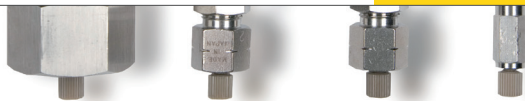




TOSOH



# TSKgel<sup>®</sup> HPLC Column Instruction Manual

Important! Do not throw away!

*Thank you for purchasing a  
TSKgel liquid chromatography column!*

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## WHAT TO DO FIRST

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First, inspect your shipment. Immediately notify Tosoh Bioscience's customer service department if your shipment is damaged or incomplete. Make sure you received the following with your column:

- Data sheet containing a chromatogram, the Quality Control test data and the conditions under which the QC test was performed (guardcolumns are not supplied with a chromatogram).
- Operating Conditions and Specifications (OCS) sheet, listing the specifications for this column type and conditions under which you can expect optimum column performance.

After you have checked the appearance of your column, test its performance under the conditions listed in the Data Sheet using the same or similar test compounds. Calculate the performance parameters as discussed under the „Quality Control“ section of this manual. Compare your results with the specifications.

**If your column is not within specifications, please contact Tosoh Bioscience within 30 days after receiving the column.** Returns can only be accepted if the column is shipped back in the Tosoh Bioscience column box and is accompanied by the original data sheet and the Tosoh Bioscience column tag (see „Return Policy“ in this manual).

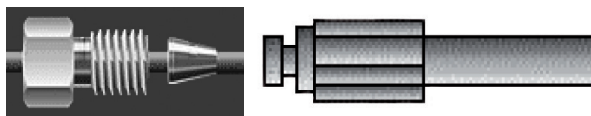
## INSTALLATION

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### Stainless steel and PEEK columns

The TSKgel column you received requires 10-32 (1/16") fittings for connecting it to any LC system (see Figure 1). We recommend that you make up two short pieces of 0.01" (0.25 mm) ID x 1/16" capillary tubing to connect the column to the guardcolumn and the detector. To prepare these connectors, use 10-32 (1/16") nuts and ferrules, plus the appropriate fittings for connecting the injector and detector. Alternatively, you can use 10-32 (1/16") fingertight fittings that are available from chromatography supply houses.

In either case, make sure to insert the capillary tubing fully into the column end fitting before setting the ferrule by tightening the nut. Repeat to make the connections at the other end of the 1/16" capillary tubing.



## **Glass columns**

The TSKgel glass column you received requires 1/4-28 fittings for connecting it to a liquid chromatography (LC) system. For glass columns, we recommend the use of plastic nuts and ferrules that can be installed without flanging the tubing. You can also use standard metric M6 nuts and ferrules.

In this case, use 1/6" Teflon® tubing with a maximum inner diameter (ID) of 0.015" or 0.4 mm to connect the column to the guardcolumn and the detector. Cut the tubing at a 90° angle with a tubing cutter designed for use on polymer tubing. Now put the ferrule on with the tapered end facing the nut. Cut the tubing flush to the base of the ferrule. Insert this into the column end fitting and hand tighten the nut.

## **Mobile phase**

Prevent air bubbles from entering the column during its installation, use and storage, since this may cause degradation of column performance through the formation of channels in the packed bed. Mobile phases must be thoroughly degassed before use. This can be accomplished by vacuum filtration, helium sparging or in-line degassing. In addition to degassing the solvent, vacuum filtration will also prevent small particles from plugging the column frit. You can use 0.20 or 0.45 micron nylon membranes to filter aqueous and aqueous/organic mobile phases.

## **Sample**

If possible, always dissolve your sample in mobile phase or the starting mobile phase when operating under gradient conditions. Alternatively, try to match the pH, salt concentration and organic solvent of the sample with those of the mobile phase and run a test to ensure that no precipitate, suspension or flocculate is formed. Finally, before making an injection, filter the sample through a 0.20-0.45 micron porosity membrane.

## **Flow direction**

The recommended flow direction through the column is indicated by the arrow on the tag. Operating the column with the flow in the reverse direction is only recommended as part of the installation procedure (see next section), cleaning, or when removing particulates from a clogged frit (see the „Troubleshooting“ section of this manual).

## **Installing the column**

After manufacturing and quality control, the column has been flushed with storage solvent and closed with caps to prevent evaporation of the solvent. As mentioned above, it is important to prevent air from entering the column. Remove the cap from the column inlet side. Solvent should be visible at the inlet fitting (if not, see below). Before starting the flow, first connect the column to the injector only. Start the flow. After liquid flows out of the column exit, you can connect the column to the detector inlet line.

