TOYOPEARL®
Affinity Type
TOYOPEARL AF-Amino-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 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.**

TABLE OF CONTENTS

January, 2002

1. Introduction

TOYOPEARL AF-Amino-650M is the activated packing material for Affinity Chromatography. This material is prepared by introducing amino groups into TOYOPEARL HW-65. Amino-activated materials can immobilize ligand with carboxyl or formyl groups.

2. Coupling Procedure of Ligand

2-1. Coupling of Ligand with carboxyl groups

a Preparation of Gel

Wash gel with distilled water and 0.5mol/L NaCl at pH of between 4.5 and 6.0 on glass filter, and prepare suction dried gel.

s Ligand Solution

Buffer with amino, carboxyl or phosphate groups cannot be applied for coupling. Hence the distilled water at pH adjusted between 4.5 and 6.0 is applied in general.

d Coupling

Mix ligand solution with suction dried gel. Then, add EDC (N-ethyl-N'-(3-dimethyl amino propyl) carbodiimide hydrochloride, 30g/L gel) and shake the mixture for 24h at 25℃.

Do not stir the mixture by stirrer, otherwise the gel will be broken.

After coupling, wash the gel to remove unreacted ligand with 0.5 or 1.0mol/L NaCl solution.

f Blocking

* Block amino groups remaining on the gel with 0.2 mol/L sodium acetate (0.8 mL/mL gel) and acetic anhydride (0.4mL/mL gel).
* Shake the mixture for 30 min at 0℃.
* Add acetic anhydride (0.4mL/mL gel) and shake the mixture for 30 min at 25℃.
* Wash the gel with water, then 0.1mol/L NaOH and then water.

2-2. Coupling of Ligand with formyl groups

a Preparation of Gel

Wash gel with distilled water and coupling buffer on glass filter, and prepare suction dried gel.

s Ligand Solution

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

Example：0.1mol/L phosphate buffer (pH 7～8)

0.1mol/L $NaHCO_3$ (pH 8～9)

Optimum volume of ligand solution is between 2 and 4 mL per mL gel.

d Coupling

Mix ligand solution with suction dried gel. Then, add sodium cyanoborohydride ($NaCNBH_3$, 100-200g/L gel) and shake the mixture for overnight at 60℃.

Do not stir the mixture by stirrer, otherwise the gel will be broken.

After coupling, wash the gel to remove unreacted ligand with buffer containing 0.5 or 1.0mol/L NaCl.

Note that $NaCNBH_3$ solution contains cyanic ion and is poisonous.

The washing should be achieved with a draught equipment. Cyanic ion on the washing should be decomposed with sodium hypochlorite in alkaline condition.

Then, the washing should be treated as waste fluid.

f Blocking

Blocking is the same procedure as described in 2-1 f .

2-3. Storage

Gel with unstable ligand like protein or enzyme should be stored with neutral pH buffer containing 0.02% sodium azide at 4℃.

3. Packing to Column

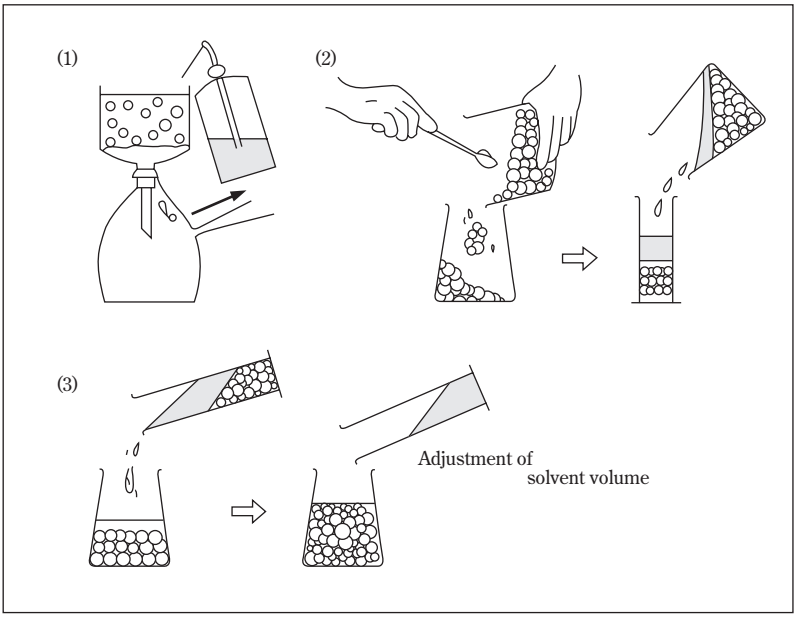
3-1. Preparation of Gel Slurry

Remove small particles by decantation.

(1) Pour the gel slurry containing 1.2 times column volume gel into a glass filter.

(2) Wash the gel 3-5 times with water to remove ethanol.

(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 Methed

Column Sizes mm(ID) × cm(L)	Packing Velocities (mL／min)	(mL／hcm²)	Suitable Velocities* (mL／hcm²)
10 × 5	5 － 12	400 － 800	30 － 130
22 × 10	55 － 65	800 － 1000	30 － 130

* Suitable velocities for chromatographic separation

4. Storage

Store TOYOPEARL AF-Amino-650M with 20% aqueous ethanol at 25℃.

TOSOH CORPORATION

BIOSCIENCE DIVISION

Shiba-Koen First Bldg.
3-8-2 Shiba, Minato-ku, Tokyo 105-8623, Japan
Phone: +81-3-5427-5180 Fax: +81-3-5427-5220
Web site: http://www.separations.asia.tosohbioscience.com/
HPLC database: www2.tosoh.co.jp/hlc/hlcdb.nsf/StartE?OpenForm

TOSOH BIOSCIENCE LLC

3604 Horizon Drive Suite 100,
King of Prussia, PA 19406, USA
Phone: +1-800-366-4875 Fax: +1-610-272-3028
E-mail: info.tbl@tosoh.com
Web site: http://www.tosohbioscience.com/

TOSOH BIOSCIENCE GmbH

Zettachring 6, 70567 Stuttgart, Germany
Phone: +49-711-132570 Fax: +49-711-1325789
E-mail: info.tbg@tosoh.com
Web site: http://www.tosohbioscience.com/

TOSOH BIOSCIENCE SHANGHAI CO., LTD.

Room 301, Plaza B, No.1289 Yi Shan Road,
Xu Hui District, Shanghai 200233, China
Phone: +86-21-3461-0856 Fax: +86-21-3461-0858
E-mail: info@tosoh.com.cn
Web site: http://www.separations.asia.tosohbioscience.com/

TOSOH ASIA PTE. LTD.

63 Market Street #10-03 Singapore 048942
Phone: +65-6226-5106 Fax: +65-6226-5215
E-mail: info.tsas@tosoh.com
Web site: http://www.separations.asia.tosohbioscience.com/

Printed in Japan