



TOSOH

Instruction Manual



Toyopearl® AF-Carboxy-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-Carboxy-650M is an activated packing material for Affinity Chromatography. This material is prepared by introducing carboxyl groups onto Toyopearl HW-65 resin. Carboxyl-activated materials can immobilize ligands with amino groups.

2. Ligand Coupling Procedure

2-1. Gel Preparation

Wash the gel with distilled water or 0.5mol/L NaCl at a pH between 4.5 and 6.0 on a sintered glass filter to prepare suction dried gel.

2-2. Ligand Solution

Buffers with amino, carboxyl and phosphate groups cannot be used during the the coupling reaction, hence, use distilled water at a pH adjusted between 4.5 and 6.0.

2-3. Ligand Solution

Mix the ligand solution with the suction dried gel and add EDC (N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride, 30g/L gel) and shake the mixture for 24h at 25°C. Do not stir the mixture using a stirrer or the gel may break causing fines to be generated. After the coupling reaction is complete, wash the gel with 0.5 or 1.0mol/L NaCl solution to remove unreacted ligand.

2-4. Blocking

Block unreacted carboxyl groups remaining on the gel with 0.5mol/L ethanolamine (5g/L gel) and EDC (30g/L gel) for 5h at 25°C.

2-5. Storage

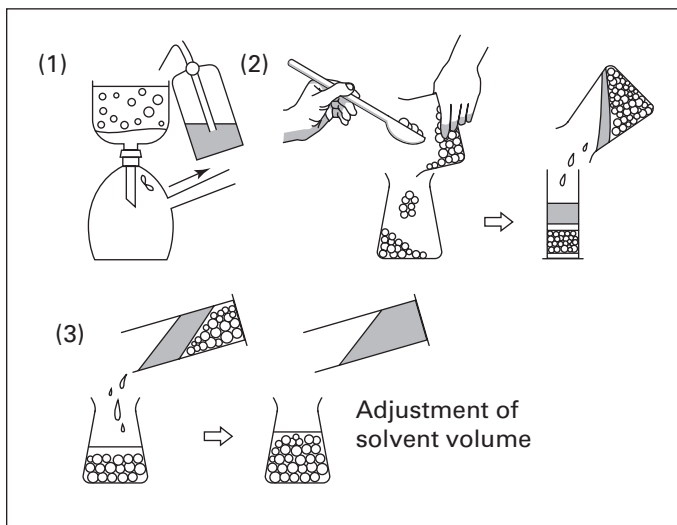
Gel with unstable ligands like proteins or enzymes should be stored in a neutral pH buffer containing 0.02% sodium azide at 4°C.

3. Column Packing

3-1. Preparation of Gel Slurry

Remove small particles by decantation as follows:

Pour the gel slurry containing approximately 1.2 times the expected column volume of gel into a sintered glass filter. Wash the gel 3-5 times with water to remove any residual buffers in the slurry. Transfer the gel into a beaker and add the packing solvent (usually the final elution buffer to be used) to make a 30-40% (volume) gel slurry.



How to prepare gel slurry

3-2. Column 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)	Packing Velocity (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.

4. Storage

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

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