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TOYOPEARL®
Affinity Type

TOYOPEARL® AF-rProtein A-650F
TOYOPEARL® AF-rProtein A HC-650F

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.

[Notational 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, or 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 for its 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 target 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 AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F are shipped in 20 % aqueous ethanol.

First Aid	Inhalation	・Move the person to an area with fresh air and rinse the mouth with plenty of water. ・Call immediately for medical attention.
	Skin exposure	・Wash the exposed area with plenty of soap and water.
	Eye exposure	・Open the eyes as wide as possible and rinse with clean water for at least 15 minutes. ・Call immediately for medical attention.
	Ingestion	・Rinse the mouth with plenty of water. ・Call immediately 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 a 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 of this product, keep away from fire or open heat sources.
	Storage temperature	・Avoid storing this product at very low temperatures (＜ 0 ℃) to prevent product from freezing.
Waste Disposal	Disposal methods	・Dispose in accordance with local laws and regulations.
	General considerations	・Please pay attention to all safety precautions with respect to the handling and storage of this product.

Precautions: TOYOPEARL Chromatographic Media

First Aid	Inhalation	• Move the person to an area with fresh air and rinse the mouth with plenty of water. • Call immediately for medical attention.
	Skin exposure	• Wash the exposed area with plenty of soap and water.
	Eye exposure	• Open the eyes as wide as possible and rinse with clean water for at least 15 minutes. • Call immediately for medical attention.
	Ingestion	• Rinse the mouth with plenty of water. • Call immediately 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 a 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 of this product, keep away from fire or open heat sources.
	Fire precautions	• Do not expose this chromatographic resin to fire or open heat sources.
Waste Disposal	Disposal methods	• Dispose in accordance with local laws and regulations. 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	• This product can be safely incinerated. • Appropriate sulfur and nitrogen oxides exhaust emission precautions should be taken specifically for TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F.

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

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Revised, in June, 2013

1. Introduction

TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F are the affinity resin for purification of human IgG or mouse IgG. consisting of a porous and spherical polymer (particle size: 30 - 60 μm). TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F have the following features.

- The quantity of gel listed on the container represents the volume of gravity settled resin and not the total liquid volume.
- The change of gel volume when packed into a chromatographic column is negligible in buffers at various pH or salt concentrations.
- Applicable to fast flow-rate on column chromatography.
- Resistant for microbial growth.
- Applicable to most HPLC systems.

2. Procedure for Chromatography

2-1 Removal of Fines

(1) Transfer gel into a beaker.

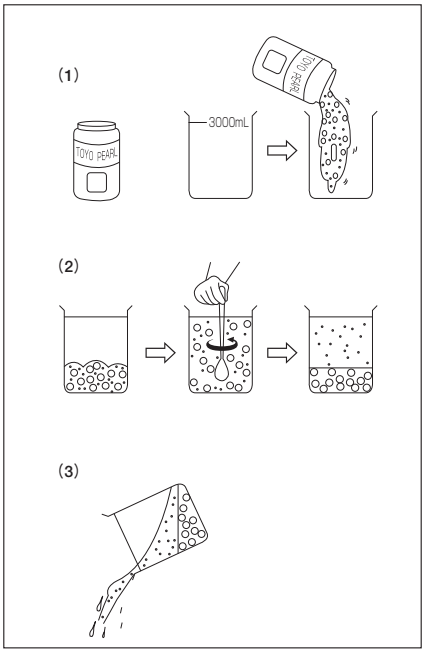
(2) Add four times volume of distilled water to a beaker, stir and leave until the gel settles.

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

Grade	Settling Time (recommended)
TOYOPEARL AF-rProtein A-650F TOYOPEARL AF-rProtein A HC-650F	60 - 90 min.

(3) Decant and discard the supernatant (containing fines).

(4) Repeat this process (2) and (3) at least three times.



Removal of Fines

2-2 Cleaning

TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F are packed with 20 % aqueous ethanol.

The washing of the gel is necessary prior to use.

Pour the gel slurry on a glass filter and wash with distilled water of three times of the gel volume.

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 - 0.3 MPa (recommended)) is recommended.

In this most case 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. Initial packing using a gravity-settled bed can be applied, however, applying pressure from flow rate or dynamic axial compression results in the best packed columns. For this resin, best results are obtained when the packing pressure is as high as possible up to a limit of 0.1 MPa - 0.3 MPa.

2-4 Equilibration and Performance Testing

After packing, the column should be equilibrated with 3 to 5 column volume of buffer. The column should then be tested for packing integrity using a standard performance test.

2-5 Sample Loading and Elution

The sample being purified is typically adsorbed onto the column using a neutral pH buffer. The sample is usually desorbed from the column using with a decreasing pH gradient.

2-6 Regeneration

The chromatographic resin can be regenerated after use by one of the following procedures.

2-6-1 Batch Method

Pour the gel into a beaker and suspend using 3 - 5 times gel volume of an appropriate cleaning solvent. Stir and let the gel settle for approximately 60 - 90 minutes. 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 conditions.

* General cleaning method
  First wash the gel with pH2.5 buffer solution using the procedure mentioned above. Then equilibrate the gel with the loading buffer.
* Severe cleaning method
  Wash the gel with in less than ten minutes with 0.1 mol/L NaOH. Then equilibrate the gel with the loading buffer.
* Extremely severe cleaning method
  Wash the gel with denaturing solution within less than one hour (6 mol/L Urea or 6 mol/L Guanidine-HCl), then wash with in less than ten minutes

with 0.1 mol/L NaOH, followed by regeneration with the loading buffer.
Wash the gel with 10 - 30 vol% ethanol aqueous solution, and then equilibrate the gel with the loading buffer.

2-6-2 Column Cleaning Method

The chromatographic resin in a packed column can be regenerated easily by flowing the cleaning solvents through the column. The solvents for the column cleaning are the same as those used in the Batch Method.

[Advantages of Column Cleaning Method]

- ＊ Simple Handling — Removing the gel from the column and repacking of the chromatographic resin into the column are not necessary.
- ＊ Good Reproducibility — Cleaning times are very consistent and reproducible.
- ＊ Quick Cleaning — By using a pump the cleaning times become shorter than that used by the Batch Method.
- ＊ Effective Cleaning — The gel can be regenerated very well with small amount of solvents compared with the Batch Method.

3. Storage

The gel should be stored in an aqueous solution containing 20 % ethanol at +2 to +8℃.

4. Remarks

4-1 Removal of Fines

As described in Section 2, remove fines before use. When the fines are not removed completely, there is a possibility that micro-particles may leach from column during chromatography. Leaching of the micro-particles, however, should stop after a short period of time.

4-2 Clogging of Filter

Increasing of pressure-drop or decreasing flow-rate is typically caused by filter (frit) clogging.
When this happens, remove the chromatographic resin from the column and clean the fitting and screens. Once the hardware is completely clean, repack the

chromatographic resin into the column as described above.

4-3 Packing Method

Tosoh Corporation recommends packing the resin into the column using a pressure-packing method.

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

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