TOYOPEARL®
Affinity Type
TOYOPEARL AF-Blue HC-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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November, 2001

1. Introduction

TOYOPEARL AF-Blue HC-650M is the media for dye-ligand affinity chromatography. It has been prepared by introducing Cibacron Blue 3GA into TOYOPEARL HW-65 and it can be applied to the separation and/or purification of NADP-dependent enzymes(e.g.dehydrogenases, reductases, kinases) or blood compounds(e.g. Serum albumin, blood coagulation factors).

TOYOPEARL AF-Blue HC-650M has a binding capacity of more than 18mg/ml-gel human serum albumin and a very low level of ligand leakage.

This resin is available in small quantities as well as in bulk amounts for large scale purifications of proteins.

2. Column Packing Procedure

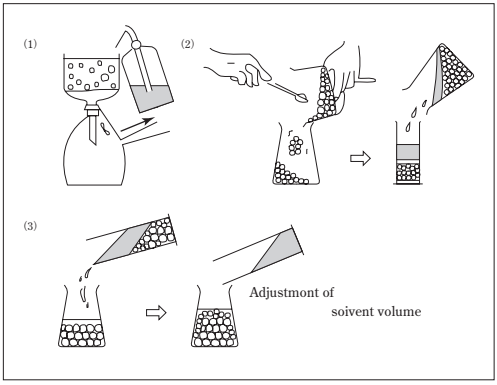
2-1. Preparation of Gel Slurry

Remove smaIl particles by decantation.

Pour the gel Slurry containing l.2 times the column volume of gel into a glass filter.

Wash the gel with 4 times the column volume of water 3-5 times 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. 30-50%(volume) concentration.



How to prepare gel slurry

2-2. Packing

Select a packing procedure.(flow constant, pressure constant, gravitational packing)

Any packing procedure can be applied.

The best results can be obtained by using a pump.

Note that TOYOPEARL AF-Blue HC-650M is pressure-stable up to sub-MPa. The optimal performance can usually be obtained with a packing pressure of 0.05-0.2MPa.

Examples of Optimum Packing Velocities on Constant Velocity Packing Method

Column Sizes (mmI.D. x cm)	Packing Velocity (ml/min)	Packing Velocity ($ml/h \cdot cm^2$)	Suitable Velocity* ($ml/h \cdot cm^2$)
10 x 5	5-12	400- 800	30-130
22 x 10	55-65	800-1000	30-130

*Recommendable velocity for obtaining better resolutions

3. Procedure for Chromatography

3-1. Cleaning

Wash the column with 1 mol/L NaCl or KC1 solution even for the first use. Then, equilibrate with the initial buffer.

3-2. Equilibration

Equilibrate the column with 3 to 5 times the column volume of buffer (pH6-8, ionic strength not more than 0.1 mol/L) without salt.

3-3. Sample Preparation

The sample should be prepared with the initial buffer. When the sample solution contains salt, the sample should be dialyzed or diluted with the initial buffer.

In the case the sample cannot be adsorbed on the column, the following treatment might be effective:

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

3-4. Elution

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

There are two elution methods in affinity chromatography,i.d. non-specific and specific elution. Table1 shows the characteristics of the two methods.

Table1 Characteristics on elution method

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

ln the non-specific elution procedure, the protein is usually eluted by using high salt concentrations(2 mol/L HCl or 3 mol/L NaCl) or a pH-step or after compounds. For highly bound proteins, add one of the solvents listed in Table2.

In the specific elution procedure, coenzymes like NADH or NADPH, and substrates for enzymes can be applied. Most proteins can be eluted with the buffer containing coenzymes up to 10m mol/L.

Table2 Elution solvents

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

Note: Albumin can be adsorbed with 0.1 mol/L phosphate buffer (pH7.0) and be eluted with the same buffer containing 3 mol/L NaCl. 4 mol/L urea or 0.3 mol/L sodium thiocyanate solution is also useful for the elution of albumin.

3-5. Cleaning and Regeneration

Wash the column with 2 mol/L KCl or 3 mo1/L NaCl solution to remove all

adsorbed components. Tightly bound lipids or other components can be eluted with 4 mol/L urea solution.

4. Storage

The gel Should be stored at +2 to +8℃ in 20% aqueous ethanol containing 1 mol/L NaCl(KCl).

5. Application

5-1. Negative Affinity Chromatography

In negative affinity chromatography, not aimed protein, but contaminants are adsorbed on the column. For example, the 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. Albumin can be easily removed from by the treatment with TOYOPEARL AF-Blue HC-650M(e.g. only albumin can be adsorbed to the medium with 0.1 mol/L, pH7.0 phosphate buffer containing 0.5mol/L NaCl)

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