TOSOH

Instruction Manual

Toyopearl® AF-Amino-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-Amino-650M is the activated packing material for affinity chromatography. This material is prepared by introducing amino groups onto Toyopearl HW-65. Amino-activated material can immobilize ligands with carboxyl or formyl goups.

2. Ligand Coupling Procedure

2-1. Coupling of Ligand with Carboxyl Groups

(1) Gel Preparation
Wash the gel with distilled water and then 0.5mol/L NaCl at a pH between 4.5 and 6.0 using a sintered glass filter. Suction dry the gel.

(2) Ligand Solution
Buffers with amino, carboxyl or phosphate groups can not be used in this coupling reaction. In general, distilled water at a pH between 4.5 and 6.0 is recommended.

(3) Coupling Reaction
Mix the ligand solution with the suction dried gel. Add EDC (N-ethyl-N'-(3-dimethyl amino propyl) carbodiimide hydrochloride, 30mg/mL gel) and shake the mixture for 24h at 25°C. Do not stir the mixture with a magnetic stirrer to prevent resin breakage. After coupling, wash the gel with 0.5 to 1.0mol/L NaCl to remove unreacted ligand.

(4) Blocking
- Block the remaining amino groups on the gel with 0.2mol/L sodium acetate (0.8mL/mL-gel) and acetic anhydride (0.4mL/mL-gel).
- Shake the mixture for 30min at 0°C.
- Add acetic anhydride (0.4mL/mL-gel) and shake the mixture for 30min at 25°C.
- Wash the gel sequentially with water, 0.1N NaOH and then water again.

2-2. Coupling of ligands with formyl group

(1) Gel Preparation
Wash the gel with distilled water and then coupling buffer on a sintered glass filter. Suction dry the gel.

(2) Ligand Solution
For best coupling, the buffer solution should not contain any amino groups. Buffers that can be used include 0.1mol/L phosphate buffer (pH 7-8) or 0.1mol/L $NaHCO_3$ (pH 8-9). The optimum volume of ligand solution is between 2 and 4mL per mL of gel.

(3) Coupling Reaction
Mix the ligand solution with the suction dried gel. Add sodium cyanoborohydride ($NaCNBH_3$, 100-200mg/mL gel) and shake the mixture overnight at 60°C. Do not stir the mixture with a magnetic stirrer to prevent resin breakage. After coupling, wash the gel with buffer containing 0.5 or 1.0mol/L NaCl. to remove unreacted ligand

NOTE: $NaCNBH_3$ contains cyanide ion and is poisonous. Washing should be done in a hood and waste should be disposed according to local regulations. Cyanide ion in the washing can be deactivated with sodium hypochlorite in alkaline solution. The waste fluid can then be treated normally.

(4) Blocking

Blocking is the same procedure as described above for carboxyl groups 2.1(4).

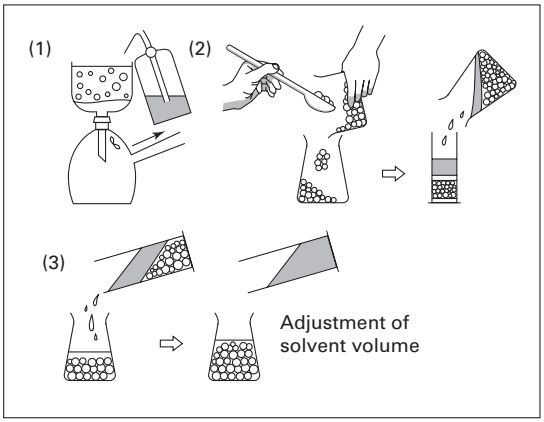
2-3. Storage

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

3. Column Packing

3-1. Gel Slurry Preparation

Define 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 (preferably with warm 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 an appropriate packing method. 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-Amino-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 gel in distilled water containing 0.02% sodium azide at 4°C.

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