Packed Columns for High Performance
Ion Exchange Chromatography
TSKgel IEC Type

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 Type columns have been optimized for high performance ion exchange chromatography developed by TOSOH CORP.

These columns have been designed for analytical and preparative separation of biopolymers such as proteins and nucleic acids.

Their specifications are given in Table 1.

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

Table 1 TSKgel IEC Type columns

Types	Base Materials	Particle Sizes	Functional Groups & Counter-ions	Column Sizes (mmID×cm)
		μm		
TSKgel SCX	Polystyrene Gel (TSKgel G2000H)	5	$-SO_3^-Na^+$	6.0×15
	ditto	5	$-SO_3^-H^+$	7.8×30
TSKgel SAX	ditto	5	$-N(CH_3)_3^+Cl^-$	6.0×15
TSKgel CM-2SW	Silica Gel (TSKgel G2000SW)	5	$-CH_2COO^-Na^+$	4.6×25
	ditto	10		7.8×30
TSKgel SP-2SW	ditto	5	$-C_3H_6-SO_3^-Na^+$	4.6×25
TSKgel DEAE-2SW	ditto	5	$-C_2H_4N^+(C_2H_5)_2HCl^-$	4.6×25
	ditto	10		7.8×30
TSKgel CM-3SW	Silica Gel (TSKgel G3000SE)	10	$-CH_2COO^-Na^+$	7.5×7.5
TSKgel DEAE-3SW	ditto	10	$-C_2H_4N^+(C_2H_5)_2HCl^-$	7.5×7.5
TSKgel SP-5PW	Hydorophilic polymer gel (TSKgel G5000PW)	10 13	$-C_3H_6-SO_3^-Na^+$	7.5×7.5 21.5×15
TSKgel DEAE-5PW	ditto	10 13	$-C_2H_4N^+(C_2H_5)_2HCl^-$	7.5×7.5 21.5×15
TSKgel CM-5PW	ditto	10 13	$-CH_2COO^-Na^+$	7.5×7.5 21.5×15

2. Typical Applications

Typical Applications of TSKgel IEC Type columns are listed in Table 2.

TSKgel SCX and TSKgel SAX are applied mostly to small ionic compounds while TSKgel CM-3SW, DEAE-3SW, SP-5PW, CM-5PW and DEAE-5PW are used for separation of large biopolymers such as proteins and nucleic acids.

The other silica-based grades are available from small to fairly large compounds.

Table 2 Typical Applications

Types	Typical Samples
TSKgel SCX (6.0×15)	Amino acids, Polyamines, Nucleosides, Peptides
TSKgel SAX	Carboxylic acids
TSKgel SCX (7.8×30)	Saccharides, Organic acids
TSKgel CM-2SW	Catecholamines, Tryptophan methabolites, Peptides
TSKgel SP-2SW	Catecholamines, Tryptophan methabolites, Peptides
TSKgel DEAE-2SW	Nucleosides, Organic acids in urine, Bile acids
TSKgel CM-3SW	Basic proteins, Neutral proteins
TSKgel DEAE-3SW	Acidic proteins, Neutral proteins, Nucleic acids
TSKgel SP-5PW	Basic proteins, Neutral proteins
TSKgel DEAE-5PW	Acidic proteins, Neutral proteins, Nucleic acids
TSKgel CM-5PW	Basic proteins, Neutral proteins

3. Unpacking

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

Fig. 1 Appearance of Package

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

1 copy Instruction Manual
1 copy Inspection Data

4. Column Parts

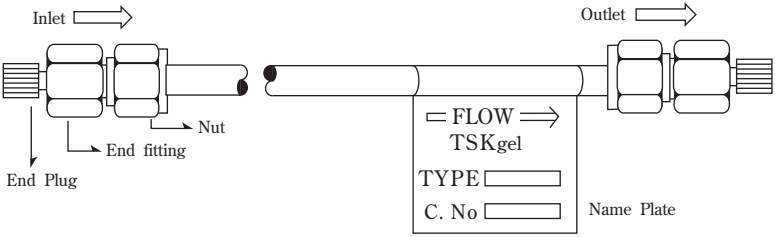


Fig. 2 Column Parts

5. Installation and Safety Considerations

5-1 Connection

All connections are of the swage lock type and of inch dimensions.

