

Rev. BU044010

TOYOPEARL
Mixed-mode Type

TOYOPEARL MX-Trp-650M

FOR IN VITRO USE ONLY / FOR PACKED-BED USE ONLY

INSTRUCTION MANUAL





TOSOH

TOSOH CORPORATION

Safety Precautions

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

[Notation Conventions]

Notation	Explanation
 WARNING	Indicates a hazard with a medium level of risk which, if not avoided, could result in death or serious injury.
 CAUTION	Indicates a hazard with a low level of risk which, if not avoided, could result in minor or moderate 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, and corrosion. Wear appropriate protective gear when cleaning up a spill.

■ **Wear protective eye gear and gloves**

Organic solvents and acids should not come into 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 use**

This product is intended for the separation and purification of small molecules and proteins. Do not use it for any other purpose.

■ **Make sure compounds are safe**

Check that the compounds in the feedstock and all 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 MX-Trp-650M is shipped in 20 % aqueous ethanol.

First Aid	Inhalation	<ul style="list-style-type: none">• Move the person to an area with fresh air and rinse the mouth with plenty of water.• Call immediately for medical attention.
	Skin exposure	<ul style="list-style-type: none">• Wash the exposed area with plenty of soap and water.
	Eye exposure	<ul style="list-style-type: none">• Open the eyes as wide as possible and rinse with clean water for at least 15 minutes.• Call immediately for medical attention.
	Ingestion	<ul style="list-style-type: none">• Rinse the mouth with plenty of water.• Call immediately for medical attention.
Handling and Storage	Ventilation	<ul style="list-style-type: none">• Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	<ul style="list-style-type: none">• Container may break if not handled with care.
	Wear appropriate protective equipment	<ul style="list-style-type: none">• Use solvent-resistant gloves and protective eye gear when using this product. Use of a gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	<ul style="list-style-type: none">• If any flammable solvents are used for shipping or storage of this product, keep away from fire or open heat sources.
	Storage temperature	<ul style="list-style-type: none">• Avoid storing this product at very low temperatures ($< 0\text{ }^{\circ}\text{C}$) to prevent product from freezing.
Waste Disposal	Disposal methods	<ul style="list-style-type: none">• Dispose in accordance with local laws and regulations.
	General considerations	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none"> • Move the person to an area with fresh air and rinse the mouth with plenty of water immediately. • Call immediately for medical attention.
	Skin exposure	<ul style="list-style-type: none"> • Wash the exposed area with plenty of soap and water.
	Eye exposure	<ul style="list-style-type: none"> • Open the eyes as wide as possible and rinse with clean water for at least 15 minutes. • Call immediately for medical attention.
	Ingestion	<ul style="list-style-type: none"> • Rinse the mouth with plenty of water. • Call immediately for medical attention.
Handling and Storage	Ventilation	<ul style="list-style-type: none"> • Provide adequate air ventilation to keep organic vapor concentrations below approved level.
	Container handling	<ul style="list-style-type: none"> • Container may break if not handled with care.
	Wear appropriate protective equipment	<ul style="list-style-type: none"> • Use solvent-resistant gloves and protective eye gear when using this product. Use of a gas mask, additional protective clothing or rubber boots could be appropriate when handling this product.
	Hazardous substance storage	<ul style="list-style-type: none"> • If any flammable solvents are used for shipping or storage of this product, keep away from fire or open heat sources.
	Fire precautions	<ul style="list-style-type: none"> • Do not expose the chromatographic resin to fire or open heat sources.
Waste Disposal	Disposal methods	<ul style="list-style-type: none"> • Dispose in accordance with local laws and regulations.
	General considerations	<ul style="list-style-type: none"> • Please pay attention to all safety precautions with respect to the handling and storage of this product.
	Disposal precaution	<ul style="list-style-type: none"> • This product can be safely incinerated. • Appropriate nitrogen oxides exhaust emission precautions should be taken specifically for TOYOPEARL MX-Trp-650M.

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

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1. Introduction

TOYOPEARL Mixed-mode Type resin has been specifically designed for packed-column use, and is based on TOYOPEARL HW-65 (Protein Exclusion Limit 5×10^6 Da), which are porous spherical polymers. TOYOPEARL Mixed-mode Type resin has both ion exchangers and hydrophobic functional groups as mixed-mode ligand. TOYOPEARL resins have the following advantages:

- Volume changes are negligible in buffers with various pH or salt concentrations.
- Able to withstand fast flow rates typically used in column chromatography.
- Resistant to microorganisms.
- Applicable to HPLC system when run at lower pressures.

