

For High Performance Liquid Chromatography Develosil Series Care and Use Manual

Thank you for purchasing Develosil series. Please contact us at info@develosil.us if you have any problems regarding your Develosil column.

To ensure sufficient performance of this product and its long-term use, please read and understand this Care and Use Manual thoroughly before using the product. The product is shipped after strict performance check to provide a reliable column.

1 · Checking contents

- When the product is delivered to you, please check that there are no defects and damage.
- Please check that you have received the HPLC/UHPLC column that you have ordered. Check the product name, column size, connection type of Develosil series you have ordered.
- This packaging includes the following items.
 - Column · Test report · Certification of analysis
- Develosil series are managed with product numbers and batch numbers. Therefore, please keep the test report.

2 · Handling

- Any shocks such as dropping and striking to the product may result in damage to its structure and performance.
- As a guideline column pressure use less than 20 MPa (2900 Psi, 200 bar).
 - ※Develosil HSR series = 25 MPa (3625 psi, 250 bar)
 - Develosil HB series = 50 MPa (7251 psi, 500 bar)

3 · Connection

- Before installing the column, replace the equipment and the piping with mobile phase.
- Flow through according to the arrow marked on the column body.
- Selection of pipe inner diameter greatly affects column performance, so please use appropriate tubing.
- When removing the column, please do after the pressure display becomes "0".

Recommended column inner diameter and tube inner diameter

Column i.d.	1.0 mm	2.0 mm	3.0 mm	4.6 mm	10 mm	20 mm
Flow	0.05 mL/min	0.2 mL/min	0.4 mL/min	1.0 mL/min	5.0 mL/min	19 mL/min
Flow cell	Semi-micro	Semi-micro	Analytical	Analytical	Analytical	Analytical
Tube i.d.	0.05 mm	0.10 mm	0.15-0.30 mm	0.15-.30 mm	0.15-0.30 mm	0.50-1.0 mm

4 · Analysis

- Column is shipped in the test report. When feeding a mobile phase, make sure that the compatibility of the solvents as well as salt precipitation.
- Make sure to filtrate the mobile phase using a 0.45 µm membrane filter before using the column.
- When high water content mobile phase is used, extreme change in the retention time may be observed when the flow of the mobile phase is stopped. In such a case, wash the column with mobile phases containing 60% Acetonitrile or Methanol, and then repeat the analysis.
- As a guideline column temperature use less than 50°C.
- Sample should be dissolved in the mobile phase or solvent weaker than mobile phase, which helps avoid sample precipitation at the column inlet/head and inconsistent retention values.
- The Develosil series has a pH range of use, but the life of the column will change depending on conditions such as the type of buffer solution used, temperature, mobile phase composition, etc. For long-term, stable analysis it is recommended to analyze with a composition containing low concentration buffer solution, additives and organic solvents. Please refer to "pH range used" for the pH range used.

Recommended type of buffer solution

pH range	Type of buffer	Concentration
1-2	TFA, Acetic acid, Formic acid, etc.	0.1-0.5%
2-8	Ammonium acetate, Ammonium formate, etc.	5-50 mM
9-10	TEA, Tris, etc.	5 mM or less

5 · Column washing and storage method

A. Columns for Reverse-Phase

i. Column washing procedure :

When using solvents containing buffer solutions or ion pair reagents, it is necessary to wash them so as not to leave them in the column. After use, wash with solvent of the same composition.

Example: Analytical eluent- MeOH/25 mM (NH₄)₂HPO₄ (pH7.0) =50/50

Washing eluent- Methanol/Water=50/50

Flush the column ten times the column volume at a reduced flow rate (approximately 50% lower than the normal flow rate).

ii. Storing of columns :

- After washing, seal the column with the plugs provided and store at temperature stable place.
- For water 100% mobile phase conditions, replace with acetonitrile / water or methanol / water before storing. Please keep away from long-term storage only with water.

iii. Applicable Columns:

ODS-K, ODS-N, ODS-P, ODS-T, ODS-A, TMS, C8, Ph, PhA, CN, NH₂, 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, HSR C18 Peptide, 300ODS-HG, 300ODS-UG, 300C8-HG, 300C4-HG, ANIDIUS, HB series

B. Columns for Normal-Phase

i. Column washing procedure :

When using additives such as acetic acid, it is necessary to wash them so as not to leave them in the columns. After use, wash with solvents of the same composition.

