HPLC
IEC Column
TSKgel IEC Type Glass Series

INSTRUCTION MANUAL

TOSOH CORPORATION

Safety Precautions

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

[Notational Conventions]

Notation	Explanation
⚠WARNING	Alerts the user to the potential for serious injury or death.
⚠CAUTION	Alerts the user to the potential for damage to hardware or bodily harm.

⚠ WARNING

■ **Keep away from fire.**

Take proper precautions when using flammable solvents. There is the potential for 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. When cleaning up a spill, wear appropriate protective gear.

■ **Wear eye protection and protective gloves.**

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

■ **Handle package with care.**

Inappropriate handling may cause rupturing and splattering.

■ **Only use this product as intended.**

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

■ **Confirm compounds are safe.**

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

■ **Proper disposal.**

Dispose of in accordance with local laws and regulations.

NOTE

Keep this manual for future reference.

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1. Introduction

TSKgel IEC Glass columns have been optimized for high performane ion exchange chromatography.

TSKgel DEAE-5PW is a week anion xchanger, TSKgel CM-5PW is a weak cation exchanger and TSKgel SP-5PW is a strong cation exchanger.

These columns have been designed for analytical and preparative separation of bio-polymers such as proteins and nucleic acids. There specification are given in Table 1. TSKgel IEC Glass column consists of a high precision glass tube with plastic end-pieces, and is fully bio-compatible.

Guardgel and kits are available for each functional group.

Table 1 TSKgel IEC-PW Type columns

Types	Functional Groups & Counter-ions	Column Dimension (mmID×cmL)	Application
TSKgel SP-5PW Glass	$-C_3H_6-SO_3^-Na^+$	5.0× 5.0 8.0× 7.5 20.0×15.0	Proteins, Peptide
TSKgel DEAE-5PW Glass	$-C_2H_4N^+(C_2H_5)_2HCl^-$	5.0× 5.0 8.0× 7.5 20.0×15.0	Acidic proteins, Nutral proteins, Nucleic acids
TSKgel CM-5PW Glass	$-CH_2COO^-Na^+$	5.0× 5.0 8.0× 7.5 20.0×15.0	Basic proteins, Nutral proteins

2. Unpacking

Check that nothing is the matter with the appearance of package or the column.

Fig. 1 Appearance of the package

Then check that the following documents are attached to the column :

1 copy Instruction Manual

1 copy Inspection Data

3. Installation

3-1. Connection Parts

The column and column parts are shown in Fig. 2. All columns can be connected with 1/4"-28 UNF screws.

3-2. Flow Direction

Use the column in the direction shown by the arrow on the tag in Fig. 2.
Operating the column with the flow in the reverse direction for a long time will cause degradation of column performance.

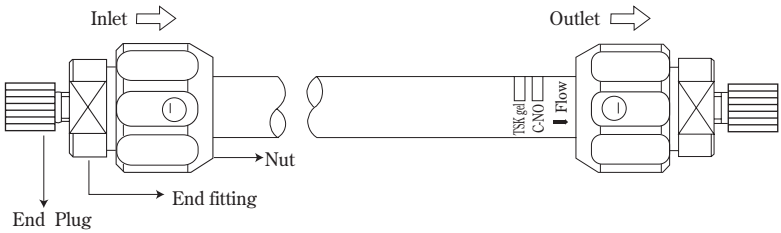

Fig. 2 Column Parts

3-3. Prevention of Bubbles

Be careful not to admit any bubbles into the column during its installation or removal from the equipment. Always remove all bubbles from all pipings before installing the column.
Admitting bubbles into the column will cause degradation of its performance through the occurrence of channeling, etc.

3-4. Prevention of Pulsatory flow

This type of column is easily affected by pulsatory flow of the solvent.
Preferably, a pump with no fluctuation should be used.
If a pump with pulsation must be used, connect a pulse damper (accumulator) to the outlet side of the pump in order to compensate for the pulsation.
The damper must be highly resistant to corrosion.

