

# Instruction manuals

## Develosil® Packed column for HPLC

Thank you for purchasing a Develosil® HPLC packed column. Please read and follow this instruction manual carefully to ensure that your column provides reliable chromatography. Failure to comply with this instruction manual may void the column warranty.

### After receiving the product

- Please check that there are no abnormalities in the column appearance and packaging.
- Please check that the product name, column size, connection joint, etc. are correct.
- A shipping inspection report is always attached to the column. This report contains the filler lot (batch) number, manufacturing number, etc., so please keep it carefully until the end.
- The solvent for testing is sealed in the column (described in the report), so please be careful of precipitation when replacing the solvent.

### Precautions for use

- Do not drop or otherwise subject the column to shock. This can seriously affect column performance.
- Please note that the seal may be damaged due to impact such as dropping and may become unusable.
- Please use the column under 20MPa (2900psi, 200bar).

### Column connections

- Before installing the column, be sure to thoroughly replace the inside of the equipment and piping with the solvent to be used.
- Follow the arrow marked on the column body for the direction of liquid flow through the column.
- The selection of piping inner diameter greatly affects column performance, so please use appropriate piping.
- Be sure to wait until the pressure display reaches "0" before removing the column.

### Recommended column inner diameter and piping inner diameter

Column inner diameter	1.0mm	2.0mm	3.0mm	4.6mm	10mm	20mm
Flow rate	0.05mL/min	0.2mL/min	0.4mL/min	1.0mL/min	5.0mL/min	19mL/min
Flow cell	semi-micro	semi-micro	For analysis	For analysis	For analysis	For preparative separation
Recommended piping	0.05mm	0.10mm	0.15	0.15	0.15	0.50
Inner diameter			-0.30mm	-0.30mm	-0.30mm	-1.0mm

## Analysis

- Impurities in the mobile phase may cause phenomena such as ghost peaks. To avoid this, please use highly pure solvents and perform treatments such as filtration as necessary.
- When using a buffer solution as the mobile phase, be sure to confirm that no precipitate is generated.
- To remove impurities, use a mobile phase that has been filtered through a membrane filter of 0.45 µm or less.
- If the mobile phase contains a large proportion of water, the retention time may be extremely shortened by stopping the liquid flow. In that case, run the analysis again after passing a mobile phase containing about 60% acetonitrile or methanol.
- When heating the column, please be careful as air bubbles will be generated near the boiling point of the solvent, which may cause noise.
- Please set the column temperature to 50°C or less.
- Dissolve the sample in the mobile phase as much as possible.
- Although the Develosil series has a specified pH range, the column life will vary depending on conditions such as the type of buffer solution used, temperature, and mobile phase composition. In order to perform stable analysis over a long period of time, we recommend analyzing with a composition that contains a low concentration of buffer solution, additives, and organic solvent. For the usable pH range, please see "List of usable pH ranges".

## Recommended buffer solution type

Usable pH range	Recommended buffer solutions and additives	Recommended concentration
1-2	TFA, acetic acid, formic acid, phosphoric acid, etc.	0.1-0.5%
2-8	Acetic acid, formic acid, ammonium phosphate, etc.	5-50mM
9-10	Sodium phosphate, triethylamine, etc.	up to about 5mM

## How to clean and store columns

### 【Reverse phase system】

#### Washing:

When using solvents containing buffer solutions or ion pair reagents, it is necessary to wash the column to ensure that no solvents remain inside the column. After use, clean with a solvent of the same composition.

Example: Analysis conditions Methanol/25mM ammonium phosphate (pH7.0)=50/50

For washing time, set the flow rate to half that of analysis, and use a volume of 10 times the column volume as a guide.

#### Storage:

- After cleaning, immediately close the container tightly with the included plug and store it in a place with minimal temperature changes.
- Under 100% water mobile phase conditions, replace with acetonitrile/water or methanol/water before storage.

Please avoid storing only in water for long periods of time.

Applicable column: ODS, ODS-K, ODS-N, ODS-P, ODS-T, ODS-A,TMS, C8, Ph, PhA, CN, NH<sub>2</sub>, ODS-HG, ODS-UG, ODS-MG, ODS-SR, PAHS,C30-UG, RPAQUEOUS, RPFULLERENE, Combi-RP, RPAQUEOUS-AR,TMS-UG, C8-UG, Ph-UG, CN-UG, XG-C18M, XG-C18LC, XG-C30M, XG-C1, XG-CN, HSR C18, HSR AQ C18, HSR C1, 300 ODS-HG, 300 ODS-UG, 300 C8-HG, 300 C4-HG, ANIDIUS, HB series

**【Normal phase system】****Washing:**

When using additives such as acetic acid, it is necessary to clean the column so that no additives remain inside the column. After use, clean with a solvent of the same composition.

Example: Analysis conditions hexane/ethanol/acetic acid = 99/1/0.1

Cleaning method Hexane/ethanol=99/1

For washing time, set the flow rate to half that of analysis, and use a volume of 10 times the column volume as a guide.

When a highly adsorbed sample is present, it is effective to increase the composition of ethanol etc. for cleaning.

**Storage:**

- After cleaning, immediately close the container tightly with the included plug and store it in a place with minimal temperature changes.

Applicable columns: 30, 60, 100

NH<sub>2</sub>, CN, CN-UG, XG-CN, 100 Diol, 300 Diol, ANIDIUS used in normal phase mode

**【Column for gel filtration】****Washing:**

Gel filtration chromatography often uses highly concentrated buffer solutions. Therefore, please be careful as acids and salts tend to remain.

Example: Analysis conditions 0.1M phosphate buffer solution + 0.2M NaCl (pH6.8)

Cleaning method Water

For washing time, set the flow rate to half that of analysis, and use a volume of at least 10 times the column volume as a guide.

**Storage:**

- After cleaning, immediately close the container tightly with the included plug and store it in a place with minimal temperature changes.

- If stored for a long time, replace with 0.05% sodium azide before storing.

Applicable columns: 100 Diol, 300 Diol

**Switching from reversed phase solvent to normal phase solvent**

Develosil NH<sub>2</sub>, CN, CN-UG, XG-CN, 100 Diol, 300 Diol, ANIDIUS can also be used in normal phase mode.

When switching solvents, please follow the steps below.

Example: Switching Develosil® NH<sub>2</sub> from acetonitrile/water = 60/40 to hexane/ethanol = 99/1

1) Replace the inside of the column with ethanol

In order to prevent a sudden increase in pressure, set the flow rate to a low level, and aim to pass 10 times the column volume.

2) Pass hexane/ethanol = 99/1.

Although ethanol is used in the above example, please choose a solvent that mixes with both the reversed phase solvent and the normal phase solvent.