5-2 Flow Direction

Use the column in the direction shown by the arrow on the name plate in Fig.2.

Operating the column with the flow in the reverse direction for the long time will cause degradation of the column performance.

5-3 Prevention of Bubbles

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

5-4 Installation

If solvent leaks from the end fitting when the cap on the inlet side of the column removed, connect the column to the equipment carefully, as mentioned above, so that no bubbles will be introduced into the column.

If no solvent leaks from the inlet side of the column, connect the outlet side to the equipment and feed solvent through the column in the reverse direction with the feed pump in order to expel the air.

Feed the solvent slowly in this case below the flow rates in Table 3.

Since rapid pressurization or solvent feeding may cause degradation of column performance. After confirming bubble-free solvent leakage at the inlet side of the column, arrange the column in the direction of normal flow, and connect the inlet side to the injector.

Table 3 Flow Rates for Solvent Replacement

Column Size (mmID×cm)	Flow Rates (mL/min)
4.6×25	0.4
6.0×15	0.6
7.5× 7.5	0.5
7.8×30	0.6 ※
7.8×30	1.5 ※※
21.5×15	3.0

※ for Polystyrene based gel
※※ for Silica based gel

5-5 Prior to Measurement

After installation of the columns, measurement can begin. As mentioned above, rapid pressurization or solvent feeding must be avoided, since these may cause degradation of column performance. Be especially careful when applying a feed pump with a rapid pressure rise.

5-6 Prevention of Pulsatory Flow

This type of column is easily affected by pulsatory flow of 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.

5-7 Measurement at High Temperature

Don't stop the pump immediately after finishing the measurement. Continue to feed solvent until the column temperature lower to the room temperature.
If the pump is stopped while the column is hot, air may be sucked into the column by contraction of the solvent.

5-8 Routine Use

If the column is being used daily, the column need not to be removed from the equipment and the buffer may be left in the column overnight.

5-9 Stort-term Storage

When the column will be used again fairly soon, remove the column from the equipment and seal the both ends of the column with the end plugs.

5-10 Long-term Storage

When the columns are not to be used again soon the column treatment mentioned above is unsatisfactory, since corrosion by a corrosive buffer may result in column degradation. For long-term storage, replace the solvent in the columns with distilled and deionized water, below the flow rates described in Table 3. For the silica based grade replace the d. d. water with MeOH.
Then remove the column from the equipment and seal both ends of each column with the end plugs.

6. Column Storage

6-1 Storage Method

Refer to Section 5-9 & 5-10.

6-2 Storage Temperature

Store the columns at room temperature. The columns may freeze and their efficiency degrade if they are left where the temperature is below 0℃.

6-3 Exposure to Direct Sunlight

Avoid exposing the columns to direct sunlight.

6-4 Corrosive Gasses

Store the column in a place safe from corrosive gasses.

7. Solvents

7-1 Replacement of Solvents

The solvents of these columns for shipment are shown in Table 4.

Table 4 Solvents for Shipment

Types	Solvents
TSKgel SCX TSKgel SAX TSKgel DEAE-5PW TSKgel SP-5PW TSKgel CM-5PW	Distilled and deionized Water
TSKgel DEAE-2SW, 3SW TSKgel SP-2SW TSKgel CM-2SW, 3SW	Methanol

In case Methanol is used for shipment, replace it with d. d. water at first, then replace the water again with the solvent to be used for the analysis.

The flow rates for solvents replacement must be applied below them as shown in Table 3.

TSKgel SCX, SAX, SP-5PW CM-5PW & DEAE-5PW are swelled or contracted by the organic solvents quite easily, so frequent solvents replacement, must be avoided.

7-2 pH Range

Keep pH range within the following range :

IEC H Series and IEC PW Series	2.0 — 12.0
IEC SW Series	2.0 — 7.5

The pH range should be determined from the stability of both packing material and the column itself. Below pH 2.0, the bonded phase of the silica-base support is subject to attack, while above pH 7.5 the silica backbone becomes soluble, leading to rapid column failure.

