HPLC COLUMN

INSTRUCTIONS

Contents

Introduction

Thank you for choosing an SGE analytical HPLC column. This HPLC column has been thoroughly tested to ensure optimum performance, and has been carefully packaged to ensure that it reaches you in good condition. If the column box shows significant damage, notify the carrier and your supplier at once and retain the evidence of shipping damage so that a claim can be made if the column does not meet performance criteria.

Operating Instructions

Testing Your Column

Each SGE column is tested separately and comes supplied with its own test report. This report details the conditions under which the column was tested. SGE recommends that this column be tested under identical conditions to confirm that it meets similar performance criteria. Only unmodified pumps and detectors are used for testing. SGE does use optimal conditions for testing HPLC columns so results within 20% of the reported values are acceptable, especially if an autosampler is used to introduce the sample.

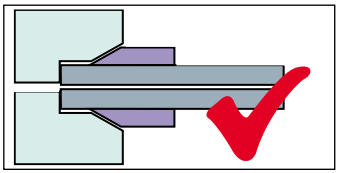


Figure 1. Pre-cut tubing, no dead volume, connected using a peek ferrule

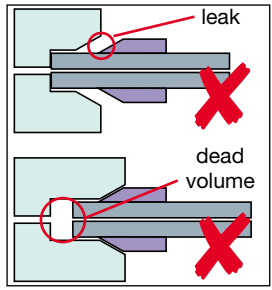


Figure 2. Stainless steel ferrules do not adjust for different manufacturers fittings leading to leaks or dead volume

HPLC Connections

⚠ ***WARNING:*** **Stainless Steel ferrules must not be used to connect Glass Lined Tubing (GLT) columns, as they can destroy the column. Use only PEEK ferrules or PEEK connectors (supplied with each column) to make connections.**

When connecting HPLC components with tubing it is vital that there is no dead volume in the connection. To achieve this it is important to press the tubing firmly into the HPLC component while tightening the nut (**figure 1**). This ensures that the tubing is properly seated thereby minimizing dead volume. The selection of ferrule is also important as manufacturers use different inserts into which the tubing and ferrule is seated. If stainless steel ferrules (not suitable for GLT column connections see **figure 2**) are used, the ferrule becomes permanently “swaged” or fused to the tubing making it useful only for connections to the one manufacturer’s components. For that reason SGE supplies only PEEK ferrules or PEEK one piece connectors. These fittings connect to any manufacturer’s components and when loosened, can be moved along the tubing to accommodate any other HPLC components. PEEK ferrules grip tubing at pressures in excess of 5000psi making them suitable for almost all HPLC applications.

⚠ *WARNING:* **If the LC system has not been operated for some time or if new tubing has been installed, make sure that the lines are flushed free of particles and contaminants before attaching the column.**

Tubing Selection

For optimum chromatography, it is recommended that minimal lengths of tubing be used to connect HPLC columns to the injector and detector. The appropriate HPLC tubing Interior Diameters (IDs) are listed in **table 1**.

TABLE 1 - Selecting Tubing ID

Column ID	Tubing ID	
mm	microns	inches
1-2	100	0.004
2-4.6	175	0.007
4-7	220	0.009

Protecting Your Column

SGE HPLC columns are designed and manufactured to give long service. To ensure long operating life, SGE recommends in-line filters and/or guard cartridges are connected before the column as illustrated in **figures 1-3**. SGE in-line filters and guard cartridges can easily be coupled to HPLC columns or components using a column coupler **(P/N 200009)**. SGE components are designed to be compatible with HPLC components from other manufacturers so it is easy to connect SGE guards and in-line filters to other manufacturer's columns as required.

