TOYOPEARL®
Affinity Type
TOYOPEARL AF-Chelate-650M

INSTRUCTION MANUAL

TOSOH

TOSOH CORPORATION

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 globes.**
Organic solvents or acid is harmful in contact with skin.

■ **Handle package with care.**
Inappropriate handling may cause rupture and spattering.

■ **Do not use for unintended use.**
This product is for separation and purification, do not use for any other purpose.

■ **When packing the columns, keep appropriate pressure.**
Overpressure may cause rupture and spattering. Wear suitable protective equipments while packing.

■ **Make sure of the safety of the obtained compound and solution after separation and purification.**

■ **Dispose of in an authorised way.**
Dispose of in the conventional procedures in compliance with local, state and federal regulations.

NOTE

■ **Keep this manual with the product.**

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1. Introduction

TOYOPEARL AF-Chelate-650M is a packing material for Metal Chelate Affinity Chromatography(MCAC). This material is prepared by introducing iminodiacetic acid into TOYOPEARL HW-65.

Metal ion chelated to this medium interacts with histidine, cystein and tryptophane residues on proteins.

TOYOPEARL AF-Chelate-650M is applicable for separation of plasma proteins and other enzymes.

2. Packing to Column

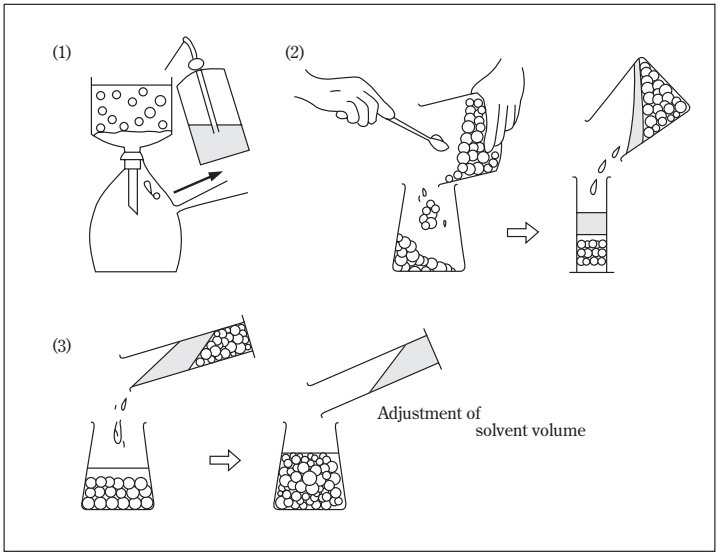
2-1. Preparation of Gel Slurry

Remove small particles by decantation.

Pour the gel slurry containing 1.2times column volume gel into a glass filter.

Wash the gel 3-5 times with water to remove ethanol in the slurry.

Transfer the gel into a beaker and add the packing solvent (usually, the final elution buffer to be used) so as to make ca. 30～40% (volume) gel concentration.



How to prepare gel slurry

2-2. 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.
Note that TOYOPEARL AF-Chelate-650M is pressure-durable up to 0.5～0.6MPa. The column of the best performance can usually be obtained under the packing pressure of 0.05～0.2MPa.

Optimum Packing Velocities on Constant Velocity Packing Method

Column Sizes mm(ID) × cm(L)	Packing Velocities (ml／min)	(ml／h・cm²)	Suitable Velocities* (ml／h・cm²)
10 × 5	5 — 12	400 — 800	30 — 130
22 × 10	55 — 65	800 — 1000	30 — 130

* Suitable velocities for chromatographic separation

3. Procedure for Chromatography

3-1. Standard Procedure

First of all, load metal ion disolved in water by passing ca 40 μmol/L solution through column, and saturate the column with metal ion.
After washing with initial buffer, apply a sample to the column.
Elute the sample by decreasing pH or increasing salt concentration (glycine, imidazole etc.). Finally, wash the column with 50mmol/L EDTA solution to remove metal ion. Then, load the metal ion again.

3-2. Selection of Metal Ion

Copper(Cu^{2+})or zinc(Zn^{2+})ion is generally utilized. Copper ion shows strong interaction with proteins. On the other hand, zinc ion shows weak interaction with proteins. Adsorption of protein is dependent on not only the type of ion but also the amount of chelated metal ion in the column.
Nickle(Ni^{2+}) and cobalt(Co^{2+}) are also applicable for MCAC.

3-3. Equilibration

Column is equilibrated with initial buffer after chelating of metal ion.
Phosphate buffer or Tris buffer is efficient since adsorption of protein occurs at pH of between 7 and 9. Buffer containing amino groups like Tris buffer inhibits the adsorption of proteins occasionally.
Chelating reagent like EDTA, EGTA or citric acid should not be contained in the buffer. Buffer should be contained 0.5-1.0mol/L salt to supress ionic interaction between packing materials and proteins during chromatography.

3-4. Elution of Proteins

There are several elution methods. The elution by decreasing pH is standard.

(1) pH gradient

Protein can be eluted by gradient of pH from 7 to 3 in the eluent.

(2) Antagonist

Protein can be eluted by the gradient of salt in eluent as follows:

Salt	Gradient
glycine	0 - 0.2mol/L
imidazole	0 - 20mmol/L
histidine	0 - 0.2mol/L
ammonium chloride	0 - 0.2mol/L

Since elution of protein with imidazole does not desorb metal ion, chromatography can be repeated several times without regeneration.

(3) Chelating reagent

Chelating reagent like EDTA and EGTA can desorb tightly bound proteins. Proteins, however, can not be separated and are eluted a one peak since chelating reagents also remove metal ion from the column.

3-5. Regeneration

After chromatography, chelated metal ion on column is once removed by washing the column with the solution containing 50mmol/L EDTA.
Then, the column is regenerated with ca. 40 μmol/L metal ion solution.

3-6. Durability

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

4. Storage

Store TOYOPEARL AF-Chelate-650M with 20% aqueous ethanol at ambient(4～35℃)

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