

**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

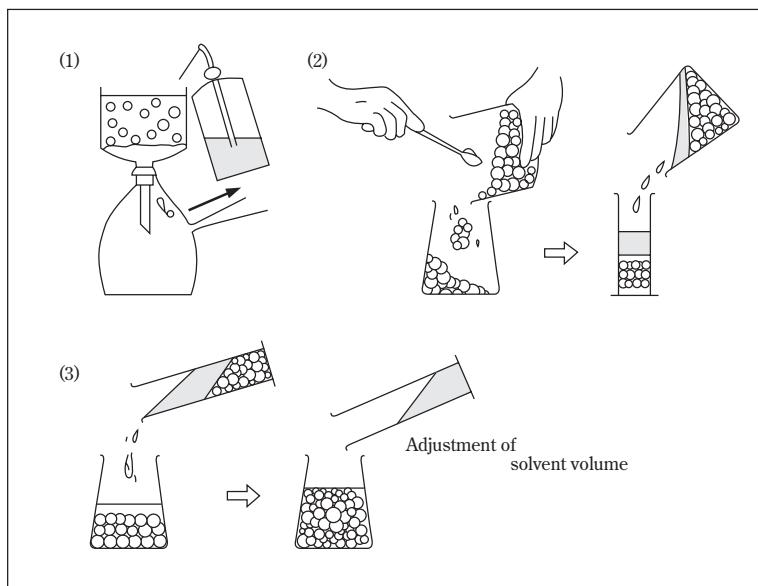
### 2-1. Preparation of Gel Slurry

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 (ml / min) (ml / h · cm <sup>3</sup> )	Suitable Velocities* (ml / h · cm <sup>3</sup> )
10 × 5	5 - 12	400 - 800
22 × 10	55 - 65	800 - 1000

\* 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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