TOYOPEARL®
HIC Type

650 Series	TOYOPEARL® Ether-650S, M
	TOYOPEARL® Phenyl-650S, M, C
	TOYOPEARL® Butyl-650S, M, C
	TOYOPEARL® Hexyl-650C
600 Series	TOYOPEARL® PPG-600M
	TOYOPEARL® Phenyl-600M
	TOYOPEARL® Butyl-600M
550 Series	TOYOPEARL® SuperButyl-550C

INSTRUCTION MANUAL

TOSOH CORPORATION

Safety Precautions

To help protect you and/or your property from potential damage, please read this manual thoroughly before using the product.

Notation Conventions

Notation	Explanation
⚠WARNING	Indicates a potentially hazardous situation which could result in death or serious injury.
⚠CAUTION	Indicates a potentially hazardous situation which could result in injury.

⚠ WARNING

■ **Keep away from fire**

Not taking proper precautions when using flammable solvents could result in 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 shock, poisoning, injury, or corrosion.
Wear appropriate protective gear when cleaning up a spill.

■ **Wear protective eye gear and gloves**

Organic solvents and acids should not come in direct contact with the skin.

■ **Handle the package with care**

Inappropriate handling may cause rupturing and/or splattering of the product.

■ **Only use this product as intended**

This product is for separation and purification. Do not use for any other purpose.

■ **Make sure compounds are safe**

Check that obtained compounds and solutions after separation and purification are safe.

■ **Proper disposal**

Dispose in accordance with local laws and regulations.

NOTE

■ Keep this manual with the product for future reference.

Precautions: Shipping Solvents

TOYOPEARL® HIC type products are shipped in 20 % aqueous ethanol.

First Aid	Inhalation	・Move the person to an area with fresh air. Immediately rinse the mouth with plenty of water. ・Call for medical attention immediately.
	Skin exposure	・Wash exposed area with plenty of soap and water.
	Eye exposure	・Open eyes as wide as possible and rinse with clean water for at least 15 minutes. ・Call for medical attention immediately.
	Ingestion	・Wash the mouth with plenty of water and immediately call for medical attention.
Handling and Storage	Ventilation	・Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	・Container may break if not handled with care.
	Wear appropriate protective equipment	・Use solvent-resistant gloves and protective eye gear when using this product. Use of gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	・If any flammable solvents are used for shipping or storage, keep away from fire and open heat.
Waste Disposal	Disposal methods	・Follow local guidelines for disposal. This product can be incinerated safely.
	General considerations	・Please pay attention to all safety precautions with respect to the handling and storage of this product.

Precautions: TOYOPEARL® Brand Chromatographic Media

First Aid	Inhalation	・Move the person to an area with fresh air. Rinse the mouth with plenty of water immediately. ・Call for medical attention immediately.
	Skin exposure	・Wash the exposed area with plenty of soap and water.
	Eye exposure	・Open eyes as wide as possible and rinse with clean water for at least 15 minutes. ・Immediately call for medical attention.
	Ingestion	・Rinse the mouth with plenty of water and call for medical attention immediately.
Handling and Storage	Ventilation	・Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	・Container may break if not handled with care.
	Wear appropriate protective equipment	・Use solvent-resistant gloves and protective eye gear when using this product. Use of gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	・If any flammable solvents are used for shipping or storage, keep away from fire and open heat.
	Fire precautions	・Do not expose the chromatographic resin to fire or open heat sources.
Waste Disposal	Disposal methods	・This product can be incinerated or buried for easy disposal. See below for additional precautions.
	General considerations	・Please pay attention to all safety precautions with respect to the handling and storage of this product.
	Disposal precaution	・Dispose in accordance with local laws and regulations. This product can be incinerated safely.

□TOYOPEARL® products contain combustible packings based on a methacrylate polymer

Table of Contents

1. Introduction

TOYOPEARL® Ether-650, Phenyl-650, Butyl-650, Hexyl-650, SuperButyl-550, PPG-600, Phenyl-600 and Butyl-600 are the packing materials for Hydrophobic Interaction Chromatography (HIC). These are prepared by introducing the hydrophobic ligand into TOYOPEARL® HW-65 (650 Series, Protein Exclusion Limit 5×10^6), TOYOPEARL® HW-55 (550 Series, Protein Exclusion Limit 7×10^5) or TOYOPEARL® HW Type resin which has medium pore size between HW-65 and HW-55 (600 Series).

These HIC packing materials can adsorb water-soluble proteins at relatively lower concentration of salt in buffer.

