TOSOH

Instruction Manual

Toyopearl® AF-Red-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

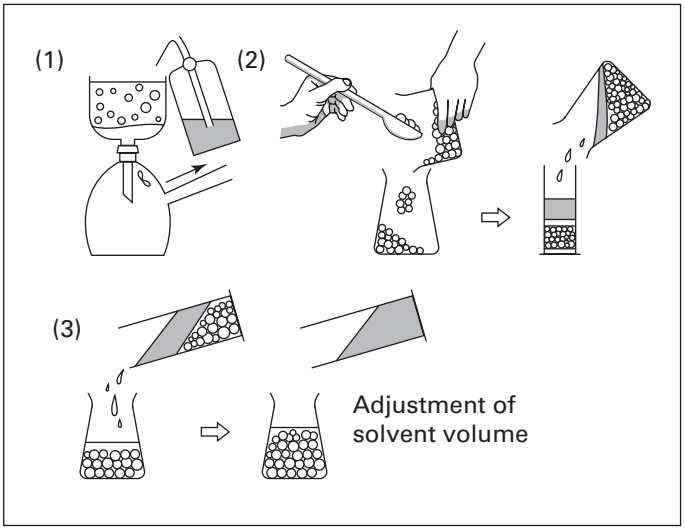
1. Introduction

Toyopearl AF-Red-650M is a media for dye-ligand Affinity Chromatography. Toyopearl AF-Red-650M is prepared by introducing Reactive Red 120 onto HW-65. It requires no further chemical modifications. Toyopearl AF-Red-650M is ideal for the chromatographic separation of blood components.

2. Column Packing

2-1. Preparation of Gel Slurry

Remove small particles by decantation:
Pour the gel slurry containing approximately 1.2 times the expected column volume of gel into a sintered 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) to make a 30-40% (volume) gel slurry.



How to prepare gel slurry

2-2. Packing

Select an appropriate packing method according to your particular requirements. Any conventional packing method can be applied including gravitational packing, however, using a pump for packing gives best results. Note that Toyopearl AF-Red-650M is best packed and run at pressures between 0.5 – 3.0 bar.

Optimum Packing Velocities for a Constant Velocity Packing Method

Column Sizes (mm I.D. x cm)	Packing Velocity (mL/min)	(cm/hr)	Recommended Operating Velocity* (cm/hr)
10 x 5	5 -12	400-800	30 - 130
22 x 10	55 - 65	800-1000	30 - 130

*Recommended velocity for best chromatographic resolution.

3. Chromatographic Procedure

3-1. Cleaning prior to first use

Wash and clean the packed column with 1mol/L NaCl or KCl solution prior to the first use. Then, equilibrate with binding buffer. Note that the dye coupled to the media leaks minimally by hydrolysis during storage. Therefore, cleaning the resin prior to first use is necessary before using the column.

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

3-2. Sample Preparation

Sample should be prepared using the binding buffer. When the sample solution contains salt, the sample should be dialyzed or diluted with the binding buffer. If the sample cannot be adsorbed onto the column, the following treatment might be effective:

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

3-3. Elution

After the sample is adsorbed onto the column wash with approximately 5 column volumes of binding buffer to remove any unbound components. There are two elution methods typically used in affinity chromatography, i.e. non-specific and specific elution. Table 1 shows the characteristics of the two methods.

Table 1 Elution Method Characteristics

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

In non-specific elution, a salt gradient in the binding buffer is used as the elution buffer. Most proteins can be eluted with a binding buffer containing 2mol/L KCl or 3mol/L NaCl. For tightly bound proteins or other impurities, the elution solvents shown in Table 2 may be useful.

In specific elution, cofactors or coenzymes such as NADH or NADPH, or enzyme substrates may be used. Most proteins can be eluted with the buffer containing up to 10mmol/L of the desired cofactor.

Table 2 Typical Elution solvents

2mol/L	potassium chloride
3mol/L	sodium chloride
4mol/L	urea
4.2mol/L	ammonium sulfate (saturated)
1mol/L	sodium thiocyanate
0.1mol/L	sodium hydroxide
1%	Triton X-100
75%	ethylene glycol
50-50%(v/v)	chloroform-methanol

3-4. 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 impurities can be eluted with a 4mol/L urea solution.

4. Storage

The gel should be stored in 20% aqueous ethanol containing 1mol/L NaCl (or KCl) at 2-8°C.

5. Application

5-1. Choice of Media

The conformational structure of both blue and red dye-affinity ligand chromatography resins resemble that of NAD^+ and $NADP^+$. Enzymes will show a different affinity between the blue and red dye media and, therefore, it is important to select the best media for the separation of the target enzyme by taking into account the resin's ability to meet capacity, purity, and recovery requirements.

5-2. Negative Affinity Chromatography

In negative affinity chromatography, only impurities are adsorbed onto the column. For example, the purification of trace amounts of a blood component may be difficult due to the interference of albumin which is a major protein component in serum. Hence, one effective technique is to remove albumin from serum in the first step of the purification using Toyopearl AF-RED 650M prior to isolation of the desired protein.

TOSOH BIOSCIENCE

IM12

Tosoh Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.separations.
us.tosohbioscience.com
email: info.tbl@tosoh.com