

**TOYOPEARL®**  
**Affinty Type**  
**TOYOPEARL AF-Carboxy-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. Coupling Procedure of Ligand	1
2-1. Preparation of Gel	1
2-2. Ligand Solution	1
2-3. Coupling	1
2-4. Blocking	1
2-5. Storage	1
3. Packing to Column	2
3-1. Preparation of Gel Slurry	2
3-2. Packing	3
4. Storage	3

## 1. Introduction

TOYOPEARL AF-Carboxy-650M is the activated packing material for Affinity Chromatography. This material is prepared by introducing carboxyl groups into TOYOPEARL HW-65. Carboxyl-activated materials can immobilize ligand with amino groups.

## 2. Coupling Procedure of Ligand

### 2-1. Preparation of Gel

Wash gel with distilled water or 0.5mol/L NaCl at pH of between 4.5 and 6.0 on glass filter, and prepare suction dried gel.

### 2-2. Ligand Solution

Buffer with amino, carboxyl and phosphate groups cannot be applied for coupling. Hence the distilled water at pH adjusted between 4.5 and 6.0 is applied in general.

### 2-3. Coupling

Mix ligand solution with suction dried gel. Then, add EDC (N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride, 30g/L gel) and shake the mixture for 24 h at 25°C. Do not stir the mixture by stirrer, otherwise the gel will be broken.

After coupling, wash the gel to remove unreacted ligand with 0.5 or 1.0mol/L NaCl solution.

### 2-4. Blocking

Block carboxyl groups remaining on the gel with 0.5mol/L ethanolamine (5g/L gel) and EDC (30g/L gel) for 5 h at 25°C.

### 2-5. Storage

Gel with unstable ligand like protein or enzyme should be stored with neutral pH buffer containing 0.02% sodium azide at 4°C.

### 3. Packing to Column

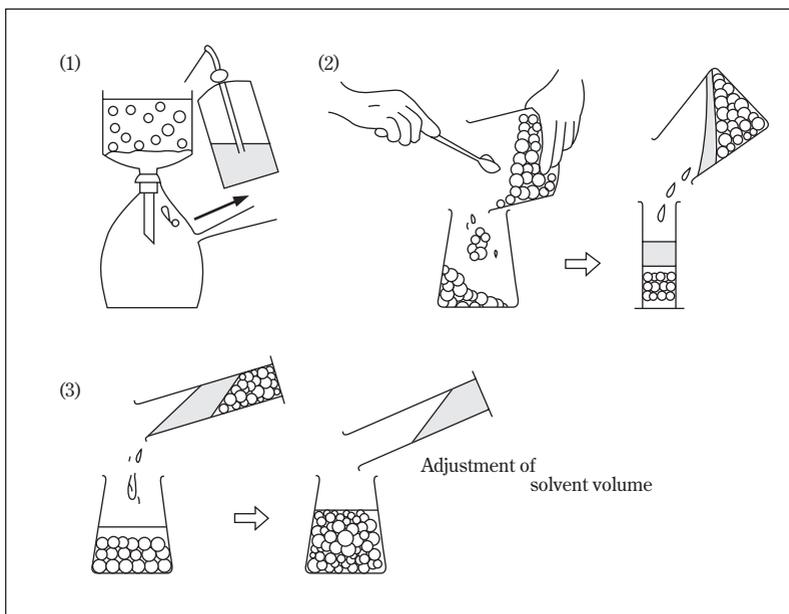
#### 3-1. Preparation of Gel Slurry

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) gel concentration.



How to prepare gel slurry

### 3-2. 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-Carboxy-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

### 4. Storage

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



**TOSOH**

---

## **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/>

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