If the column inlet fitting appears dry, we recommend that you first disconnect the bottom cap and hook up the column exit to the injector. Then slowly start the flow in this reversed flow direction until a few drops of mobile phase exit from the column. Turn off the flow, let the pressure go to zero, and disconnect the column from the system. Turn the column around and hook it up so that the flow is now in the direction of the arrow. Start the flow at a low setting and stop it as soon as the mobile phase exits from the bottom fitting. Now you can hook up the column to the detector inlet and increase the flow to the desired setting. Be sure to set the flow within the recommended range shown on the OCS sheet.

## COLUMN PROTECTION

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In addition to filtering the sample and the mobile phase, the best way to protect the separation column is to install a guardcolumn in front of it. This will trap highly adsorptive sample components and residual particulates in the sample, the mobile phase or from instrument components, such as pump and injector seals.

There are two types of guard products for protecting your TSKgel column. The most convenient option, which is available for some column types, is to purchase a prepacked TSKgel guard column or cartridge. Alternatively, you can purchase a guardgel Kit, which consists of the necessary glass or stainless steel hardware and packing material to pack your own guard column. Consult the OCS sheet for the part number of the appropriate guardgel Kit for your separation column. The kits come with a separate instruction sheet for assembling, packing and installing the guard column.

Helpful hints about guardcolumn replacement can be found in the "Troubleshooting" section of this manual and in the OCS sheets that are shipped with each TSKgel column and guard column product.

## QUALITY CONTROL

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The performance of each TSKgel column has been tested under the conditions described in the enclosed data sheet. Table 1 lists suggested suppliers for standard compounds to perform the QC test.

Column performance is judged by the following characteristics:

1. Theoretical plates (efficiency)
2. Asymmetry factor
3. Resolution
4. Retention time

Standards for quality control of TSKgel columns

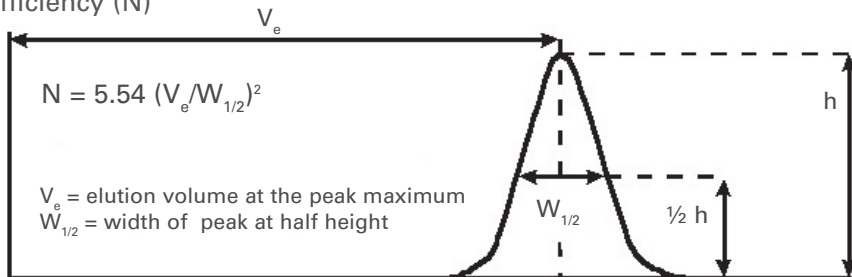
<b>Standard</b>	<b>Supplier</b>	<b>Catalogue #</b>
Protein Standard Mix 1.5 - 600 kDa	Sigma-Aldrich	69385
Recommended to be used for all TSKgel 3000SW, G3000SWxl, Super SW3000 Super SW mAb , QC-PAK GFC-300 and UP-SW30000 columns		
p-Aminobenzoic acid	Sigma-Aldrich	A9878
Bombesin	Sigma-Aldrich	B4272
Bovine serum albumin	Sigma-Aldrich	A4503
Chymotrypsin	Sigma-Aldrich	C7762
Chymotrypsinogen	Sigma-Aldrich	C4879
Cytidine	Sigma-Aldrich	C4654
Cytidine-5-monophosphate	Sigma-Aldrich	C1006
Dopamine.HCl	Sigma-Aldrich	H8502
Ethylene glycol	Fluka	03750
Ferritin	Sigma-Aldrich	F4503
γ-Globulin	Sigma-Aldrich	G5009
Lysozyme	Sigma-Aldrich	L6876
d-Mannitol	Sigma-Aldrich	M4125
Ovalbumin	Sigma-Aldrich	A2512
Polyethylene oxide (kit)	Tosoh Bioscience	05773
Polystyrene (kit)	Tosoh Bioscience	06476
Ribonuclease A	Sigma-Aldrich	R5503
Thyroglobulin	Sigma-Aldrich	T1001
Trypsin	Sigma-Aldrich	T7409
Trypsin inhibitor	Sigma-Aldrich	T9003
Trypsinogen	Sigma-Aldrich	T1143
Tryptamine. HCl	Sigma-Aldrich	246557
Uric acid	Sigma-Aldrich	U2625

