

**TOYOPEARL®**  
**Affinity Type**  
**TOYOPEARL AF-Heparin-650M**

# **INSTRUCTION MANUAL**





**TOSOH CORPORATION**

## 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
 <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.**

When using with flammable solvents, it can cause 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 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. Packing to Column	1
2.1. Preparation of Gel Slurry	1
2.2. Packing	2
3. Procedure for Chromatography	3
3.1. Column Washing	3
3.2. Equilibration	3
3.3. Elution	3
3.4. Regeneration	3
4. Storage	4

## 1. Introduction

TOYOPEARL AF-Heparin-650M is a packing material for group specific Affinity Chromatography. This material is prepared by introducing porcine intestinal mucosa heparin into TOYOPEARL HW-65. This material can be applied to the separation of blood coagulation factors, complement, DNA or RNA polymerase, lipoprotein etc.

## 2. Packing to Column

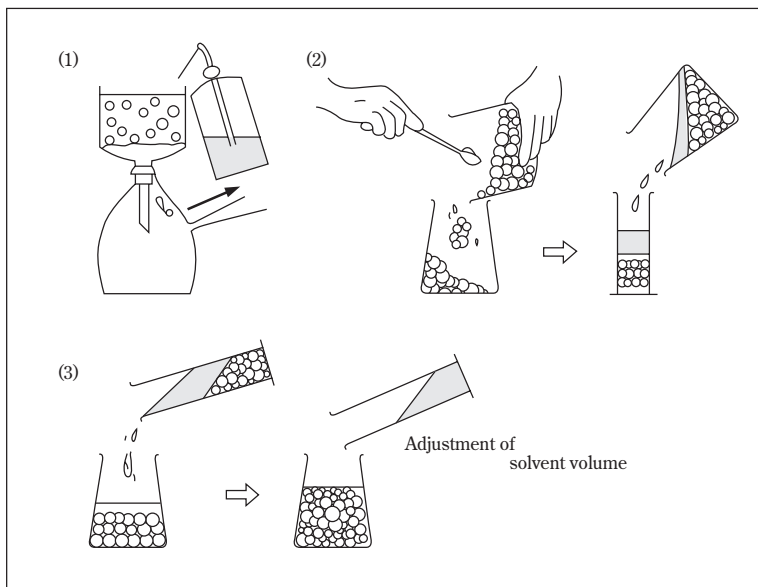
### 2-1. Preparation of Gel Slurry

Remove small particles by decantation.

Pour the gel slurry containing 1.2 times column volume of gel into a glass filter.

Wash the gel 3-5 times with water to remove ethanol in the slurry.

Transfer the gel into a beaker and add the packing solvent (usually, final elution buffer to be used) so as to make ca. 30~40% (volume) concentration.



How to prepare gel slurry

## 2-2. Packing

Select packing method according to your situation.

Any conventional packing method can be applied.

Besides the gravitational packing, the packing method using a pump can be applied, giving better result.

Note that TOYOPEARL AF-Heparin-650M is pressure-durable up to 0.5~0.6MPa. The column of the best performance can usually be obtained under the packing pressure of 0.05~0.2MPa.

**Optimum Packing Velocities on Constant Velocity Packing Method**

Column Sizes mm(ID) × cm(L)	Packing Velocities		Suitable Velocities* (ml / h · cm <sup>2</sup> )
	(ml / min)	(ml / h · cm <sup>2</sup> )	
10 × 5	5 - 12	400 - 800	30 - 130
22 × 10	55 - 65	800 - 1000	30 - 130

\* Suitable velocities for chromatographic separation

### **3. Procedure for Chromatography**

#### **3-1. Column Washing**

Wash column with the solution containing 1mol/L NaCl.

#### **3-2. Equilibration**

Equilibrate column with the initial buffer at pH of between 5 and 10.

#### **3-3. Elution**

Adsorbed protein can be eluted with gradient of salt like NaCl up to 2mol/L in general. Protein can be also eluted with the buffer containing 2g/L of heparin.

#### **3-4. Regeneration**

TOYOPEARL AF-Heparin-650M can be regenerated with the solution containing 3mol/L NaCl in general use. The solution containing 6mol/L urea or guanidine HCl is applicable to remove tightly bound protein to the packing materials.

#### 4. Storage

Store TOYOPEARL AF-Heparin-650M with 20% aqueous ethanol at refrigerate (2~8°C).



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## **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](http://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](mailto: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](mailto: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](mailto: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](mailto:info.tsas@tosoh.com)

Web site: <http://www.separations.asia.tosohbioscience.com/>

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