

**TOYOPEARL®**  
**Affinity Type**  
**TOYOPEARL AF-Formyl-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-Formyl-650M is the activated packing material for Affinity Chromatography. This material is prepared by introducing formyl group into TOYOPEARL HW-65. Formyl-activated materials can immobilize ligand with amino groups.

## 2. Coupling Procedure of Ligand

### 2-1. Preparation of Gel

This product contain 1% glutaraldehyde in the packing solution.

Wash gel with distilled water and coupling buffer on glass filter and prepare suction-dried gel.

### 2-2. Ligand Solution

It is necessary for coupling to use the buffer at neutral pH without amino groups.

Example : 0.1mol/L phosphate buffer (pH7~8)

0.1mol/L NaHCO<sub>3</sub> (pH8~9)

Optimum volume of ligand solution is between 2 and 4ml per ml gel.

For coupling of protein, adequate concentration of protein is between 10 and 20mg per ml gel.

### 2-3. Coupling

Mix ligand solution with suction-dried gel. Then, add sodium cyanoborohydride ( $\text{NaCNBH}_3$ , 30g/L gel) and shake the mixture for overnight 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 the buffer containing 0.5 or 1.0mol/L NaCl.

Note that  $\text{NaCNBH}_3$  contains poisonous cyanic ion.

Washing should be achieved with a draft equipment. Cyanic ion in the washing should be decomposed with sodium hypochlorite in alkaline ( $>\text{pH}12$ ) condition.

Then, the washing solvent should be treated as waste fluids.

### 2-4. Blocking

Block formyl groups remaining on the gel with 4ml of 1.0mol/L Tris-HCl buffer (pH7.8) and 7mg of  $\text{NaCNBH}_3$  per ml gel for 1h at 25°C.

Washing should be achieved as described above.

### 2-5. Storage

The 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

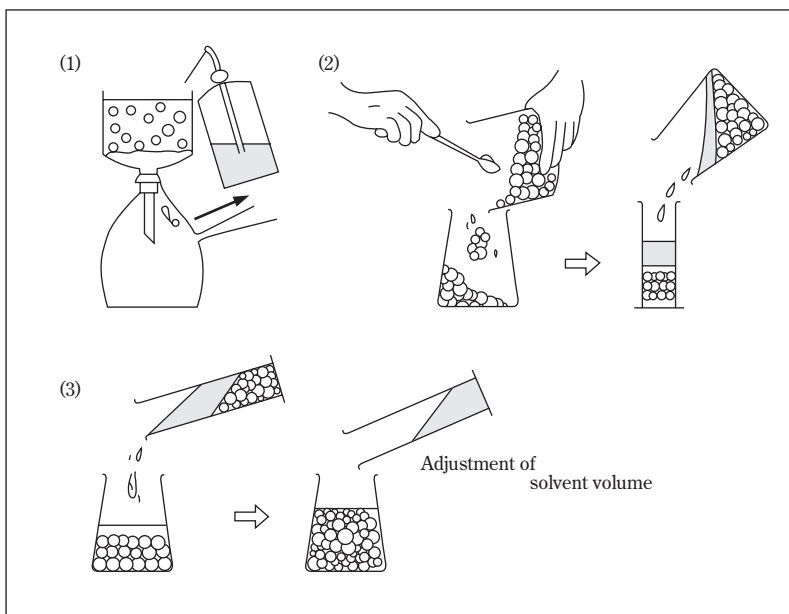
#### 3-1. Preparation of Gel Slurry

Remove small particles by decantation.

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

Wash the gel 3-5 times with water to remove a preservative.

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 pump can be applied, giving better result.

Note that TOYOPEARL AF-Formyl-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

Store the unused gel of TOYOPEARL AF-Formyl-650M with 1% glutaraldehyde at refrigerate (2~8°C).



**TOSOH**

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