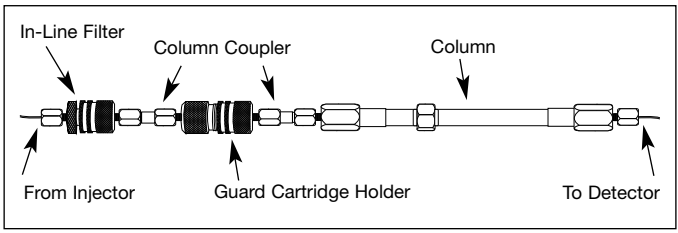


Figure 3. How to set up your HPLC Column

• *Pressure and Temperature Limits*

SGE HPLC columns are all silica based. This means that they have an upper pressure limit of 6000psi. To ensure optimal column life, operating pressures of 3000psi or lower are recommended. Column pressures will usually increase as the column ages, though sudden increases in pressure are usually a result of a blocked frit (See column maintenance). Pressure will vary with different mobile phases. For example Water/Methanol mixtures will generally give higher back pressure than Water/Acetonitrile mobile phases. SGE HPLC columns can be used at temperatures of up to 60°C. For optimum reproducibility columns should be thermostated at between 30°C and 40°C.

• *Solvent Filter*

A solvent filter **(P/N 204000)** should always be connected to the inlet lines inside the solvent bottles containing mobile phase solvents. All mobile phases should be pre filtered prior to use. SGE solvent filters are non metal making them fully bio compatible.

• *In-Line Filter*

SGE in-line filters **(P/N 204002)** are designed to capture particulates that may come off the pump or be introduced by samples. In-line filters generally contain 0.5 micron frits to catch fine particulates that would otherwise collect in the column over time causing blockages. The frits in SGE in-line filters are made of titanium making them suitable for most bio applications. In-line filters should be replaced when the in-line filter contributes excessive back pressure to the HPLC system.

• *Guard Cartridges*

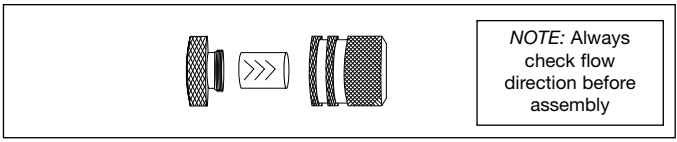


Figure 4. Assembly of In-Line Filter / Guard Cartridge holder

⚠ ***WARNING:*** **Stainless Steel ferrules must not be used to connect SGE guard cartridges, as they can destroy the cartridges. Use only PEEK ferrules or PEEK connectors (supplied with each column) to make connections.**

Guard cartridges are used to capture impurities that may otherwise lodge on the HPLC column. Guard cartridges are especially useful with samples from biological sources as these may contain lipids and proteins that pass through frits

but can quickly block columns. Guard cartridges also protect columns from particulates but not as effectively as in-line filters. Guard cartridges should have the same phase as the column they are protecting. SGE guard cartridges are made from GLT and are fitted with titanium frits making them suitable for most bio applications. SGE guard cartridges require a holder **(P/N 205000)** for connection to HPLC systems. Guard cartridges should be replaced when the chromatography begins to deteriorate or when the guard cartridge contributes excessive back pressure to the HPLC system. High throughput laboratories replace guards at fixed times or after certain numbers of injections, depending on the sample.

Flow Rate Selection

HPLC columns have optimum flow rates at which they have higher efficiencies. These flow rates depend on the interior diameter of the column as shown in **table 2**.

TABLE 2 - Optimum Flow Rates

Column ID	Optimum Flow Rate
4.6mm	1mL / min
4.0mm	0.75mL / min
2.0mm	0.20mL / min

Mobile Phase Selection

SGE HPLC columns are compatible with all typical mobile phases used with most HPLC columns.

- *pH Limits*

- Exsil columns have an operating pH range of 2-7.
- Accurasil™ columns have an operating pH range of 2-7.
- Wakosil II 5C18 AR has a pH range of 1.4-9.4. All other Wakosil columns have a pH range of 2-7.5.
- Nucleosil ODS can be used in a pH range of 1-9 if organic buffers are used, though pH range of 2-7 is recommended.