4. Column Storage

Store the columns at constant temperature in the range of 4℃ to 30℃.
Don't store these columns below 4℃ not to freeze the solvent in the column.
Avoid exposing the columns to direct sunlight, and store the columns in a place safe from corrosive gases.
If the column is being used daily, you need not remove the column from the equipment and the buffer may be left in the column overnight, as long as the buffer salt has good solubility. When the columns are to be stored for a few days, remove the column from the instrument and seal both ends with the end plugs. For long-term storage, the column must be protected from growth of microorganisms.
Thus, aqueous buffers should be replaced with distilled and deionized water.

5. Sample and Eluent Preparation

5-1. Replacement of Solvent

TSKgel IEC Glass series columns are filled with distilled and deionized water for shipment.
Replace this solvent with an appropriate solvent, using one-half of an appropriate flow rate shown in Table 1. Since frequent solvent replacement accelerates degradation of column efficiency, use the same solvent as far as possible.

5-2. pH

TSKgel IEC Glass series gels are hydrophilic polymer-based and should be operated within a pH range of 2.0-12.0.

5-3. Filtration and Degass

Use only HPLC grade solvents that have been filtered through an appropriate 0.5 μm filter.
Filter all buffered solution before using them. This reduces the problem of plugged filters and preserves column life.
Vacuuming or ultrasonic wave radiation may be used to remove dissolved gasses which could affect your solvent delivery system.

5-4. Counter-ion Selection

Since counter-ions have varying affinities for the ion exchange support, resolution can be affected by them. For anion exchangers, the retention sequence of commonly used counter-ions is citrate^{2-} > SO_4^{2-} > PO_4^{2-} > Cl^- > formate$^-$ > acetate$^-$ > OH, and for cation exchangers, that is $K^+ \cong NH_4^+ > Na^+ \cong$ Tris$^+$ > $Li^+ \cong H^+$, respectively.

5-5. Ionic Strength

Ionic strength gradients are very effective and popular in ion exchange chromatography. Although the required range of ionic strength varies, the following range is typically adopted in the separation of proteins : Starting buffer 0.05-0.1mol/L and final buffer 0.2-0.5mol/L.

The ionic strength can be adjusted by addition of chloride, sulfate or acetate salts.

5-6. Organic Solvents

Organic solvents miscible with water are often used as a modifier to reduce hydrophobic interactions between sample molecules and the support. Typical concentration range of organic modifiers below 20%.

The organic modifier should be premixed with the aqueous buffer to ensure that precipitation of buffer salts does not occur.

5-7. Surfactants or Denaturating agents

These series columns can be used with a solvent containing surfactants denaturating agents in the separation of biological polymers such as proteins and peptides.

5-8. Sample

The sample should be dissolved in the starting eluent and filtered through a 0.5 μm filter to protect the column from accumulation of impurities.

6. Flow Rate

Factors such as resolution, analytical time, and column life should be carefully considered in selecting flow rates. A higher flow rate results in a shorter analytical time. While a lower flow rate results in improved column efficiency and tends to extend column life.

Standard flow rates for phosphate or other aqueous buffers are recommended between 0.5-0.8 mL/min. The flow rates and pressure drops for hese column types are suggested below:

Table 2 Recommended flow rates

Cat. No.	Types	Column Sizes (mmID×cmL)	Max. Flow Rate (mL×min)	Recommended Flow Rate (mL×min)	Max. Pressure Drop (MPa)
13061	TSKgel DEAE-5PW Glass	5.0× 5.0	1.0	0.5—0.8	1.5
08802	TSKgel DEAE-5PW Glass	8.0× 7.5	1.2	0.5—1.0	1.0
14016	TSKgel DEAE-5PW Glass	20.0×15.0	8.0	4.0—6.0	1.5
13062	TSKgel SP-5PW Glass	5.0× 5.0	1.0	0.5—0.8	1.5
08803	TSKgel SP-5PW Glass	8.0× 7.5	1.2	0.5—1.0	1.0
14017	TSKgel SP-5PW Glass	20.0×15.0	8.0	4.0—6.0	1.5
14010	TSKgel CM-5PW Glass	5.0× 5.0	1.0	0.5—0.8	1.5
14011	TSKgel CM-5PW Glass	8.0× 7.5	1.2	0.5—1.0	1.0
14012	TSKgel CM-5PW Glass	20.0×15.0	8.0	4.0—6.0	1.5