(TOYOPEARL Mixed-mode Product)

TOYOPEARL MX-Trp-650M Particle sizes : 50 - 100 μ m

2. Procedure for Chromatography

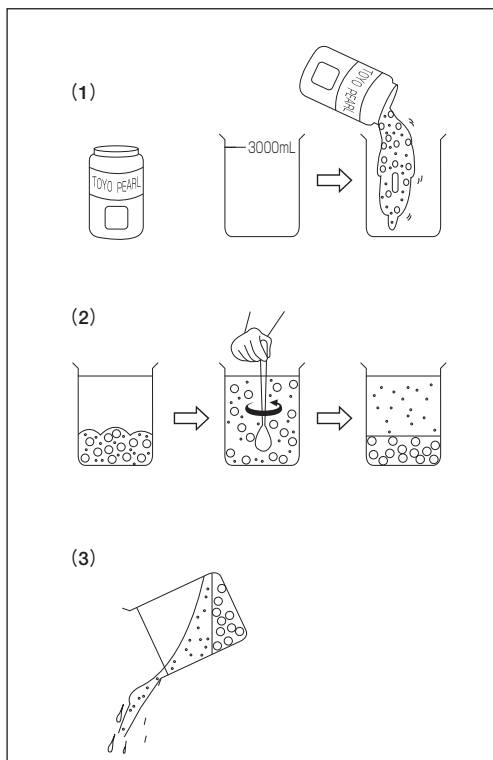
2-1 Removal of Fines

- (1) As an example, pour 500 mL of the gel into a 3000 mL beaker (The capacity should be about six times the gel volume).
- (2) Add 2000 mL of distilled water (four times the gel volume) to the beaker, stir and let the gel settle.

Note: The recommended settling times of the gel with this particle sizes is as follows.

Grade	Settling Time (recommended)
TOYOPEARL MX-Trp-650M	30 - 45 minutes

- (3) Decant and discard the supernatant (containing fines).
- (4) Repeat steps (2) and (3) of this process at least three times.



Removal of Fines

2-2 Cleaning

TOYOPEARL Mixed-mode Type resins are shipped or stored in an aqueous solution containing 20 % ethanol.

The shipping or storage solvent should be removed prior to use.

Pour the gel slurry onto a sintered glass funnel and wash with at least a three-fold excess of distilled water.

2-3 Preparation of Gel Slurry and Packing

After removing fines from the gel by decantation (Section 2-1), wash the gel with packing buffer. The packing buffer should contain the highest salt concentration that the column will be exposed during normal use, cleaning or storage. Transfer the gel into a beaker and add packing buffer to make an approximately 30 %-50 % (V/V) (recommended) slurry.

Packing the column under pressure (0.05 MPa-0.3 MPa) is recommended. In most cases a pump and a reservoir are necessary to pack the column. Usually the packing flow rate is at least two times faster than that of the operating flow rate which will generate higher pressures.

Gravitational packing methods are not recommended.

2-4 Equilibration

After packing, the column should be equilibrated with 3 to 5 column volume of buffer.

2-5 Elution

Adsorbed samples are eluted by increasing the salt concentration up to 1 mol/L or by changing the pH of the buffer.


2-6 Regeneration

The gel can be regenerated using the following procedures.

2-6-1 Batch method

Pour the gel into a beaker containing the cleaning solvent, stir and leave the gel until it settles. Discard the supernatant by decantation.

Repeat this process 2 or 3 times.

 **Caution :** The Extremely Severe Cleaning method that is described below uses an HCl solution. Please note that some proteins may aggregate in acidic solutions.

TOYOPEARL MX-Trp-650M

*** General cleaning**

First wash the gel with 0.5 mol/L - 1.0 mol/L NaCl solution by the procedure mentioned above, and then equilibrate the gel with buffer.

*** Severe cleaning**

Wash the gel with 0.1 mol/L - 0.5 mol/L NaOH followed by washing with 0.1 mol/L - 0.5 mol/L NaCl solution. Equilibrate the gel with buffer.

*** Extremely severe cleaning**

Wash the gel with 0.1 mol/L - 0.5 mol/L NaOH. Wash with water until the pH is mostly neutralized and then 0.1 mol/L - 0.5 mol/L HCl. Finally wash with 0.1

mol/L - 0.5 mol/L NaCl followed by equilibrium with buffer.

2-6-2 Column Method

The gel, packed into a column, can be regenerated easily by using standard chromatography cleaning solvents.

The procedure and the list of solvents for the cleaning are the same as that for the Batch Cleaning Method.

[Advantages of Column Cleaning Method]

- * Simple Handling There is no need to remove the resin from the column in order to clean.
- * Good Reproducibility
- * Quick Cleaning Using a pump to flow the cleaning reagents over the column bed reduces the amount of time necessary to clean the column compared to batch cleaning methods.
- * Effective Cleaning Typically column cleaning requires less solvent than batch cleaning.

3. Storage

The gel should be stored in an aqueous solution containing 20 % ethanol at ambient temperatures (4-35 °C). In addition, the gel should be kept away from oxidizing agent, protected from light and stored in the dark.

4. Remarks

4-1 Removal of Fines

As described in Section 2, fines should be removed prior to use. When fines are not removed completely, there is a possibility that micro-particles may elute from column during normal chromatographic use. However, the level of fines should gradually diminish as the column is used.

4-2 Clogging of Screens and Frits

Increased pressure-drop or decreased flow-rates are typically caused by clogged screens or frits. When this happens, remove the chromatographic resin from the column and clean the fittings, screens or frits. Once the hardware is completely clean, repack the chromatographic resin into the column as described above.

4-3 Adsorption of Protein

When the protein sample does not adsorb onto the resin using the initial buffer conditions, it may be necessary to dialyze or desalt the sample prior to applying to the column.

4-4 Packing Method

TOSOH recommends packing the resin into the column using a flow and pressure method.

Packing the column using suction or using gravity settling is not recommended, particularly for columns more than 10 cm in length.

4-5 notes

The gel should be kept away from oxidizing agent and protected from light.



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