TOSOH

Instruction Manual

Toyopearl® AF-Epoxy-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

Table of Contents

1. Introduction

Toyopearl AF-Epoxy-650M is an activated resin for Affinity Chromatography. This resin is prepared by introducing epoxy groups onto Toyopearl HW-65. Epoxy-activated resin can immobilize ligands with amino groups, thiol groups or hydroxy groups.

2. Care and Handling

1) Resin
   The resin is shipped in dry form and when hydrated swells about 3.5 times its size in water.
2) Density of Epoxy Functionality
   The density of epoxy functionality is about 800μmol/g-dry gel.
3) Stability
   Unused resin in the dry form is stable.
   If the resin is hydrated and swollen in water, please store at low temperature (4°C) and use within one week. The immobilized resin is very stable. It can be used with all water soluble organic solvents and it is stable between pH 2-12.

3. Coupling Procedure of Ligand

3-1. Coupling of Glycine (-NH_2)

1) Preparation of Gel
   Wash the hydrated and swollen gel with pure water and prepare 5g of suction dried gel.
2) Ligand Solution
   Add 1.5g of glycine to 2mol/L NaOH aq. solution and adjust pH to 11.
   Make the final volume approximately 10mL of ligand solution.
3) Coupling
   Mix the ligand solution and the suction dried gel. Shake the reaction for 8h at 45 °C. To remove the excess ligand solution at the end of the reaction, wash the gel several times with water, followed by 1mol/L NaCl aq. solution and finally water again.
4) Blocking
   To block the glycine groups remaining on the gel, place the gel in 1mol/L ethanolamine and shake overnight.
   Using the above procedure, about 1μmol/L of glycine will be attached to 1mL of gel.

3-2. Coupling of Glutathione (–SH)

1) Preparation of Gel
   Wash the hydrated and swollen gel with pure water and prepare 0.5g of suction dried gel. Wash the gel with 0.1mol/L phosphate buffer and then suspend the gel in 4mL of the above buffer.
2) Ligand Solution
   Dissolve 100μg of glutathione in a small amount of pure water, adjust the pH of the solution to 7 with KOH and make the final volume of the ligand solution about 1mL.
3) Coupling
   Mix the ligand solution and the gel by shaking the solution for 24h at 37°C. To remove excess ligand, wash the gel with water, with 1mol/L NaCl aq. solution and finally pure water, again.
4) Blocking
   To block glutathione groups remaining on the gel, put the gel in 1mol/L ethanolamine and set aside overnight. Using the above procedure, about 200μmol/L of glutathione will be attached to 1g of suction dried gel.

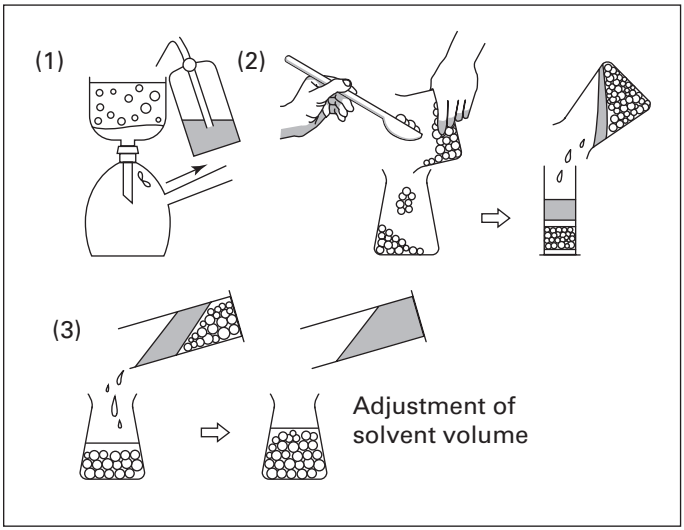
3-3. Coupling of β-Cyclodextrin (-OH)

1) Preparation of Gel
   Wash the gel with water and prepare 1.0g of suction dried gel.
2) Ligand Solution
   Dissolve 150mg of β-cyclodextrin in 3mL of 0.1mol/L NaOH aq. solution.
3) Coupling
   Mix the ligand solution and the gel and shake the solution for 16h at 45°C. To remove excess of ligand, wash the gel with water at 45°C, then with 1mol/L NaCl aq. solution at 45°C and finally with water at 45°C again.
4) Blocking
   To block the glutathione groups remaining on the gel, place the gel in 1mol/L ethanolamine and shake overnight. Using the above procedure, about 2.5μmol/L of β-cyclodextrin will be attached to 1g of dry gel.

4. Column Packing

4-1. Preparation of Gel Slurry

Remove small particles by decantation as follows:
Stirr gel in the water for 1- 2 minutes and decantate after settling for at least 30 minutes. Repeat this process 3 times. Transfer the gel into a beaker and add the packing buffer (usually, the final elution buffer to be used) to make a 30-40% (volume) gel slurry.



How to prepare gel slurry

4-2. Packing

Select an appropriate packing method according to your particular requirements. Any conventional packing method can be used such as gravitational packing, however, packing with a pump gives best results. Use a packing method that generates a pressure between 0.5 and 3 bar.

Optimum Packing Velocities for a Constant Velocity Packing Method

Column Sizes (mm I.D. x cm)	Packing Velocity (mL/min)	(cm/hr)	Recommended Operating Velocity* (cm/hr)
10 x 5	5 -12	400-800	30 - 130
22 x 10	55 - 65	800-1000	30 - 130

*Recommended velocity for best chromatographic resolution.

5. Storage

The gel should be stored in 20% aqueous ethanol at a temperature between 4-35°C.

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