Rev. BU040000

TSKgel®
HIC Type

TSKgel® Phenyl-5PW (20)
TSKgel® Phenyl-5PW (30)
TSKgel® Ether-5PW (20)
TSKgel® Ether-5PW (30)

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

TSKgel® HIC Type products are shipped in an aqueous solution containing 20 % 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: TSKgel® Brand 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.

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

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1. Introduction

TSKgel® HIC Type is an HIC chromatographic resin consisting of a porous polymer.

<Products Line-up>

Grade	Particle Size
TSKgel® Phenyl-5PW (20) TSKgel® Ether-5PW (20)	15 μm - 25 μm
TSKgel® Phenyl-5PW (30) TSKgel® Ether-5PW (30)	20 μm - 40 μm

2. Procedure for Chromatography

2－1 Removal of Fines

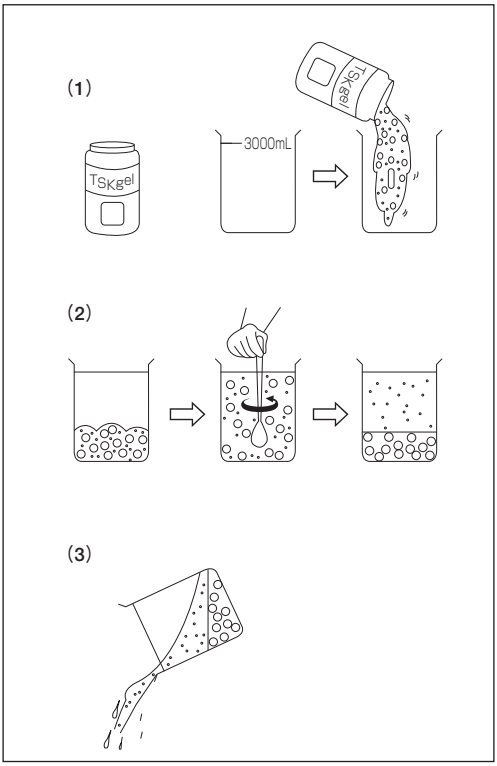
(1) As an example, pour the gel of 500 mL in the beaker of 3000 mL. (The capacity has six times of the gel.)

(2) Add distilled water to a total of 2000 mL (four times of the gel) in the beaker, stir and leave until the gel settles.

Grade	Settling Time (recommended)
TSKgel® Phenyl-5PW (20) TSKgel® Ether-5PW (20)	≥120 minutes
TSKgel® Phenyl-5PW (30) TSKgel® Ether-5PW (30)	90-120 minutes

(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

TSKgel® HIC Type is shipped or stored in an aqueous solution containing 20 % 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, wash the gel with packing solvent. The packing buffer should contain the highest salt concentration that the column will be exposed during normal use, cleaning and storage. Transfer the gel into a beaker and add the packing buffer to make an approximately 30 %-50 % (V/V) (recommended) slurry.

Packing the column under pressure (max. 2 MPa (recommended)) is recommended.

In this 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 2 MPa.

2－4 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.

2－5 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.

2－6 Regeneration

Most proteins can be eluted with distilied 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 mol/L-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 gel should be stored in an aqueous solution containing 20 % ethanol at ambient temperatures (4 ℃-35 ℃).

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 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

TOSOH 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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