*Note: Common chemical standards are not included in this list.*

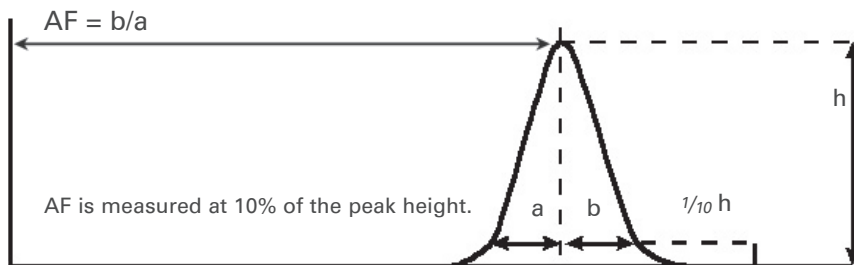
To use a sample component, you must establish baseline data when the column is new and performing well. After establishing that the column is performing properly, using standard test probes, calculate the asymmetry factor, theoretical plates and resolution of one or more of your sample components. Also note the retention time. This becomes your „baseline test mix“ with which you can later compare.

Consult **Figure 1** to calculate the quality control parameters that were determined after the column was manufactured.

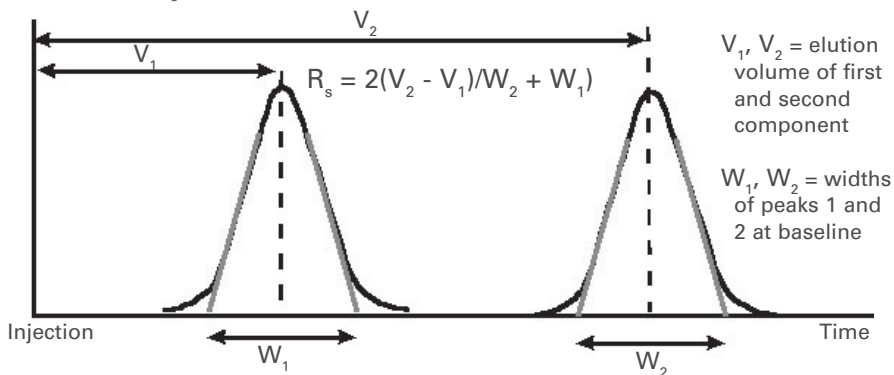
Efficiency (N)



Asymmetry factor (AF)



Resolution ( $R_s$ )



≡ FIGURE 1

## OPERATING CONDITIONS

The operating conditions and specifications (OCS) sheet lists the following parameters:

- shipping solvent
- maximum and optimum flow rates
- pressure limits
- pH limitations
- organic solvent compatibility

- maximum salt concentration
- maximum temperature
- recommendations for protecting, cleaning and storing the column
- column specifications

The following paragraphs provide additional detail about the role of temperature, column cleaning and storage.

## Temperature

All TSKgel columns should be operated above 0°C. They are usually operated at ambient temperature or at a higher controlled temperature. The maximum temperature your column can tolerate is listed on the OCS sheet.

For all columns the use of elevated temperature may change column efficiency and resolution, compared to measurements performed at room temperature or lower. When performing measurements at elevated temperature, we recommend that you do not stop the pump immediately after finishing the experiment. Instead, continue to pump mobile phase while allowing the column to equilibrate to room temperature. Otherwise, air may be sucked into the heated column due to contraction of the solvent.

## Cleaning

Occasionally, samples are run that adsorb onto the packing material. When this occurs, it is time to clean your column. You do not necessarily have to run a standard test probe to monitor your column. If you have a well-resolved peak, establish baseline data when the column is new and operating correctly. If one of the performance characteristics of your column changes by 10% or more, it is prudent to clean your column.

When you clean the column, there are a few basic rules to follow. These rules apply regardless of what type of TSKgel column you are running.

1. Clean your column in the **reverse flow** direction.
2. While cleaning, **do not connect the column to the detector**.
3. Run the column at **half of the maximum recommended flow rate**. Take special care to monitor the pressure, as the cleaning solutions may be of different viscosities than your normal mobile phase.
4. If you are cleaning with a high or low pH solution, make certain that the rest of your chromatographic system (pump, pump seals, injector, etc.) is compatible.

Each type of TSKgel column has a recommended set of cleaning solutions specific to the column. Choose a cleaning solution based upon the column and sample type. For example, a low pH salt solution will remove basic proteins. Organics will remove hydrophobic proteins. Chaotropic agents will remove strongly adsorbed materials (e.g., via hydrogen bonding).

