Rev. BU012001

TOYOPEARL®
AFC Type

TOYOPEARL AF-Epoxy-650M

INSTRUCTION MANUAL

TOSOH

TOSOH CORPORATION

Safety Precautions

To help protect you and/or your property from potential damage and ensure personal safety, please read this manual thoroughly before using the product.

[Notational Conventions]

Notation	Explanation
⚠ **WARNING**	Indicates a hazard with a medium level of risk which, if not avoided, could result in death or serious injury.
⚠ **CAUTION**	Indicates a hazard with a low level of risk which, if not avoided, could result in minor or moderate injury.

⚠ WARNING

■ **Keep away from fire**

Not taking proper precautions when using flammable solvents could result in 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 shock, poisoning, injury, or corrosion.
Wear appropriate protective gear when cleaning up a spill.

■ **Wear protective eye gear and gloves**

Organic solvents and acids should not come into direct contact with the skin.

■ **Handle the package with care**

Inappropriate handling may cause rupturing and/or splattering of the product.

■ **Only use this product for its intended use**

This product is intended for the separation and purification of small molecules and proteins. Do not use it for any other purpose.

■ **Make sure compounds are safe**

Check that the target compounds and solutions after separation and purification are safe.

■ **Proper disposal**

Dispose in accordance with local laws and regulations.

NOTE

Keep this manual with the product for future reference.

Precautions: TOYOPEARL® Brand Chromatographic Media

First Aid	Inhalation	・Move the person to an area with fresh air and rinse the mouth with plenty of water. ・Call immediately for medical attention.
	Skin exposure	・Wash the exposed area with plenty of soap and water.
	Eye exposure	・Open the eyes as wide as possible and rinse with clean water for at least 15 minutes. ・Call immediately for medical attention.
	Ingestion	・Rinse the mouth with plenty of water. ・Call immediately for medical attention.
Handling and Storage	Ventilation	・Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	・Container may break if not handled with care.
	Wear appropriate protective equipment	・Use solvent-resistant gloves and protective eye gear when using this product. Use of a gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	・If any flammable solvents are used for shipping or storage of this product, keep away from fire or open heat sources.
	Fire precautions	・Do not expose this chromatographic resin to fire or open heat sources.
Waste Disposal	Disposal methods	・Dispose in accordance with local laws and regulations.
	General considerations	・Please pay attention to all safety precautions with respect to the handling and storage of this product.
	Disposal precaution	・This product can be safely incinerated. ・Fumes produced during incineration may contain carbon oxides.

□ TOYOPEARL® products contain combustible chromatographic packings based on a methacrylate polymer.

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Revised in December, 2010

1. Introduction

TOYOPEARL AF-Epoxy-650M is the activated resin for Affinity Chromatography. This resin is prepared by introducing epoxy groups into TOYOPEARL HW-65. Epoxy-activated resin can immobilize ligand with amino groups, thiol groups and hydroxyl groups.

2. Care for Handling

1) Resin

The resin is shipped in dry form which swells about 3.5 times in water.

2) Density of Epoxy Functionality

Density of epoxy functionality is about 800 μmol/g-dry gel.

3) Stability

Unused resin in dry form is stable.

In case the resin is swelled in water, please store it at low temperature (4℃) and use it within a week.

The immobilized resin is very stable, so it can be used with all water-soluble organic solvents and it is stable in the pH range 2-12.

3. Coupling Procedure of Ligand

3-1 Coupling of Glycine ($-NH_2$)

1) Preparation of Gel

Wash gel with pure water and prepare 5 g of suction dried gel.

2) Ligand Solution

Add 1.5 g of glycine to 2 mol/L NaOH aqueous solution and adjust pH to 11 and make about 10 mL of solution.

3) Coupling

Mix ligand solution and suction dried gel.

Shake the solution for 8 hours at 45℃.

To expel the excess of ligand, wash the gel well with pure water, then with 1 mol/L NaCl aqueous solution and with pure water again.

4) Blocking

To block epoxy groups remaining on the gel, put the gel in 1 mol/L ethanolamine and shake them for overnight.

By the above procedure, glycine will be attached to 1 mL of gel about 100 μmol.

3-2 Coupling of Glutathione (-SH)

1) Preparation of Gel

Wash gel with pure water and prepare 0.5 g of suction dried gel.

Wash the gel with 0.1 mol/L phosphate buffer then suspend the gel in 4 mL of the above buffer.

2) Ligand Solution

Solve 100 μg of glutathione in small amount of pure water, then adjust pH of the solution to 7 with KOH and make 1 mL of solution.

3) Coupling

Mix ligand solution and the gel.

Shake the solution for 24 hours at 37℃.

To expel the excess of ligand, wash the gel well with pure water, then with 1 mol/L NaCl aqueous solution and with pure water again.

4) Blocking

To block epoxy groups remaining on the gel, put the gel in 1 mol/L ethanolamine and leave them for overnight.

By the above procedure, glutathione will be attached to 1 g of suction dried gel about 200 μmol.

3-3 Coupling of β-Cyclodextrin (-OH)

1) Preparation of Gel

Wash gel with pure water and prepare 1.0 g of suction dried gel.

2) Ligand Solution

Solve 150 mg of β-cyclodextrin in 3 mL of 0.1 mol/L NaOH aqueous solution.

3) Coupling

Mix ligand solution and the gel.

Shake the solution for 16 hours at 45℃.

To expel the excess of ligand, wash the gel well with pure water at 45℃ then with 1 mol/L NaCl aqueous solution at 45℃ and with pure water at 45℃ again.

4) Blocking

To block epoxy groups remaining on the gel, put the gel in 1 mol/L ethanolamine and shake them for overnight.

By the above procedure, about 2.5 μmol of β-cyclodextrin will be attached to 1 g of dry gel.

4. Packing to Column

4-1 Preparation of Gel Slurry

Remove small particles by decantation.

Decantation is done as follows.

Agitate gel in the water for 1-2minutes, then decantate it after leaving for 30 minutes. Repeat this process for 3 times.

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.

4-2 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.

The column of the best performance can usually be obtained under the packing pressure of 0.05 MPa-0.2 MPa.

Optimum Packing Velocities on Constant Velocity Packing Method

Column Sizes mm(I.D.)×cm(L)	Packing Velocities (mL/min)	(mL/h·cm^2)	Suitable Velocities* (mL/h·cm^2)
10×5	5-12	400-800	30-130
22×10	55-65	800-1000	30-130

*Suitable velocities for chromatographic separation

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Printed in Japan

T1101-200