wide as possible and rinse with clean water for at least 15 minutes. ・Immediately call for medical attention.
	Ingestion	・Rinse the mouth with plenty of water and call for medical attention immediately.
Handling and Storage	Ventilation	・Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	・Container may break if not handled with care.
	Wear appropriate protective equipment	・Use solvent-resistant gloves and protective eye gear when using this product. Use of gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	・If any flammable solvents are used for shipping or storage, keep away from fire and open heat.
	Fire precautions	・Do not expose the chromatographic resin to fire or open heat sources.
Waste Disposal	Disposal methods	・This product can be incinerated or buried for easy disposal. See below for additional precautions.
	General considerations	・Please pay attention to all safety precautions with respect to the handling and storage of this product.
	Disposal precaution	・Dispose in accordance with local laws and regulations. This product can be incinerated safely. ・Assure that appropriate countermeasures is taken when incinerating TOYOPEARL GigaCap Q-650S, M and TOYOPEARL GigaCap DEAE-650M. Exhausts may contain nitrogen oxides. ・Appropriate sulfur exhaust emission precaution should be taken for TOYOPEARL GigaCap S-650S, M.

□ TOYOPEARL products contain combustible packings based on a methacrylate polymer

Table of Contents

Revised in April, 2013

1. Introduction

TOYOPEARL GigaCap series Ion exchanger has been specifically designed for packed-column use, and is based on TOYOPEARL HW-65 (650 Series, Protein Exclusion Limit 5 × 10^6 Da), 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 microorganism
* Applicable to HPLC system.

〈Products Line-up〉

650 Series	Strong Cation	TOYOPEARL GigaCap S-650S, M
	Strong Anion	TOYOPEARL GigaCap Q-650S, M
	Weak Cation	TOYOPEARL GigaCap CM-650M
	Weak Anion	TOYOPEARL GigaCap DEAE-650M

* Note (Particle sizes)
S : Superfine 20 - 50 μm
M : Medium 50 - 100 μm

2. Procedure for Chromatography

2-1 Removal of Fines

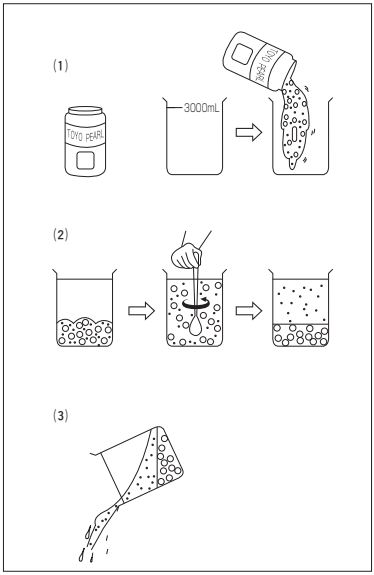
(1) As an example, pour 500 mL of the gel into a 3000 mL beaker (The capacity should be about six times the gel volume).

(2) Add 2000 mL of distilled water (four times the gel volume) to the beaker, stir and let the gel settle.
Note: The recommended settling times of the gel with this particle sizes is as follows.

Grade	Settling Time (recommended)
TOYOPEARL GigaCap S-650M TOYOPEARL GigaCap Q-650M TOYOPEARL GigaCap CM-650M TOYOPEARL GigaCap DEAE-650M	30 - 45 min.
TOYOPEARL GigaCap S-650S TOYOPEARL GigaCap Q-650S	60 - 90 min.

(3) Decant and discard the supernatant (containing fines).

(4) Repeat steps (2) and (3) of this process at least three times.



Removal of Fines

2-2 Cleaning

TOYOPEARL Ion Exchangers are packed with 20 % aqueous 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 (Section 2-1), wash the gel with packing buffer. The packing buffer should contain the highest salt concentration that the column will be exposed during normal use, cleaning or storage. Transfer the gel into a beaker and add packing buffer to make an approximately 30 - 50 % (V/V) (recommended) slurry.

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

In this most cases 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 which will generate higher pressures.

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 the pH of the buffer.

2-6 Regeneration

The gel can be regenerated using the following procedures.

2-6-1 Batch method

Pour the gel into a beaker containing the cleaning solvent, stir and leave the gel until it settles. Discard the supernatant by decantation.

Repeat this process 2 or 3 times.

⚠Caution : The Extremely Severe Cleaning method that is described below uses an HCl solution. Please note that some proteins may aggregate in acidic solution.

TOYOPEARL GigaCap S-650S, M, TOYOPEARL GigaCap CM-650M

* General cleaning

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

* Severe cleaning

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

＊Extremely severe Cleaning

Wash the gel with 0.1 - 0.5 mol/L NaOH. Wash with until the pH is mostly neutralized and then 0.1 - 0.5 mol/L HCl. Finally wash with 0.1 - 0.5 mol/L NaCl followed by equilibrium with buffer.

TOYOPEARL GigaCap Q-650S, M, TOYOPEARL GigaCap DEAE-650M

＊General cleaning

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

＊Severe cleaning

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

＊Extremely severe Cleaning

Wash the gel with 0.1 - 0.5 mol/L HCl. Wash with until the pH is mostly neutralized and then 0.1 - 0.5 mol/L NaOH. Finally wash with 0.1 - 0.5 mol/L NaCl followed by equilibrium with buffer.

2-6-2 Column Method

The gel, packed into a column, can be regenerated easily by using standard chromatography cleaning solvents.

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

[Advantages of Column Cleaning Method]

＊Simple Handling	There is no need to remove the resin from the column in order to clean.
＊Good Reproducibility	
＊Quick Cleaning	Using a pump to flow the cleaning reagents over the column bed reduces the amount of time necessary to clean the column compared to batch cleaning methods.
＊Effective Cleaning	Typically column cleaning requires less solvent than batch cleaning.

3. Storage

The gel should be stored in an aqueous solution containing 20 % aqueous ethanol at ambient temperatures (4 - 35 ℃)

4. Remarks

4-1 Removal of Fines

As described in Section 2, fines should be removed prior to use. When fines are not removed completely, there is a possibility that micro-particles may elute from column during normal chromatographic use. However, the level of fines should gradually diminish as the column is used.

4-2 Clogging of Screens and Frits

Increasing of pressure-drop or decreased flow-rates are typically caused by clogged screens or frits. When this happens, remove the chromatographic resin from the column and clean the fittings, screens or frits once the hardware is completely clean, repack the chromatographic resin into the column as described above.

4-3 Adsorption of Protein

When the protein sample does not adsorb onto the resin using the initial buffer conditions, it may be necessary to dialyze or desalt the sample prior to applying to the column.

4-4 Packing Method

Tosoh Corporation recommends packing the resin into the column using a flow and pressure method. Packing the column using suction or using gravity settling is not recommended, particularly for columns more than 10 cm in length.

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