Additionally, the stainless steel of the column is subject to corrosion at low pH. Particularly, use of halides at a low pH is dangerous from the viewpoint of corrosion.

7-3 Counter-ion Selection

Since counter-ions have varying affinities for the ion exchange support, resolution can be affected by them. The retention sequence of commonly used counter-ions is as follows ; for anion exchangers, citrate > SO_4^{2-} > PO_4^{3-} > Cl^- > Formate > acetate > OH^-, for cation exchangers, K^+ > NH_4^+ > Na^+ > Li^+ > H^+, respectively.

From the viewpoint of column maintenance, it is recommended to avoid Cl ions as much as possible, although they are the most common ions.

7-4 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 using IEC SW Series and IEC PW Series columns : Starting buffer of ionic strength is 0.05-0.1mol/L and final buffer of it is 0.2-0.5mol/L

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

7-5 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 :

IEC SW Series : below 30%

IEC PW Series : below 20%

IEC H Series : below 20%

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

7-6 Surfactants or Denaturating agents

IEC SW Series and IEC PW Series columns can be used with a solvent containing surfactants senaturating agents in the separation of biological polymers such as proteins and peptides.

7-7 Filtration

The solvent should be filtered through a 0.45 μ m filter in order to prevent the accumulation of particle matter.

7-8 Degassing

Solvent should be degassed to ensure a continuous flow through the system.

8. Flow Rates

8-1 Choice of Flow Rates

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. On the contrary, a lower flow rate result in improved column efficiency.

Furthermore, a lower flow rate tends to extend column life and to decreases the occurrence of top-off.

Suitable flow rates are shown in Table 5.

Do not use these coloumn with pressure-drops and flow rates over the maximums shown in Table 5.

Table 5 Suitable Flow Rates

Types	Column Sizes (mmID×cm)	Suitable Flow Rates (mL/min)	Max. Flow Rates (mL/min)	Max. Pressure-drop/column (MPa)
TSKgel SCX TSKgel SAX	6.0×15	0.5—1.0	1.2	15
TSKgel SCX	7.8×30	0.5—1.0	1.2	7
TSKgel SP-2SW TSKgel CM-2SW TSKgel DEAE-2SW	4.6×25	0.6—0.8	1.0	15
TSKgel CM-3SW TSKgel DEAE-3SW	7.5×7.5	0.5—1.0	1.2	2.5
TSKgel SP-5PW TSKgel DEAE-5PW TSKgel CM-5PW	7.5×7.5	0.5—1.0	1.2	1.5
TSKgel CM-2SW TSKgel DEAE-2SW	7.8×30	1.0—2.0	3.0	6
TSKgel SP-5PW TSKgel DEAE-5PW TSKgel CM-5PW	21.5×15	4.0—6.0	8.0	2.5

9. Operating Temperature

The optimal operating temperature for these columns, both analytical and preparative, is between 10℃ and 45℃ Below 10℃ use a lower flow rate to protect the columns.

10. Preparation of Sample Solution

10-1 Sample Solution Composition

Adjust the pH and concentrations of salt and organic solvent in a sample as closely as possible to those of the eluent.

This is especially essential when the injection volume of a sample is large.

A Sample solution should never be applied if it will form insoluble matter when mixed with the eluent.

10-2 Elimination of Insoluble Matter

Always purify the sample solution either by centrifugation or preferably by micro-pore filtratopn (of e.g. 0.45 μ m pore size.)

11. Measurement of Column Efficiency

The number of theoretical plates (N), the asymmetry factor (As) and their chromatographic conditions are as shown in the Inspection Data.