For columns or column types not listed below, please contact Tosoh Bioscience Technical Service for the appropriate solutions.

### **Cleaning solutions**

Generally, 3-5 column volumes (CV) of cleaning solution is sufficient. Rinse well with 3-5 CV of distilled, deionized water between each solution. **Only use chaotropic agents when neutral salt or organic has not improved performance characteristics.**

#### **SW and SWxL, UP-SW, UP-SW LS, Super SW**

1. Concentrated neutral salt (e.g., 0.5 M) at low pH (e.g., pH 3.0)
2. Water soluble organic (MeOH, ACN, EtOH, 10%-20%) in aqueous buffer

#### **PW and PWxL**

1. High concentration neutral salt (e.g., 0.5 M-1.0 M) of your normal run buffer
2. Buffered solutions at low pH (e.g., 2-3) or high pH (e.g., 11-12)
3. Water soluble organic (MeOH, ACN, EtOH, 10%-20%) in aqueous buffer

#### **Ion Exchange, SW Type**

1. High concentration neutral salt (e.g., 0.5 M-1.0 M) of your normal run buffer
2. Buffered solutions at low pH (e.g., 2-3)
3. Water soluble organic (MeOH, ACN, EtOH, 10%-20%) in aqueous buffer

#### **Ion Exchange, PW Type**

1. 0.1 M-0.2 M NaOH
2. 20%-40% aqueous acetic acid
3. Water soluble organic (MeOH, ACN, EtOH, 10%-20%) in aqueous buffer

#### **Hydrophobic Interaction**

1. 0.1 M-0.2 M NaOH
2. 20%-40% aqueous acetic acid

#### **Reversed Phase, silica-based**

1. Acetonitrile or methanol
2. Gradient from 10%-100% acetonitrile in 0.05% trifluoroacetic acid

#### **Reversed Phase, PW Type**

1. Acetonitrile or methanol
2. 0.1 M-0.2 M NaOH
3. 20%-40% aqueous acetic acid

#### **Hydrophilic Interaction, Amide-80**

1. 45% Acetonitrile
2. 50 mM phosphate buffer pH 6.0/ acetonitrile 50/50

Choose cleaning solvent 1. to desorb compounds, 2. for ionic compounds

#### **Protein-A Affinity, PW Type**

1. Up to 2 mL of 0.1 M NaOH solution



## **FcR-III Affinity, NPR and PW Type**

1. Up to 0.5 - 2 mL of a buffer containing 0.5 mol/L NaCl or 20 % ethanol

*As a last resort, buffered solutions of SDS (0.1%), urea (8 M) or guanidine (6 M) can be used to clean the column. We only recommend this method if all other methods fail.*

## **Storage**

Consult the OCS sheet for the recommended storage solvent and storage temperature or temperature range for this column.

After your experiments, you can leave the column overnight in the LC system at low flow rates if you plan to use it the next day. If not, flush the system and the column with the storage solvent and screw the caps in both ends of the column. For long-term storage, the column must be protected from the growth of microorganisms by incorporating a bacteriostat such as 0.05% NaN<sub>3</sub> or 10%-20% acetonitrile or ethanol into the storage solvent. Avoid exposing the column to corrosive solvents and gasses and avoid exposure to direct sunlight. Refer to the OCS sheet for column-specific information.

## **Rehydration**

Dehydration of TSKgel liquid chromatography columns can result from improper use or during long-term storage, for example, if the plugs are not tightened or if air inadvertently is pumped into the column during use. It is easier to detect dehydration in glass columns because the dry packing will appear to pull away from the column walls. This condition can be remedied by using the following procedure:

1. Connect the column to your LC system in reverse flow direction.
2. Do not connect the column to the detector.
3. Pump a filtered mobile phase of 20% acetonitrile in distilled, deionized water over the column at half of the recommended maximum flow rate. Please note that reversed phase columns require 60% methanol.
4. Continue this procedure until you are confident that the column has been rehydrated. Rehydration can take several hours, depending on the column size. Rinse well with distilled, deionized water (3-5 CVs).
5. Connect the column to your LC system in proper flow direction.
6. Equilibrate with your normal mobile phase.
7. Perform the recommended QC tests to ensure that the column is performing properly.