<Products Line-up>

650 Series	TOYOPEARL® Ether-650S,M TOYOPEARL® Phenyl-650S,M,C TOYOPEARL® Butyl-650S,M,C TOYOPEARL® Hexyl-650C
600 Series	TOYOPEARL® PPG-600M TOYOPEARL® Phenyl-600M TOYOPEARL® Butyl-600M
550 Series	TOYOPEARL® SuperButyl-550C

* Note (Particle Sizes)
S : Superfine 20-50 μm
M : Medium 40-90 μm
C : Coarse 50-150 μm

2. Procedure for Chromatography

2−1 Removal of Fines

(1) Transfer 500 mL of the HIC packing material into a 3000 mL beaker.

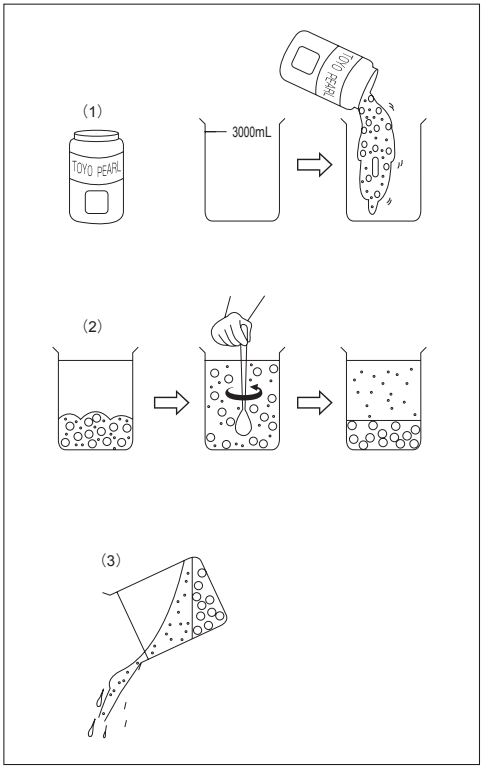
(2) Add distilled water up to 2000 mL, stir and leave it quietly to settle down.

Note The necessary standing times for the HIC packing materials with the different particle sizes are as follows.

- ＊ Coarse Grade : 15−30 min.
- ＊ Medium Grade : 30−45 min.
- ＊ Superfine Grade : 60−90 min.

(3) Discard the supernatant (containing fines) by decantation.

(4) Repeat (2) and (3) process three or more times.



Removal of Fines

2-2 Preparation of Packing Material Slurry and Packing

After removing fines from the packing material by decantation, wash it with packing solvent, then transfer it into a beaker and add the packing solvent so as to make ca. 30-50 % (V/V) slurry.

The packing method under pressure (fifty to a few hundreds kPa) is desirable.

In this case a pump and a reservoir are necessary for the packing.

Usually the flow rate of packing is two times faster than that of operation.

The gravitational packing method is often applied as conventional one.

2-3 Adsorption of Protein

Equilibrate a column with the buffer containing salt like ammonium salfate or sodium chloride.

High concentration of salt in the buffer tends to adsorb proteins by the hydrophobic interaction between the packing materials and the proteins.

2-4 Elution

Protein can be eluted by decreasing salt concentration in buffer.

When protein cannot be eluted by salt gradient method, try to add organic solvent like alcohol or detergent, otherwise to change pH in the final buffer.

2-5 Regeneration

Most proteins can be eluted with distilled water and the column can be used repetitively. However, more hydrophobic protein, lipid and detergent cannot be eluted with distilled water in some cases. In such case, wash the packing materials with dilute alkali or acid solutions (0.1-0.5 mol/L), or with organic solvent like alcohol or acetone.

Washing of the packing materials can be performed in a column or in a beaker.

3. Storage

The HIC packing material should be stored with 20 % aqueous ethanol at ambient (4-35 ℃).

4. Remarks

4−1 Removal of Fines

As described in section 2−1, remove fines before use.

When the removal of fines is not performed completely, microparticles leak from column during chromatography.

4−2 Clogging of Filter

Increasing of pressure-drop or decreasing of flow-rate is caused by clogging of filter.

In this case, take out the packing material from the column, then clean the filter and fitting and repack the packing material into the column.

4−3 Adsorption of Protein

When protein is not adsorbed well to the column with the initial buffer, add salt in the buffer to raise ionic strength. Change of pH in the buffer close to isoelectric point of protein is also effective.

4−4 Packing Method

Packing of column by suction method can not be recommended for the column length more than 10 cm. Because particle size of the packing material is small, so the density of packed material becomes different between the upper-side and lower-side of the column.

In this case the HIC packing materials must be packed to a column by pressure-packing method.

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