11-1 Method of Calculating the Number of Theoretical Plates

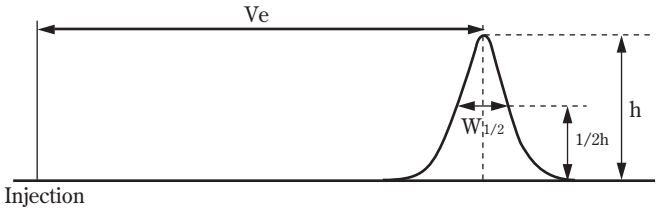


Fig. 3 Method of Calculating the Number of Theoretical Plates

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

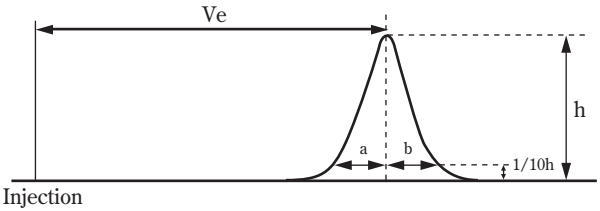
$N = 5.54(Ve/W_{1/2})^2$

Ve : Elution volume

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

h : Peak height

11-2 Method of Calculating the Asymmetry Factor



Fig, 4 Method of Calculating the Asymmetry Factor

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

As = b ／ a

11-3 Dead Volime

If the dead volume of the equipment or the injection volume of a sample solution becomes too large, the number of theoretical plates may decrease.

12. Guard Column

Fundamental key to problem prevention have been outlined in section 5 to 10. But when impurities that tend to be absorbed by the packing material present in the sample, they are absorbed on the inlet side of the column and accmulate gradually, causing reduction of column efficiency.

In such case the original column efficiency can be maintained by connecting a guard column before the column and replacing it if efficiency becomes degraded by the adsorption of material to the guard column.

For maximum insurance against such problems, a guard column should be used as much as possible we prepared the "guard gel kit" as a guard column for TSKgel IEC Type columns.

Please refer to "INSTRUCTION NANUAL for TSKguardgel for HPLC"

Table 6 Guaed Gel kit

Cat. No.	Types	Applicable column (mmID×cm)
07210	TSKgel guardgel DEAE-5PW kit	TSKgel DEAE-5PW (7.5×7.5)
07211	TSKgel guardgel SP-5PW kit	TSKgel SP-5PW (7.5×7.5)
13069	TSKgel guardgel CM-5PW kit	TSKgel CM-5PW (7.5×7.5)
07644	TSKgel guardgel SP-SW kit	TSKgel SP-2SW (4.6×25)
07646	TSKgel guardgel QAE-SW kit	TSKgel QAE-2SW (4.6×25)
07648	TSKgel guardgel DEAE-SW kit	TSKgel DEAE-2SW (4.6×25) (7.8×30) TSKgel DEAE-3SW (7.5×7.5)
07650	TSKgel guardgel CM-SW kit	TSKgel CM-2SW (4.6×25) (7.8×30) TSKgel CM-3SW (7.5×7.5)

13. Troubleshooting

When using TSKgel column, some problems can be avoided by following these instructions. But some problems (such as those due to column life, adsorptive materials, production of air bubbles, dried gel, or frozen solvent) can not be corrected once they occur, so care dhould be taken in handling these columns.

13-1 Clogging of the End Fitting

In case pressure-drop increases or the flow rate decreases, the end fitting should be cleaned by reversing the flow through the column.

If the clog cat not be removed, prepare a new end fitting and replace the old end fitting by a new one, being very careful to loose any of the packed gel underneath.

13-2 Contamination

Prolonged operation with complex mixtures may lead to the gradual accumulation of strongly ionic compounds or hydrophobic compounds. This is evidenced by changes in chromatographic behavior and loss of resolution.

Adsorbed material can be stripped from the column by flushing with at least one of the following solvents. In this case the flow rates must be below them as shown in Table 5.

1) For IEC-SW series
   a) Buffer of high salt concentration (0.5-1.0mol/L)
   b) Buffer of low pH 2-3.
   c) Buffer containing an organic modifier such as methanol or acetonitrile.
   d) Beffer containing a solubilizer such as urea and non-ionic surfactants.
2) For IEC-PW Series (TSKgel SP-5PW CM-5PW and DEAE-5PW)
   a) 0.1-0.2mol/L NaOH
   b) 20-40vol.% acetic acid.
   c) Buffer containing an organic modifier such as methanol or acetonitrile.
   d) Buffer containing a solubilizer such as urea and non-ionic surfactants.