*Don't use these column with the flow rate and pressure drop over maximum shown in above table. These flow rates are attainable with the buffer or aqueous solvents having approximately the same viscosity as the shipping solvent.

Caution

Don't use the system under the condition that the pressure drop is above 3.0 MPa to prevent the damage of the glass column and the leakage at the end fitting.

7. Temperature

The optimal operating temperature for TSKgel IEC Glass columns is lower than 30℃. These columns should not be used above room temperature for extended periods of time.

For temperatures below 10℃, use a lower flow rate to protect the columns.

8. Guard Column

We recommend the use of an in-line frit filter and a guard column or guard gel kit to protect all analytical columns. This is of extreme importance when buffered mobile phases and/ or biological samples are being evaluated. The guard column and guardgel kit should contain a packing similar to the column packing it protects.

Guardgel kit consists of gel and empty column. If you want to know more information, please refer to "INSTRUCTION MANUAL for TSKguardgel kit Glass for HPLC".

Table 3 Guard columns

Cat. No.	Types	Applied Columns
08806	TSKguardgel DEAE-5PW kit Glass	TSKgel DEAE-5PW Glass (8.0×7.5)
08807	TSKguardgel SP-5PW kit Glass	TSKgel SP-5PW Glass (8.0×7.5)
14024	TSKguardgel CM-5PW kit Glass	TSKgel CM-5PW Glass (8.0×7.5)
14466	TSKguardcolumn DEAE-5PW Glass	TSKgel DEAE-5PW Glass (20×15)
14467	TSKguardcolumn SP-5PW Glass	TSKgel SP-5PW Glass (20×15)
14468	TSKguardcolumn CM-5PW Glass	TSKgel CM-5PW Glass (20×15)

*Column size : 8.0mmID × 1.0 cmL (guardgel kit) 20.0mmID×2.0cmL (guardcolum)

9. Column Efficiency

Columns are thoroughly tested in our quality control laboratories for adherence to our specifications. Since slight variations in your results will occur depending on the equipment used, test sample make up and equipment settings and condition, perform the test sample run given here for your new column and record the results (theoretical plate and the settings used) before attempting the first analysis. Use these results for comparisons throughout the life of your column.

Be sure to record results and instrument setting (and configurations) to allow exact reproduction and comparison in the future.

9-1. Calculation method

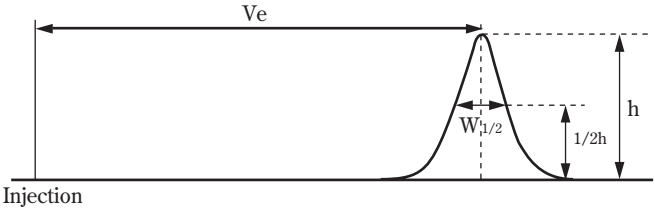

Fig. 3

The number of theoretical plates of a column (N) is calculated by the half peakwidth method shown in Fig. 3 and the following equation.

