



TOSOH

# **Instruction Manual**



## **Toyopearl® AF-Tresyl-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-Tresyl-650M is a reactive packing material for affinity chromatography. This material is prepared by introducing tresyl groups onto Toyopearl HW-65. Tresyl-activated material can immobilize ligands with free amino or thiol groups very efficiently and in a short amount of time.

## 2. Ligand Coupling Procedure

### 2-1. Gel Swelling

1g of dry Toyopearl AF-Tresyl-650M swells to about 4mL of wet gel.

### 2-2. Ligand solution

For efficient coupling the ligand solution must be at a neutral pH. Tosoh recommends 0.1mol/L phosphate buffer at a pH between 7-8 with 0.5mol/L NaCl or 0.1mol/L NaHCO<sub>3</sub> with 0.5mol/L NaCl at pH between 8-9. For efficient coupling of proteins the amount of protein should be between 10 and 20mg/mL-gel. The optimum volume of ligand solution is approximately 10mL per mL of gel.

### 2-3. Coupling Reaction

Mix the ligand solution with the dried gel and shake the mixture for 4h at 25°C or overnight at 4°C. Do not stir the mixture with a magnetic stirrer to prevent resin breakage. After coupling, wash the gel to remove unreacted ligand with buffer containing 0.5 to 1.0mol/L NaCl.

### 2-4. Blocking

Block the unreacted tresyl groups remaining on the gel with 0.1mol/L Tris-HCl buffer (pH = 8.0) containing 0.5mol/L NaCl for 1h at 25°C or 4h at 4°C. Wash as described in step 2.3.

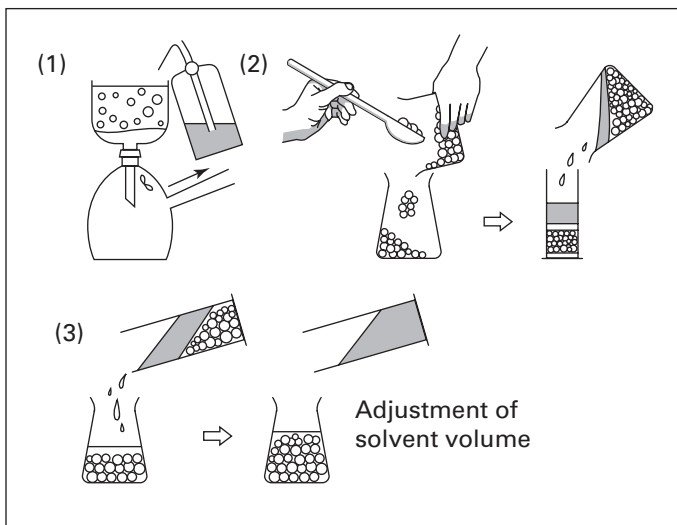
### 2-5. Storage

Gel immobilized with unstable ligands such as proteins or other enzymes should be stored in neutral pH buffer containing 0.02% sodium azide at 4°C.

### 3. Column Packing

#### 3-1. 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

#### 3-2. Packing

Select the packing method according to the situation under consideration. Any conventional packing method can be applied. Packing the column with a pump is recommended however, gravity packing has also been used successfully. NOTE: Toyopearl AF-Tresyl-650 can be run up to 3bar. Past experience suggests that the column should be packed with a pump to a pressure of 0.5-2.0bar for best results.

### 4. Storage

Store the dried-activated gel at 4°C.

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