14. Quality Specifications and Warranty

14-1 Inspection Data

The results of each inspection are described in the Inspection Data enclosed in the column package. In the Inspection Data, N is expressed as that per column.

The conditions used in determining the Inspection Data are as follows :

① Eluent : SCX — H_2O
SAX — H_2O
SP-2SW — 0.05mol/L Phosphate Buffer (pH 3.0) + 8.6% CH_3CN
CM-2SW — 0.2mol/L Acetata Buffer (pH 5.0) + 15% CH_3CN
CM-3SW — 0.2mol/L Acetata Buffer (pH 5.0) + 15% CH_3CN
QAE-2SW — 1 / 15mol/L Phosphate Buffer (pH 6.4)
DEAE-2SW — 1 / 15mol/L Phosphate Buffer (pH 6.4)
DEAE-3SW — 1 / 15mol/L Phosphate Buffer (pH 6.4)
DEAE-5PW — 0.02mol/L Tris — HCl Buffer + 0.035 mol/L NaCl (pH 8.0)
SP-5PW — 0.08mol/L Phosphate Buffer (pH 3.5)
CM-5PW — 0.01mol/L Acetata Buffer (pH 4.5)

② Sample Volume : 20 μL except for 21.5mm ID columns
100 μL for 21.5mm ID columns

③ Other Conditions

Types	Column Sizes (mmID×cm)	Samples		Flow Rates	Detection
			mg/mL	mL/min	
TSKgel SCX	6.0×15	Ethylene	2.0	1.0	(RI)
	7.8×30	Glycol	10.0	1.0	(RI)
TSKgel SAX	6.0×15	Ethylene Glycol	2.0	1.0	(RI)
TSKgel SP-2SW	4.6×25	Dopamine	0.1	0.9	(UV) 280nm
TSKgel CM-2SW	4.6×25	Tryptamine·HCl	0.1	0.9	(UV) 280
	7.8×30	ditto	0.2	2.0	(UV) 280
TSKgel DEAE-2SW	4.6×25	Uric acid	0.1	0.9	(UV) 254
	7.8×30	ditto	0.2	2.0	(UV) 254
TSKgel CM-3SW	7.5× 7.5	Tryptamine·HCl	0.1	1.0	(UV) 280
TSKgel DEAE-3SW	7.5× 7.5	Uric acid	0.1	1.0	(UV) 254
TSKgel SP-5PW	7.5× 7.5	Cytidine	0.1	1.0	(UV) 254
	21.5×15	ditto		6.0	(UV) 254
TSKgel CM-5PW	7.5× 7.5	Cytidine	0.1	1.0	(UV) 254
	21.5×15	ditto		6.0	(UV) 254
TSKgel DEAE-5PW	7.5× 7.5	Cytidine -5-monophosphate	0.1	1.0	(UV) 254
	21.5×15	ditto		6.0	(UV) 254

14–2 Quality Specifications

Table 7 Quality Specifications

Cat. No.	Types	Column Sizes (mmID×cm)	Number of Theoretical Plates (TP/Column)	As
07156 07157	TSKgel SCX TSKgel SAX	6.0×15	2,000	0.8—1.6
07158	TSKgel SCX	7.8×30	6,000	
07165 07167 07168	TSKgel SP-2SW TSKgel CM-2SW TSKgel DEAE-2SW	4.6×25	5,000	
07162 07163	TSKgel CM-3SW TSKgel DEAE-3SW	7.5×7.5	1,300	
07161 07164 13068	TSKgel SP-5PW TSKgel DEAE-5PW TSKgel CM-5PW	7.5×7.5	1,300	
07169 07170	TSKgel CM-2SW TSKgel DEAE-2SW	7.8×30	5,000	
07575 07574	TSKgel SP-5PW TSKgel DEAE-5PW	21.5×15	3,000	
14021	TSKgel CM-5PW	21.5×15	2,500	

14–3 Warranty

Immediately after reciept, check the appearance of the column and test its performance.

If the guaranteed specifications in Table 7 can not be obtained or the column has been damaged during the transportation, contact TOSOH, representative within two weeks. TOSOH will replace the column free of charge.

No column should be returned to TOSOH without its prior autherigation.

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

TOSOH CORPORATION

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