TOYOPEARL® Ion Exchanger 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

# INSTRUCTION MANUAL



# **Safety Precautions**

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

#### [Notational Conventions]

Notation	Meaning
	Alerts the user to the potential for serious injury or death.
	Alerts the user to the potential for damage to hardware or bodily harm.

#### 

#### Keep away from fire.

When using with flammable solvents, it can cause fire, explosion, or poisoning.

#### 

#### 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 shorts, poisoning, injury, and corrosion. When cleaning up the spill, wear suitable protective equipment.

#### Wear eye protection and protective globes.

Organic solvents or acid is harmful in contact with skin.

#### Handle package with care.

Inappropriate handling may cause rupture and spattering.

Do not use for unintended use.

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

#### When packing the columns, keep appropriate pressure. Overpressure may cause rupture and spattering. Wear suitable protective equipments while packing.

Make sure of the safety of the obtained compound and solution after separation and purification.

Dispose of in an authorised way. Dispose of in the conventional procedures in compliance with local, state and federal regulations.

#### NOTE

Keep this manual with the product.

### - Table of Contents -

1.	Introduction ·····1			
2.	Procedure for Chromatography2			
	2-1 Removal for Fines2			
	2-2 Cleaning			
	2-3 Preparation of Gel Slurry and Packing			
	2-4 Equilibration ······3			
	2-5 Elution			
	2-6 Regeneration ······4			
3.	Storage			
4.	Remarks			
	4-1 Removal of Fines ······6			
	4-2 Clogging of Filter			
	4-3 Adsorption of Protein			
	4-4 Packing Method			

#### 1. Introduction

TOYOPEARL Ion Exchangers are based TOYOPEARL HW-65 (650 Series, Protein Exclution Limit 5  $\times$  10<sup>6</sup>) or TOYOPEARL HW-55 (550 Series, Protein Exclution Limit 7  $\times$  10<sup>5</sup>), 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.

#### **(Products Line-up)**

	650 Sorios	Weak Anion	TOYOPEARL DEAE-650 S, M, C
		Weak Cation	TOYOPEARL CM-650 S, M, C
	050 Series	Strong Cation	TOYOPEARL SP-650 S, M, C
		Strong Anion	TOYOPEARL SuperQ-650 S, M, C
550 S	550 Sortion	Strong Anion	TOYOPEARL QAE-550 C
	550 Series	Strong Cation	TOYOPEARL SP-550 C

*	Note	(Particle	Sizes)		
For	650	Series	S: Superfine	20 - 50	$\mu\mathrm{m}$
			M: Medium	40 - 90	$\mu\mathrm{m}$
			C : Coarse	50 -150	$\mu\mathrm{m}$
For	550	Series	C : Coarse	50 -150	$\mu\mathrm{m}$

#### 2. Procedure for Chromatography

- 2-1 Removal of Fines
- a Transfer 500 mL of gel into a beaker of 3000 mL.
- s Add distilled water up to 2000 mL in the beaker, stir and leave them until the gel precipitates.
  - Note: The necessary standing times of the gel with different particle sizes are as follows.
    - \* Coarse Grade : 15-30 min.
    - \* Medium Grade : 30-45 min.
    - \* Superfine Grade: 60-90 min.
- d Discard the supernatant(containing fines) by decantation.
- f Repeat the process s and d three or more 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, wash the gel with packing solvent which should be used with the highest salt concentration in the used eluents, then transfer the gel into a beaker and add the packing solvent so as to make ca. 30-50 % (V / V) slurry.

The packing method under pressure (0.05-0.3MPa) is desirable.

In this case a pump and a reservoir are necessary for the packing.

Usually the flow rate of packing is two times faster than that of operation.

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.

- a TOYOPEARL DEAE-650S, M, C, TOYOPEARL SuperQ-650S, M, C, TOYOPEARL QAE-550C
  - \* General cleaning

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

\* Severe cleaning

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

s TOYOPEARL CM-650S, M, C, TOYOPEARL SP-650S, M, C, TOYOPEARL SP-550C

\* General cleaning

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

```
* Extremely severe cleaning
Wash the gel with 0.1 mol/L NaOH, then water, then 0.1-0.5 mol/L
HCl, then 0.1-0.5 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 appling 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 (preperably 4 ${\sim}10\,;$  ).

#### 4. Remarks

#### 4-1 Removal of Fines

As described in Section 2, remove fines before use.

When the removal of fines is not performed completely, microparticles leak from column during chromatography.

Leak of microparticles, however, would be stopped in a short time.

#### 4-2 Clogging of Filter

Increasing of pressure-drop or decreasing of flow-rate is caused by clogging of filter.

In this case, take out the gel from the column, clean the fitting and repack the gel into the column.

#### 4-3 Adsorption of Protein

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

#### 4-4 Packing Method

Pack the gel to a column by pressure-packing method.

Packing of column by suction method can not be recommended for the column more than 10 cm long.



# **TOSOH CORPORATION**

**BIOSCIENCE DIVISION** 

Shiba-Koen First Bldg. 3-8-2 Shiba, Minato-ku, Tokyo 105-8623, Japan Phone: +81-3-5427-5180 Fax: +81-3-5427-5220 Web site: http://www.separations.asia.tosohbioscience.com/ HPLC database: www2.tosoh.co.jp/hlc/hlcdb.nsf/StartE?OpenForm

# **TOSOH BIOSCIENCE LLC**

3604 Horizon Drive Suite 100, King of Prussia, PA 19406, USA Phone: +1-800-366-4875 Fax: +1-610-272-3028 E-mail: info.tbl@tosoh.com Web site: http://www.tosohbioscience.com/

# TOSOH BIOSCIENCE GmbH

Zettachring 6, 70567 Stuttgart, Germany Phone: +49-711-132570 Fax: +49-711-1325789 E-mail: info.tbg@tosoh.com Web site: http://www.tosohbioscience.com/

## TOSOH BIOSCIENCE SHANGHAI CO., LTD.

Room 301, Plaza B, No.1289 Yi Shan Road, Xu Hui District, Shanghai 200233, China Phone: +86-21-3461-0856 Fax: +86-21-3461-0858 E-mail: info@tosoh.com.cn Web site: http://www.separations.asia.tosohbioscience.com/

# TOSOH ASIA PTE. LTD.

63 Market Street #10-03 Singapore 048942 Phone: +65-6226-5106 Fax: +65-6226-5215 E-mail: info.tsas@tosoh.com Web site: http://www.separations.asia.tosohbioscience.com/

HLC, TSK-GEL, TSKgel, TSKgel SuperMultipore, BioAssist, Enantio, PStQuick, Enviropak, TOYOPEARL, ToyoScreen, TOYOPEARL GigaCap, TOYOPEARL MegaCap, TOYOPEARLPAK and TOYOPAK are registered trademarks of TOSOH CORPORATION in Japan and other countries.

This manual may not be reprinted or copied in whole or in part without written consent of TOSOH CORPORATION. The contents of the manual are subject to change without notice.

Printed in Japan