## **TROUBLESHOOTING**

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### **Instrumentation**

Although liquid chromatography is a highly reliable qualitative and quantitative technique, there are things that can go wrong, such as excessive back pressure, poor peak shape, or changes in retention or selectivity. The problem is either with

the column, the sample, the mobile phase or the instrumentation. Use a process of elimination to find out where the problem is. Does the problem still occur when you inject a standard (see Table 1) and another batch of mobile phase? If so, take the guardcolumn out of the system and again inject standards. If the problem is still there, then also take out the column. Directly connect the injector to the detector. If the problem still persists, then you have determined that the instrumentation is the problem. Otherwise, the column should be investigated.

## **Column**

The useful column lifetime is a function of factors such as: the cleanliness and composition of the mobile phase and the sample; the flow rate and pressure used; and the temperature. Refer to the section above about „Cleaning“, however, is not effective when the column is damaged by irreversible sample adsorption, channeling, exposure of the packing material to excessive heat, particle fracturing due to freezing of the mobile phase, or large pressure pulsations.

Column problems due to clogging of the top frit and a depression of the packed bed may be remedied by following the procedures described below. Also take a look at one of the troubleshooting guides, textbooks or expert programs that are on the market.

### **Clogged frit**

The inlet frit may become (partially) clogged by particulate matter derived from the sample, mobile phase or components in your liquid chromatography system. If completely clogged, this will result in increased column back pressure. The pressure does not increase in the case of partial clogging of the frit, although this can result in peak tailing or splitting due to uneven sample distribution.

Backflushing is often successful in cleaning the top frit. Follow these steps: stop the flow and let the pressure return to zero. Disconnect the column from injector and detector. Reconnect the column exit to the injector. Do not connect the column to the detector! Start the flow and flush the column for at least 30 minutes at half the standard flow rate. Monitor the pressure to be sure it does not exceed the maximum. Then, install the column again with the flow going in the direction of the arrow. Establish that the procedure has resulted in a lower pressure drop or in improved peak shape in the case of partial clogging. If not, clean and/or replace the end fitting (available from Tosoh Bioscience) as described below.

To clean or replace the end fitting, use a vise to carefully remove the old fitting from the top of the column, making sure not to disrupt the gel packing at the column top. Soak the fitting in a detergent solution for 5-10 minutes or sonicate in 6 M nitric acid. If not successful, replace the end fitting with a new one. Follow the instructions in the section titled „Installing the column“ to displace air from the column end fitting when reassembling the column.

## Filling voids

Column packings can sometimes settle, thereby creating a void or depression at the top of the column. To fill such voids, we recommend that you use SW- or PW -type top-off gels for gel filtration columns or one of the TSK Guardgel products or other TSKgel column types.

## RETURN POLICY

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Tosoh Bioscience is fully committed to quality products and service. TSKgel column products are accompanied by a chromatogram demonstrating the performance of a test mixture on the column and by a sheet that contains information about the OCS for the column.

Despite our commitment to product quality, columns and resins occasionally perform differently than expected in a customer's application. Therefore, we ask you to inspect your TSKgel column within 30 days of receipt by using the same conditions we employed to ensure product performance. Let us know within this 30-day period if the product does not meet the specifications on the OCS sheet and QC document, or as listed in the Inspection Data Sheet.

Subject to prior authorization, we will accept for return all products that do not perform according to their specifications. If a product is authorized for return for reasons other than Tosoh Bioscience's error or because of a product defect, there will be a restocking charge of 25 Euro or 10% of the list price, whichever is greater.

Tosoh Bioscience warrants only that the product will conform to its chemical descriptions. TOSOH BIOSCIENCE MAKES NO OTHER WARRANTIES, EXPRESS OR IMPLIED, AND EXPRESSLY DISCLAIMS ANY IMPLIED WARRANTY OF FITNESS FOR A PARTICULAR PURPOSE. IN NO EVENT SHALL TOSOH BIOSCIENCE BE RESPONSIBLE FOR SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES WHETHER THE CLAIM IS IN CONTRACT, NEGLIGENCE OR OTHERWISE.

## ADDITIONAL HELP

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If you require additional technical assistance, call Tosoh Bioscience at:  
Technical hotline: +49 6155-70437-36 or e-mail: techsupport.tbg@tosoh.com

*We hope your Tosoh Bioscience TSKgel column will help you  
achieve success with your research!*

TSKgel is a registered trademark of the Tosoh Corporation.

Teflon is a registered trademark of DuPont.

TSKgel columns, guardgels, and guardgel kits contain media manufactured under one or more of U.S. patents 4,224,415; 4,256,840; 4,382,124 and 4,501,826. The purchaser is entitled to use these products under U.S. patent 4,297,220.



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