



TOSOH

Instruction Manual



Toyopearl® AF-Formyl-650M

TOSOH BIOSCIENCE

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
 WARNING	Alerts the user to the potential for serious injury or death.
 CAUTION	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 gloves.**

Organic solvents or acid are harmful when in contact with the skin.

- **Handle package with care.**

Inappropriate handling may cause rupture and spattering.

- **Do not use for unintended purposes.**

This product is for separation and purification, do not use for any other purpose.

- **When packing the columns, monitor pressure.**

Overpressure may cause rupture and spattering. Wear suitable protective equipment while packing.

- **Monitor the safety of the compounds and solution after separation and purification.**

- **Dispose of in an appropriate manner.**

Make sure that all local state and federal regulations are followed when disposing of this product.

NOTE

- **Keep this manual with the product**

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1. Introduction

Toyopearl AF-Formyl-650M is an activated packing material for affinity chromatography. This material is prepared by introducing formyl groups onto Toyopearl HW-65. Formyl-activated material can immobilize ligands with free amino groups.

2. Ligand Coupling Procedure

2-1. Coupling of Ligand with Amino Groups

(1) Gel Preparation

Wash the gel with distilled water or coupling buffer on a sintered glass filter. Suction dry the gel.

(2) Ligand Solution

For efficient coupling the ligand solution must be at a neutral pH. Tosoh recommends 0.1 mol/L phosphate buffer at a pH = 7-8 or 0.1 mol/L NaHCO_3 at pH = 8-9. The optimum volume of ligand solution is between 2 and 4 mL per mL of gel. For efficient coupling of proteins the amount of protein should be between 10 and 20 mg/mL-gel.

(3) Coupling Reaction

Mix the ligand solution with the suction dried gel. Add sodium cyanoborohydride (NaCNBH_3 , 30 mg/mL gel) and shake the mixture overnight at 25°C. Do not stir the mixture with a magnetic stirrer to prevent resin breakage. After coupling, wash the gel to remove unreacted ligand with buffer containing 0.5 to 1.0 mol/L NaCl. NOTE: NaCNBH_3 contains cyanide ion and is poisonous. Washing should be done in the hood with proper waste disposal. Cyanide ion in the washing can be deactivated with sodium hypochlorite in alkaline condition. The waste fluid can then be treated normally.

(4) Blocking

Block the unreacted formyl groups remaining on the gel with 4 mL of 1.0 mol/L Tris-HCl buffer (pH = 7.8) and 7 mg of NaCNBH_3 per mL of gel for 1 h at 25°C. Wash as described in step 2.3.

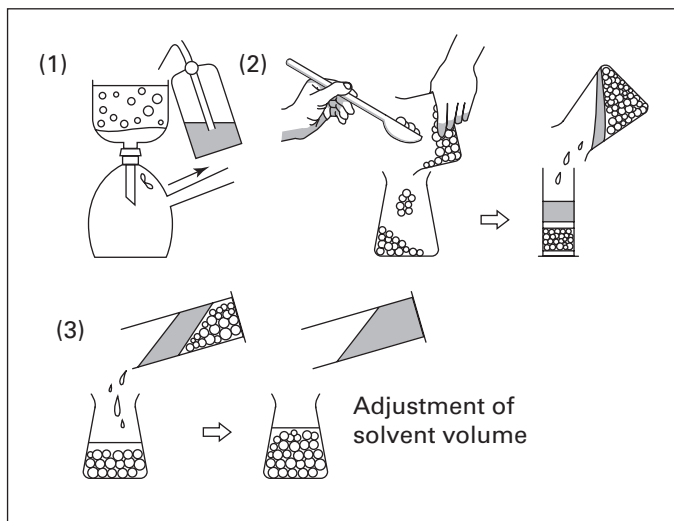
2-2. Storage

Gel immobilized with unstable ligands such as proteins or other enzymes should be stored in neutral pH buffer containing 0.02% sodium azide at 4°C.

3. Column Packing

3-1. Gel Slurry Preparation

Decant to remove any broken resin in the gel slurry. Take about 1.2 column volumes of the gel and place in a sintered glass filter. Wash the gel 3-5 times with water (preferable hot water) to remove any trace amounts of sodium azide or ethanol. Transfer the gel into a beaker and add the packing solvent (generally the final elution buffer) to make a 30-40% gel slurry.



How to prepare gel slurry

3-2. Packing

Select the packing method according to the situation under consideration. Any conventional packing method can be applied. Packing the column with a pump is recommended however, gravity packing has also been used successfully. NOTE: Toyopearl AF-Formyl-650 can be run up to 3bar. Past experience suggests that the column should be packed with a pump to a pressure of 0.5-2.0bar for best results.

4. Storage

Store the activated gel in 1% glutaraldehyde solution at 4°C.

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