- *Reverse Phase Recommendations*

Reversed phase separations are the most popular HPLC technique. It is recommended that Wakosil II columns used for isocratic separations always contain at least 5-10% organic solvent (acetonitrile or methanol is suitable). If 100% aqueous mobile phases are used with Wakosil II columns, mobile phase collapse will gradually occur leading to shifting retention times. If this occurs flushing with 100% methanol can easily regenerate the Wakosil column.

Column Maintenance

These simple precautions should be followed to ensure the longest possible column life.

1. Columns should not be subjected to mechanical or pressure shock, which can cause irreversible damage to the column.
2. Columns should be pumped in the flow direction as marked on the column. Columns can be reversed to flush frits if blockages occur.
3. Do not store the column in an aqueous buffer as this will promote microbiological contamination. First flush the column with water and then with 50/50 acetonitrile/water prior to storage.

• *Preventative Maintenance*

Weekly cleaning of the column is strongly recommended. If untreated biological samples are injected, then the column should be cleaned more frequently. Follow the specific washing procedure for your column phase. This is usually sufficient to maintain a column in near new condition.

NOTE: Excessive accumulation of impurities will lead to permanent damage of the packing.

Column Log

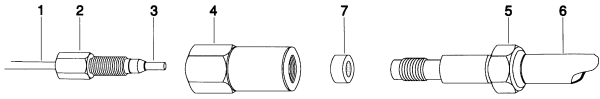
Keeping a simple log sheet facilitates column maintenance. This ensures information for trouble shooting is readily available. Suggested headings include: date, operator, sample, temperature, mobile phase(s), flow rate, storage solvent, and comments.

Back Pressure

High back pressures increase the wear and tear on a HPLC system so that increased maintenance and down time may result. For this reason it is important to make sure that any system components that contribute to higher than usual back pressure be replaced. With a properly set up HPLC system it will be guard cartridges and in-line filters that need replacement. Occasionally a column may be the source of back pressure in which case it is best to try and clear it by pumping mobile phase through the reversed columns, making sure that the column is not connected to the detector. If this is unsuccessful then the inlet frit should be replaced as described below. If the column still generates high pressure it should be washed as described in Specific Maintenance.

Frit Replacement

• *Glass Lined Columns (GLT)*



1. Disconnect 1/16” tubing **(1)** and Male Nut **(2)** from each end of the column.

2. Support the column **(6)** vertically.

3. Using two spanners, remove the stainless steel endfitting **(4)**.

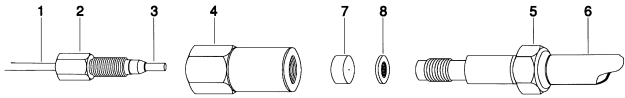
NOTE: Use the Hex sleeve nut **(5)** to ensure that the column does not rotate during endfitting removal.

4. Screw a frit assembly removal bolt (available from your SGE supplier) into the endfitting **(4)** to remove the frit assembly **(7)**.

5. Locate the new frit assembly in the endfitting **(4)** ensuring the frit **(7)** is faced against the end of the column.

6. The distance between the ferrule and the tubing end **(3)** is different for different brand HPLC columns. SGE recommends that only PEEK nuts (supplied) or ferrules can be used for column connections.

• *Stainless Steel Columns*



1. Disconnect 1/16” tubing **(1)** and Male Nut **(2)** from each end of the column.

2. Support the column **(6)** vertically.

3. Using two spanners, remove the stainless steel endfitting **(4)**.

NOTE: Use the Hex sleeve nut **(5)** to ensure that the column does not rotate during endfitting removal.

4. Screw a frit assembly removal bolt (available from your SGE supplier) into the endfitting **(4)** to remove the frit assembly **(8)**.

5. Locate the stainless steel insert **(7)** in the endfitting **(4)**, making sure that the flat face is up. Now place the frit assembly **(8)** into the endfitting and screw the endfitting back onto the column using two spanners to ensure that the endfitting does not leak.

