

**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.**

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## 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

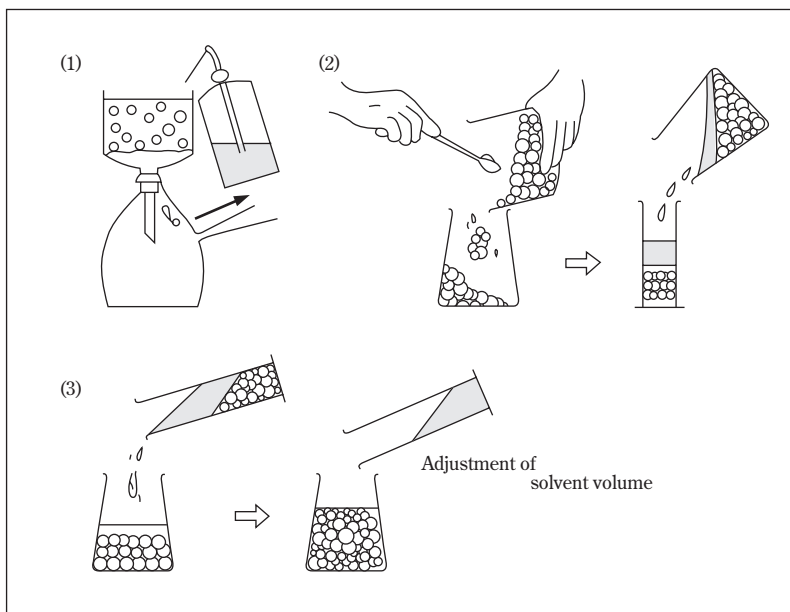
#### 3-1. Preparation of Gel Slurry

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).



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