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

Ve : Elution volume

$W_{1/2}$: Half width value of peak

h : Peak height

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

As＝b／a

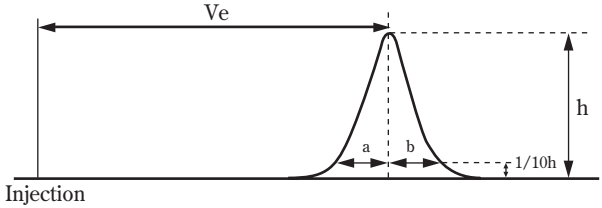

Fig. 4

9-2. Test condition

(1) Eluent

Types	Eluent
TSKgel DEAE-5PW	0.02mol/L Tris-HCl buffer＋0.035mol/L NaCl (pH 8.0)
TSKgel SP-5PW	0.08mol/L Phosphate buffer (pH 3.5)
TSKgel CM-5PW	10mmol/L Acetate buffer (pH 4.5)

(2) Flow Rate vs. Column Dimension

Flow Rates (mL/min)	Column Dimension (mmID×cmL)
0.8	5.0×5.0
1.0	8.0×7.5
6.0	20.0×15.0, 21.5×15.0

(3) Samples and their Concentrations

Types	Samples	Concentrations
TSKgel DEAE-5PW	Cytidine-5-monophosphate	0.1 mg/mL
TSKgel SP-5PW	Cytidine	0.1
TSKgel CN-5PW	Cytidine	5.0

(4) Injection Volumes vs. Column Dimension

Injection Volumes (μL)	Column Dimension (mmID×cmL)
20	5.0×5.0
20	8.0×7.5
100	20.0×15.0, 21.5×15.0

(5) Detector

Detector : UV−8000 (made by TOSOH)

Wave Length : 254nm

(6) Measuring Temperature : Room Temperature

10. Troubleshooting

Table 4 Typical column problems and Solutions

PROBLEM	CAUSE	SOLUTION
Leakage of Solvent	Loose of end fitting and nut	Turn end fitting tighten with the torque lower than 3 N·m
Excess pressure buildup	Frits plugged with particulates	Replace end fitting or clean in an Ultrasonic bath. Always filter solvents and samples.
	Sample precipitates on column.	Slowly purge with cleaning solvent (refer to "Column Cleaning Procedures")
Loss of resolution broad peaks, low plate counts.	Contaminated column.	Slowly purge with cleaning solvent (refer to "Column Cleaning Procedures")
	Column collapse and void formation	Since this may occur during the first two weeks of use, TOSOH will replace any column with this defect.

“Column cleaning Procedure”

0.1-0.2 N NaOH are very effective to wash or regenerate colums. Usually, column can be regenerated by injecting 0.1-0.2 N NaOH of 1-2 mL several times using sample loop of 1-2 mL. When this procedure did not help, wash the column by injecting 20-40% acetic acid of 1-2 mL several times.

11. Quality Specifications and Warranty

Cat. No.	Types	Column Size (mmID×cm)	No. of Theoretical Plates (TP／Column)	Asymmetry Factor (As)
13061	TSKgel DEAE-5PW Glass	5.0× 5.0	≧ 700	0.8～1.6
08802	TSKgel DEAE-5PW Glass	8.0× 7.5	≧1,300	0.8～1.6
14016	TSKgel DEAE-5PW Glass	20.0×15.0	≧3,000	0.8～1.6
13062	TSKgel SP-5PW Glass	5.0× 5.0	≧ 700	0.8～1.6
08803	TSKgel SP-5PW Glass	8.0× 7.5	≧1,300	0.8～1.6
14017	TSKgel SP-5PW Glass	20.0×15.0	≧3,000	0.8～1.6
14010	TSKgel CM-5PW Glass	5.0× 5.0	≧ 700	0.8～1.6
14011	TSKgel CM-5PW Glass	8.0× 7.5	≧1,300	0.8～1.6
14012	TSKgel CM-5PW Glass	20.0×15.0	≧2,500	0.8～1.6

11-1. Warranty

Immediately after receipt, check the appearance of the column and test its performance according to Section 9.

If the guaranteed specifications (above Table) can not be obtained, contact your TOSOH representative within two weeks.

Note that column lifetime is not guaranteed.

The specification of these columns may change without notice for their improvement.

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