

TOYOPEARL®
Affinity Type
TOYOPEARL AF-Tresyl-650M

INSTRUCTION MANUAL





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 gloves.**

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.**

TABLE OF CONTENTS

1. Introduction	1
2. Coupling Procedure of Ligand	1
2-1. Swelling of Gel	1
2-2. Ligand Solution	1
2-3. Coupling	2
2-4. Blocking	2
2-5. Storage	2
3. Packing to Column	3
3-1. Preparation of Gel Slurry	3
3-2. Packing	4
4. Storage	4

1. Introduction

TOYOPEARL AF-Tresyl-650M is the activated material for Affinity Chromatography. This material is prepared by introducing tresyl groups into TOYOPEARL HW-65. Tresyl-activated materials can immobilize ligand with amino or thiol groups efficiently in short time.

2. Coupling Procedure of Ligand

2-1. Swelling of Gel

1 g of dried gel swells to ca. 5 mL.

2-2. Ligand Solution

It is necessary for coupling to use neutral pH buffer without amino groups.

<Examples> A : 1.0mol/L phosphate buffer(pH 7)

B : 1.0mol/L phosphate buffer(pH 7.5-8)

C : 0.5mol/L phosphate buffer(pH 7)

D : 0.1mol/L carbonate buffer(pH 8-9)

containing 0.5molar NaCl

A : First choice

B : Second choice ; when ligand cannot be immobilized effectively

C : Third choice ; when ligand is insoluble with solution A

D : Fourth choice ; when the coupling cannot be achieved effectively

Optimum volume of ligand solution is 10ml per gram dried gel.

For coupling of protein, adequate concentration of protein is between 10 and 20mg per 0.2g dried gel.

2-3. Coupling

Mix ligand solution with dried gel and shake the mixture for 4 h at 25°C or overnight at 4°C. Do not stir the mixture by stirrer, otherwise the gel would be broken. After coupling, wash the gel to remove unreacted ligand with buffer containing 0.5 or 1.0mol/L NaCl.

2-4. Blocking

Block tresyl groups on the gel with 0.1mol/L Tris-HCl buffer (pH 8.0) containing 0.5molar NaCl for 1 h at 25°C or 4 h at 4°C.

2-5. Storage

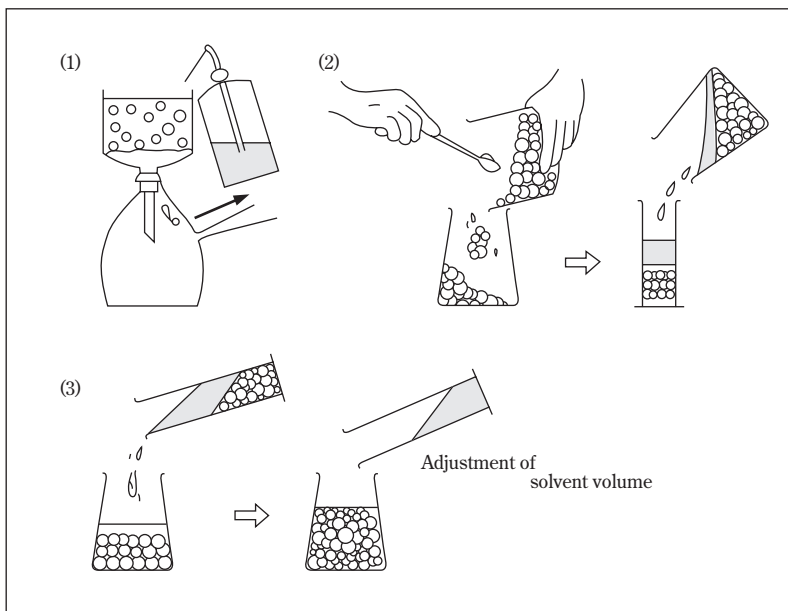
TOYOPEARL AF-Tresyl-650M with unstable ligand like protein or enzyme should be stored with neutral pH buffer containing a preservative at 4°C.

3. Packing to Column

3-1. Preparation of Gel Slurry

Remove small particles by decantation.

- (1) Pour the gel slurry containing 1.2 times column volume of gel into a glass filter.
- (2) Wash the gel 3-5 times with water to remove a preservative.
- (3) Transfer the gel into a beaker and add the packing solvent (usually, final elution buffer to be used) so as to make ca. 30~40% (volume) gel concentration.



How to prepare gel slurry

3-2. Packing

Select packing method according to your situation.

Any conventional packing method can be applied.

Besides the gravitational packing, the packing method using pump can be applied, giving better result.

Note that TOYOPEARL Affinity Type is pressure-durable up to 0.5MPa. The column of the best performance can usually be obtained under the packing pressure of 0.05~0.20MPa.

Optimum Packing Velocities on Constant Velocity Packing Method

Column Sizes mm(ID) × cm(L)	Packing Velocities		Suitable Velocities* (mL / hcm ³)
	(mL / min)	(mL / hcm ³)	
10 × 5	5 – 12	400 – 800	30 – 130
22 × 10	55 – 65	800 – 1000	30 – 130

* Suitable velocities for chromatographic separation

4. Storage

Store dried and activated TOYOPEARL AF-Tresyl-650M at 4°C



TOSOH

TOSOH CORPORATION

SCIENTIFIC INSTRUMENTS DIVISION

• Head Office

3-8-2 Shiba, Minato-ku, Tokyo 105-8623, Japan

Tel: 81-3-5427-5181 Fax: 81-3-5427-5220

• Nanyo Plant

4560 Kaisei-cho, Shinnanyo-shi, Yamaguchi 746-8501, Japan

Tel: 0834-63-9894 Fax: 0834-63-9879