Example: Analytical eluent- Hexane/Ethanol/Acetic

acid = 99/1/0.1 Washing eluent- Hexane/Ethanol=99/1

Flush the column ten times the column volume at a reduced flow rate (approximately 50% lower than the normal flow rate).

It is effective to increase the ethanol content when the sample adsorption is strong.

ii. Storing of columns :

- After washing, seal the column with the plugs provided and store at temperature stable place.

iii. Applicable Columns

Columns specific to normal phase : 30, 60, 100,

Columns that can be used for Normal-Phase: NH₂, CN, CN-UG, XG-CN, 100 Diol, 300 Diol, ANIDIUS

C. Columns for Gel Filtration

i. Column washing procedure :

High concentration of buffer solution may be used for gel filtration chromatography. After using, wash the column enough because acid and salt tend to remain.

Example : Analytical eluent- 0.1 M Buffer + 0.2 M NaCl
(pH6.8) Washing eluent- Pure water

Flush the column ten times the column volume at a reduced flow rate (approximately 50% lower than the normal flow rate).

ii. Storing of columns :

- After washing, seal the column with the plugs provided and store at temperature stable place.
- When storing the column for a long period, replace with 0.05% sodium azide aqueous solution.

iii. Applicable columns

100 Diol, 300 Diol

6 · Switching from Reversed-Phase to Normal-Phase

They can be also used with Normal-Phase (Develosil NH₂, CN, CN-UG, XG-CN, 100 Diol, 300 Diol, ANIDIUS). When switching the eluent of columns, follow the procedure below.

Example Column: Develosil NH₂

Replacing eluent from Acetonitrile/Water = 60/40 to Hexane/Ethanol = 99/1

- 1) Replace the eluent to Ethanol.
Flush the column ten times the column volume at a reduced flow rate to avoid sudden pressure increases.
- 2) Flush the column with Hexane/Ethanol = 99/1

Use the mutually miscible eluent such as ethanol when switching from Reversed-Phase to Normal-Phase.

pH range list

Packing Name	pH range
1st generation	
ODS	pH2.0-7.5
ODS-K	
ODS-N	
ODS-P	
ODS-T	
ODS-A	
TMS	pH2.0-7.5
C8	
Ph	
PhA	
CN	
NH ₂	
2nd generation	
ODS-HG	pH1.0-9.0
ODS-UG	pH2.0-10.0
ODS-MG	pH2.0-7.5
ODS-SR	pH2.0-7.5
PAHS	pH2.0-7.5
C30-UG	pH2.0-8.0
RPAQUEOUS	
RPFULLERENE	
Combi-RP	pH1.0-8.0
RPAQUEOUS-AR	
TMS-UG	pH2.0-7.5
C8-UG	
Ph-UG	
CN-UG	
3rd generation	
XG-C18M	pH1.5-8.0
XG-C18LC	
XG-C30M	
XG-C1	
XG-CN	
4th generation	
HSR C18	pH1.0-10.0
HSR AQ C18	pH2.0-9.0
HSR C1	pH2.0-8.0
Wide Pore	
300 ODS-HG	pH2.0-7.5
300 ODS-UG	
300 C8-HG	
300 C4-HG	
Gel Filtration	
100 Diol	pH2.0-7.5
300 Diol	

Packing Name	pH range
HILIC	
ANIDIUS	pH2.0-7.5
Normal Phase	
30	pH2.0-7.0
60	
100	