TOSOH

Instruction Manual

TOYOPEARL® AF-Chelate-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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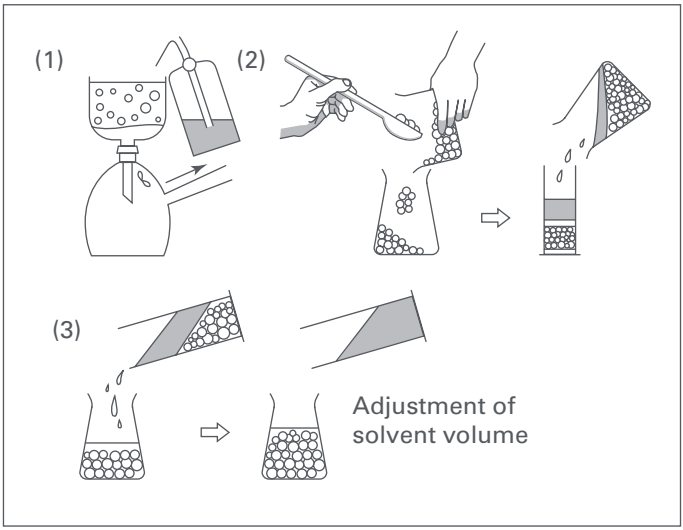
1. INTRODUCTION

TOYOPEARL AF-Chelate-650M is a metal chelating packing material for affinity chromatography. This material is prepared by introducing iminodiacetic acid onto TOYOPEARL HW-65. TOYOPEARL AF-Chelate-650M, after priming with metal ions, is useful for the affinity purification of proteins containing histidine, cysteine and tryptophan residues. This resin is particularly useful for the purification of plasma proteins and other enzymes.

2. COLUMN PACKING

2-1. 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

2-2. Packing
Select the packing method according to your particular situation. Any conventional packing method can be applied. Packing the column with a pump is recommended, however, gravity packing has also been used successfully.

3. CHROMATOGRAPHIC PROCEDURE

3-1. Standard Procedure

An aqueous metal ion solution (40 μmol/L) is used to saturate the chelating resin. After washing with initial buffer, apply a protein sample onto the column. The proteins are eluted by decreasing the pH or by increasing the salt concentration with glycine, imidazole, etc. Wash the column with a 50 μmol/L EDTA solution to remove metal ions. To run again, re-load the column with a fresh solution of aqueous metal ions.

3-2. Metal Ion Selection

Copper (Cu^{2+}) or Zinc (Zn^{2+}) are generally used. Copper ions show strong interaction with proteins. Zinc ions show a weak interaction with proteins. Nickel (Ni^{2+}) and Cobalt (Co^{2+}) are also used in metal chelate affinity chromatography although less frequently. Adsorption of proteins is dependent not only on the type of ion but also the amount of chelated metal ions on the column.

3-3. Equilibrium

The column is equilibrated with initial buffer after chelating the metal ions to the resin. Phosphate buffer is efficient since adsorption of proteins normally occurs at a pH between 7 and 9. Buffers containing amino groups like Tris-HCl can sometimes inhibit the adsorption of proteins. Chelating reagents like EDTA, EGTA or citric acid should not be used in the loading buffer but salt solutions (0.5 - 1.0 mol/L) should be included to suppress any ionic interaction during chromatography between the packing materials and the proteins.

3-4. Protein Elution

There are several choices for eluting proteins. The most common way is by decreasing the pH of the buffer.

1. pH Gradient

Proteins can be eluted by a gradient of pH from 7 to 3 using the starting buffer.

2. Competing Agent

Proteins can be eluted by a gradient of salt:

Salt	Gradient
Glycine	0 - 0.2 mol/L
Imidazole	0 - 0.02 mol/L
Histidine	0 - 0.2 mol/L
Ammonium Chloride	0 - 0.5 mol/L

Elution of proteins with an imidazole buffer does not desorb the metal ions. Thus, chromatography can be repeated several times without having to regenerate the column.

3. Chelating Reagent

Chelating reagents like EDTA and EGTA can desorb tightly bound proteins. However, the proteins and the metal ions will co-elute using these type of reagents.

4. Regeneration

After chromatography, chelated metal ions on the column are removed by washing the column with a solution containing 50 mmol/L EDTA and 0.5 mol/L qNaCl. The column must be regenerated with the aqueous 40 µmol/L metal ion solution.

3-5. Durability

TOYOPEARL AF-Chelate-650M is stable in 0.5 mol/L NaOH or HCl for at least 10 days.

4. STORAGE

Store TOYOPEARL AF-Chelate-650M with aqueous 0.5 mol/L NaCl containing 0.02% sodium azide at room temperature.

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