TOYOPEARL®
Affinity Type
TOYOPEARL AF-Blue-650ML
TOYOPEARL AF-Red-650ML

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-Blue-650ML and TOYOPEARL AF-Red-650ML are the media for Dye-ligand Affinity Chromatography. TOYOPEARL AF-Blue-650ML are prepared by introducing Ci F3GA into TOYOPEARL HW-65. TOYOPEARL AF-Red-650ML is prepared by introducing Reactive Red-120 into HW-65.

TOYOPEARL AF-Blue-650ML and TOYOPEARL AF-Red-650ML have higher resolution in the separation of nucleotide-dependent enzymes or blood components.

2. Packing to Column

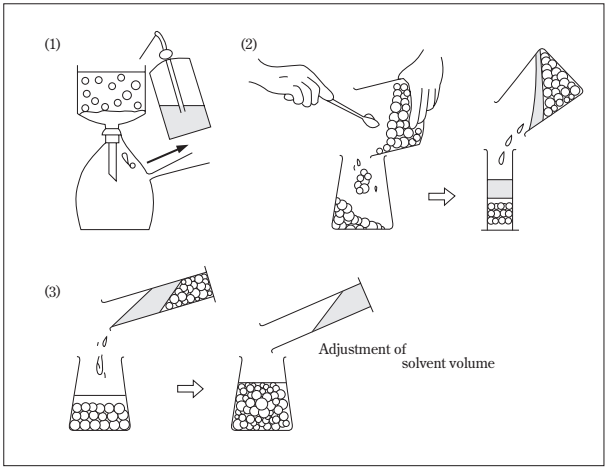
2-1. Preparation of Gel Slurry

Remove small particles by decantation.

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

Wash the gel 3-5 times with water to remove ethanol and 1mol/L sodium chloride.

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) concentration.



How to prepare gel slurry

2-2. Packing

Select the 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-Blue-650ML and TOYOPEARL AF-Red-650ML are 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 Methed

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. Cleaning for the first use

Wash and clean colum with 1mol/L NaCl or KCl solution even for the first use. Then, equilibrate with initial buffer.
Note that dye coupled to media leaks gradually by hydrolysis during storage. Therefore, cleaning of column is necessary before using the column.

3-2. Equilibration

Equilibrate the column with ca. 5 times column volume of buffer (pH 6-8, ionic strength less than 0.05mol/L without salt.

3-3. Sample Preparation

Sample should be prepared with the initial buffer. When the sample solution contains salt, the sample should be dialysed or diluted with the initial buffer.
In the case the sample cannot be adsorbed to the column, the following treatment might be effective :

1. Decrease the flow rate.
2. Decrease pH value of the initial buffer.
3. Add metal ion to the initial buffer (e.g. 10mmol/L $MgCl_2$) .
4. Add EDTA or mercaptoethanol to the initial buffer.

3-4. Elution

After sample preparation, wash the column with ca. 5 times column volume of the initial buffer and remove unbound components.

There are two elution methods on affinity chromatography, i.g. non-specific and specific elution. Table 1. shows the characteristics of the two methods.

Table 1 Characteristics on elution method

Elution method		Purification	Recovery	Economy
Non-specific	step gradient	good	good	cheap
	linear gradint	excellent	good	cheap
Specific	step gradient	excellent	excellent	expensive

In non-specific elution, the method by salt gradient in buffer are applied in general. Most proteins can be eluted with the buffer containing 2mol/L KCl or 3mol/L NaCl.

For tightly bound proteins or other components, the solvents shown in Table 2. are useful.

In specific elution, coenzyme like NADH or NADPH, and substrate for enzymes can be applied. Most proteins can be eluted with the buffer containing coenzyme up to 10mmol/L.

Table 2 Solvents useful for elution

2 mol/L	Potassium chloride
3 mol/L	Sodium chloride
4 mol/L	Urea
4.2 mol/L	Ammonium sulfate (saturated)
1 mol/L	Sodium thiocyanate
0.1 mol/L	Sodium hydroxide
1 %	Triton X-100
75 %	Ethylene glycol
50—50 %	Chloroform-methanol

3-5. Cleaning and Regeneration

Wash the column with 2mol/L KCl or 3mol/L NaCl solution to remove all adsorbed components. Tightly bound lipids or other components can be eluted with 4mol/L urea solution.

4. Storage

The gel should be stored with 20% aqueous ethanol containing 1mol/L NaCl (KCl) at refrigerate (2～8℃).

5. Application

5-1. Choice of Media

Steric structure of blue and red dye resembles to that of NAD+ and NADP+. Enzymes, therefore, show a little different affinity between blue and red dye media.

It is important to choose a better medium for the separation of enzyme with regard to capacity, purification and recovery.

5-2. Negative Affinity Chromatography

In negative affinity chromatography, not aimed protein, but contaminants are adsorbed to column. For example, purification of small amount of blood component is interfered by albumin which is majored in serum. Hence, it is effective to remove albumin from serum in the first step of the purification.

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