6. The distance between the ferrule and the tubing end **(3)** is different for different brand HPLC columns. SGE recommends that PEEK nuts (supplied) on ferrules be used for connections. If SS ferrules are to be used, a new connection should be made with fresh ferrules and connecting tubing.

Specific Maintenance

• *Reverse Phase*

– Washing Procedure for Reverse-Phase Columns

Washing the column successively with non-polar eluents will usually remove accumulated impurities. Follow the washing sequence below, using 30ml of each solvent, to thoroughly clean the column.

1. Distilled water 90%, 10% Methanol
2. 0.5M H3P04 90%, 10% Methanol (Optional)*
3. Distilled water 90%, 10% Methanol (Optional)*
4. Methanol
5. Methanol/Chloroform (1:1) (Optional)
6. Methanol or Acetonitrile (Optional)
7. Distilled water 90%, 10% Methanol
8. Eluent to recondition column

*NOTE: Whenever step 2 is used, it must be followed by step 3. Silica based packings are very quickly attacked by compounds such as Tetrabutyl ammonium phosphate. Never leave these materials in the column any longer than is necessary to complete the analysis. Flush the column with water at the completion of the analysis and store with the 50%water/50% methanol (or acetonitrile) in the column.

- Protein Contamination

Proteins can adsorb onto columns causing loss of performance. In this situation rinse the column overnight with 20% 0.1M nitric acid/80% isopropanol at a flow rate of 1/5th the usual (i.e. at 0.2 ml/min for 4.6mm ID columns).

- Lipid Contamination

If lipids or other highly hydrophobic compounds have contaminated the column use the full washing procedure except replace step 5 with 100% chloroform or dichloromethane.

- Column Storage

Before storing reverse-phase columns for extended periods they should be washed as set out under “Washing Procedure for Reverse-Phase Columns” (steps 1-6). The column should be stored filled with 50-100% methanol (or acetonitrile) and the balance water. The ends should be plugged to prevent evaporation of the solvent. For short term storage (ie. up to 3 weeks) flushing with water then methanol will suffice. Plug the column to prevent it from drying out. If a column dries out simply condition it by pumping through 30ml of methanol. Store the column in a cool place where it will not be jarred or dropped.

• *Normal Phase*

- Washing procedure for Normal-Phase Columns

Follow the washing sequence below, using 30mls of each (dry) solvent, to thoroughly clean the column.

1. Heptane
2. 1,1,1, Trichloromethane
3. Ethylacetate
4. Acetone
5. Acetonitrile
6. Eluent

- Column Storage

Before storing normal-phase columns for extended periods, they should be thoroughly washed as set out under "Washing Procedure for Normal-Phase Columns". The normal-phase column should be washed with the same solvents in reverse order (i.e. 5-1) so that heptane is the final rinse. Plug the column with heptane inside and store it in a cool place where it will not be jarred or dropped.

• *Ion Exchange*

- Washing Procedure for Ion-Exchange Columns

Follow the washing sequence below, using 30ml of each solvent, to regenerate either cationic or anionic exchange columns.

1. Distilled water
2. Complexer wash - 0.1M disodium EDTA
3. Distilled water*
4. Acetonitrile - to remove absorbed organics from the organic substrate
5. Distilled water
6. Eluent

* It is important to wash with distilled water between the acetonitrile wash and the other washes as acetonitrile may cause precipitation of the buffer salts which will block and damage the column.

- Column Storage

Before storing ion-exchange columns, wash all buffers from the column with distilled water to prevent the crystallisation of salts which could block the column. Then fill the column with 50% acetonitrile (or methanol)/ 50% water, plug the ends and store the column in a cool place where it will not be jarred or dropped.

