Rev. CO063000

Packed Column
for Ion Exchange Chromatography

TSKgel SP-NPR
(0.3 mm I.D.)

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, and 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 Solvent

First Aid	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	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.
	Storage	• If the column is left at below 0 ℃, shipping solvent may freeze and container may break.

☐ Shipping solvent: 10 mmol/L Ammonium acetate (pH 4.2)

Precautions: Packing Material

First Aid	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. • Immediately call for medical attention.
	Ingestion	• Rinse the mouth with plenty of water and call for medical attention immediately.
Handling and Storage	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.
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 precautions	• Dispose in accordance with local laws and regulations. This product can be incinerated safely. • Assure that appropriate countermeasures are taken when incinerating anion exchange packing material since it contains sulfonate functional group. Fumes produced during incineration may contain sulfur oxides.

☐ Packing material: Vinyl polymer

Contents

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1. Introduction

This instruction manual contains crucial information on how to care for and use these columns in a proper manner, so as to make the most effective use of their high performance capabilities.

Be sure to carefully read instructions in this manual prior to use of these columns.

2. Prior to Use

Be sure to inspect the packaging and the exterior of the column for any signs of damage prior to use. If any damages are evident, contact your local Tosoh sales representative at the address listed at the end of this manual.

Confirm the following documents are included in the package.

1) Instruction manual	1 copy
2) Inspection data	1 copy

3. Column Specifications

Specification of TSKgel SP−NPR columns are shown as follows.

1) Part No.	: 21942
2) Dimensions	: Internal diameter 0.3 mm, Length 5.0 cm
3) Packing	: Ion exchange resin containg sulfopropyl group
4) Particle size	: 2.5 μm
5) Functional group	: Sulfopropyl group
6) Capacity	: ca. 1.0 eq/L
7) Shipping solvent	: 10 mmol/L Ammonium acetate (pH 4.2)

4. Installation and Operation

4−1. Handling

Do not drop, bump, vend the column. Peak splitting or broadening may be caused by mutation of packing structure.

4−2. Installation

1) Connect the column to the tubing according to the direction of arrow shown on the tag attached to the column.
2) Use 1/16" tubing and handy connector (Part No. 16566) to connect the column.
3) Recommend to use PEEK sleeve (1/16" outside diameter, manufactured by Upchurch Scientific, Inc.), when fused silica tubing is used.
4) Use the nuts (Part No. 06160, Package of 5) and ferrules (Part No. 19079, Package of 10), when stainless fittings are used.

5) Use the degassed eluent and HPLC system, when the column is attached to the HPLC system.

4-3. Operation condition

1) Suitable flow rate: 4.0 ～ 6.0 μL/min
2) pH range: 2.0 ～ 12.0
3) Maximum pressure: 20 MPa
4) Temperature range: 10 ～ 45 ℃

4-4. Removing

After making sure the column reached to the room temperature, stop the pump.

After making sure not to drop the column pressure, remove the column.

4-5. Storage

Always keeps the column filled with storage solvent fastening the end plugs in order to keep the packing materials from drying and store it at room temperature.

- Storage solvent:10 mmol/L Ammonium acetate (pH 4.2)

If the column inlet or outlet dry out and separate out salts from eluent, it may cause flow error by clogging.

If the column is left at below 0 ℃, It may freeze and results in degradation of performance.

4-6. Column cleanup

Prolonged operation with complexed ingredient in various sample may lead to gradual accumulation of contaminants in the column.

This is evidenced by changes in chromatographic behavior.

Column performance may be recovered by cleaning operation as follows.

1) Flushing the column with water of HPLC grade. (Flow rate: 4.0 μL/min, Flushing time: 15 min or more)
2) Adsorbed materials can be stripped from the column by repeatedly injecting with following cleaning samples.
   ① 0.1 mol/L NaOH
   ② 20 ～ 40 % Acetic acid
   ③ Solution containing aqueous organic solvent
3) If column performance could not be recovered by repeatedly injection, it is recommended to flushing the column with 3 mol/L ammonium formate (pH 3.0) solution. (Flow rate: 2.0 μL/min, Flushing time: 30min or more)

5. Calculation of Theoretical Plate Number and Asymmetry Factor

The theoretical plate number (N) and the asymmetry factor (As) as well as their chromatographic conditions are as shown in the inspection sheet.

5-1 Method of Calculating Theoretical Plate Number

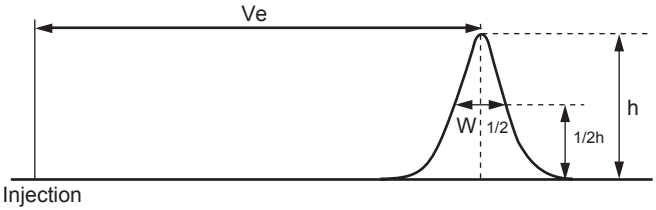

Figure 1 Method of Calculating Theoretical Plate Number

The theoretical plate number (N) of a column is calculated by the half width method shown in Figure 1 and the following equation.

$N = 5.54 (Ve/W_{1/2})^2$

Ve	: Elution time (min)
$W_{1/2}$	: Half width value of peak (min)
h	: Peak height

5-2 Method of Calculating Asymmetry Factor

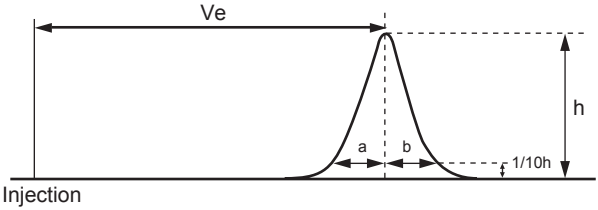

Figure 2 Method of Calculating Asymmetry Factor

The asymmetry factor (As) of a column is calculated by the 1/10h method.
As = b/a

6. Quality Specifications and Warranty

6-1 Conditions for Inspection data

Eluent: 10 mmol/L Ammonium acetate (pH 4.2)
Flow rate: 4.0 μL/min
Temperature: 25 ℃
Sample: Cytidine (0.6 g/L)
Injection vol.: 20 nL
Instrument: Capillary HPLC system

(Tubing)

Injector to column	: 30 μm I.D. × 15 cm
Column to detector	: 30 μm I.D. × 15 cm
Detector cell	: 18 nL (Response : 0.05 sec)

6-2 Quality Specifications

Table 1

Grade	TSKgel SP-NPR
Part No.	21942
Plates	1,000 or more
Asymmetry	0.9 ~ 2.2

6-3 Warranty

Check the appearance of the column and test its performance according to Section 5 within two weeks after the receipt of the column.

If the quality specifications in Table 1 can not be obtained or the column has been damaged during transportation, contact TOSOH representative within two weeks. TOSOH will replace the column free of charge.

Note that column life is not guaranteed.

No column should be returned to TOSOH without TOSOH's consent.

The specifications of these columns may be improved without notice.

TOSOH CORPORATION

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