Part Number Listing

Analytical Columns

Part No.	Column Description	
206510	Wakosil II 5C8 RS 5µm	250x4.6mm SS
206610	Wakosil II 5C8 RS 5µm	150x4.6mm SS
207130	Wakosil II 5C8 RS 5µm	250x4.0mm GL
2065052	Wakosil II 3C18 RS 3µm	250x4.6mm SS
2066052	Wakosil II 3C18 RS 3µm	150x4.6mm SS
2070262	Wakosil II 3C18 RS 3µm	250x4.0mm GL
2070252	Wakosil II 3C18 RS 3µm	150x4.0mm GL
2085052	Wakosil II 3C18 RS 3µm	50x4.0mm GL
206505	Wakosil II 5C18 RS 5µm	250x4.6mm SS
206605	Wakosil II 5C18 RS 5µm	150x4.6mm SS
208605	Wakosil II 5C18 RS 5µm	50x4.6mm SS
207026	Wakosil II 5C18 RS 5µm	250x4.0mm GL
207025	Wakosil II 5C18 RS 5µm	150x4.0mm GL
207029	Wakosil II 5C18 RS 5µm	250x2.0mm GL
207028	Wakosil II 5C18 RS 5µm	150x2.0mm GL
208205	Wakosil II 5C18 RS 5µm	50x2.0mm GL
207080	Wakosil II 5C18 RS 5µm	250x1.0mm GL
207081	Wakosil II 5C18 RS 5µm	150x1.0mm GL
207082	Wakosil II 5C18 RS 5µm	100x1.0mm GL
2065050	Wakosil II 5C18 AR 5µm	250x4.6mm SS
2066050	Wakosil II 5C18 AR 5µm	150x4.6mm SS
2070260	Wakosil II 5C18 AR 5µm	250x4.0mm GL
2070250	Wakosil II 5C18 AR 5µm	150x4.0mm GL
2065051	Wakosil II 5C18 HG 5µm	250x4.6mm SS
2066051	Wakosil II 5C18 HG 5µm	150x4.6mm SS
2065030	Exsil C8 5µm	250x4.6mm SS
2066030	Exsil C8 5µm	150x4.6mm SS
207100	Exsil C8 5µm	250x4.0mm GL
2065010	Exsil ODS 3µm	250x4.6mm SS
2066010	Exsil ODS 3µm	150x4.6mm SS
2062010	Exsil ODS 3µm	100x4.6mm SS
2086010	Exsil ODS 3µm	50x4.6mm SS

Analytical Columns

Part No.	Column Description	
206501	Exsil ODS 5µm	250x4.6mm SS
206601	Exsil ODS 5µm	150x4.6mm SS
206201	Exsil ODS 5µm	100x4.6mm SS
207001	Exsil ODS 5µm	250x4.0mm GL
207002	Exsil ODS 5µm	150x4.0mm GL
207007	Exsil ODS 5µm	250x2.0mm GL
2070091	Exsil ODS 5µm	150x2.0mm GL
207009	Exsil ODS 5µm	100x2.0mm GL
208201	Exsil ODS 5µm	50x2.0mm GL
206508	Exsil Cyano 5µm	250x4.6mm SS
206608	Exsil Cyano 5µm	150x4.6mm SS
207600	Exsil Cyano 5µm	250x4.0mm GL
2065069	Exsil Phenyl 5µm	250x4.6mm SS
2066069	Exsil Phenyl 5µm	150x4.6mm SS
206507	Exsil Amino 5µm	250x4.6mm SS
206607	Exsil Amino 5µm	150x4.6mm SS
207500	Exsil Amino 5µm	250x4.0mm GL
206509	Exsil Silica 5µm	250x4.6mm SS
206609	Exsil Silica 5µm	150x4.6mm SS
206512	Exsil SAX 5µm	250x4.6mm SS
206612	Exsil SAX 5µm	150x4.6mm SS
207400	Exsil SAX 5µm	250x4.0mm GL
206514	Exsil SCX 5µm	250x4.6mm SS
206614	Exsil SCX 5µm	150x4.6mm SS
207300	Exsil SCX 5µm	250x4.0mm GL
2065014	Nucleosil 300 ODS 5µm	250x4.6mm SS
2066014	Nucleosil 300 ODS 5µm	150x4.6mm SS
207018	Nucleosil 300 ODS 5µm	100x2.0mm GL
2082014	Nucleosil 300 ODS 5µm	50x2.0mm GL
207038	Nucleosil 300 ODS 5µm	250x1.0mm GL
207039	Nucleosil 300 ODS 5µm	150x1.0mm GL
207040	Nucleosil 300 ODS 5µm	100x1.0mm GL
2081014	Nucleosil 300 ODS 5µm	50x1.0mm GL

Analytical Columns

Part No.	Column Description	
206520	Accurasil C18 5µm	250 x 4.6 SS Pk3
206521	Accurasil C18 5µm	250 x 4.6 SS Pk1
206620	Accurasil C18 5µm	150 x 4.6 SS Pk3
206621	Accurasil C18 5µm	150 x 4.6 SS Pk1

Guard Cartridges

Part No.	Column Description	
2050192	Wakosil II 3C18 RS 3µm	10x4mm Guard Pk3
205016	Wakosil II 5C8 RS 5µm	10x4mm Guard Pk3
205019	Wakosil II 5C18 RS 5µm	10x4mm Guard Pk3
2050191	Wakosil II 5C18 HG 5µm	10x4mm Guard Pk3
2050190	Wakosil II 5C18 AR 5µm	10x4mm Guard Pk3
209205	Wakosil II 5C18 RS 5µm	10x2mm Guard Pk3
209105	Wakosil II 5C18 RS 5µm	10x1mm Guard Pk3
2050014	Nucleosil 300 ODS 5µm	10x4mm Guard Pk3
2092014	Nucleosil 300 ODS 5µm	10x2mm Guard Pk3
2091014	Nucleosil 300 ODS 5µm	10x1mm Guard Pk3
205002	Exsil Silica 5µm	10x4mm Guard Pk3
205005	Exsil SCX 5µm	10x4mm Guard Pk3
205006	Exsil SAX 5µm	10x4mm Guard Pk3
205008	Exsil Phenyl 5µm	10x4mm Guard Pk3
205001	Exsil ODS 5µm	10x4mm Guard Pk3
2050010	Exsil ODS 3µm	10x4mm Guard Pk3
205007	Exsil Cyano 5µm	10x4mm Guard Pk3
205003	Exsil C8 5µm	10x4mm Guard Pk3
205004	Exsil Amino 5µm	10x4mm Guard Pk3
209201	Exsil ODS 5µm	10x2mm Guard Pk3
2050015	Accurasil C18 5µm	10x4mm Guard Pk3

NOTE: Guard Cartridges require Guard Cartridge Holder P/N 205000

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SGE International Pty. Ltd.
Toll Free: 1800 800 167
Tel: +61 (0) 3 9873 4200
Fax: +61 (0) 3 9874 5672
e-mail: support@sge.com

SGE, Incorporated (USA)
Tel: (800) 945 6154
Fax: (512) 836 9159
e-mail: usa@sge.com

SGE Europe Ltd. (UK)
Tel: +44 (0) 1908 568 844
Fax: +44 (0) 1908 566 790
e-mail: uk@sge.com

SGE (France) Sarl
Tel: +33 (0) 1 69 29 80 90
Fax: +33 (0) 1 69 29 09 25
e-mail: france@sge.com

SGE Italia Srl.
Tel: 06 4429 0206
Fax: 06 4429 0724
e-mail: sge.italia@tin.it

SGE Deutschland GmbH
Tel: +49 (0) 6151 860486
Fax: +49 (0) 6151 860489
e-mail: germany@sge.com

SGE China
Tel: +86 (10) 6588 8666
Fax: +86 (10) 6588 6577
e-mail: sge@bj.col.com.cn

SGE Japan Inc.
Tel: +81 45 222 2885
Fax: +81 45 222 2887
e-mail: japan@sge.com

SGE India
Tel: +91 (022) 471 5896
Fax: +91 (022) 471 6592
e-mail: sgeindia@vsnl.com

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