

Column Care and Use Instructions

Pro series

YMC-Pack *Pro* C18, *Pro* C18 RS, *Pro* C8, *Pro* C4 /Hydrosphere C18

1. Introduction

Thank you for purchasing a YMC high-performance liquid chromatography (HPLC) column “*Pro* series”.

Pro series, which are based on ultra high purity silica gel and adopted the advanced endcapping technology, provide excellent separations with symmetrical peaks for a wide range of compounds, especially basic pharmaceuticals.

Pro series, which are manufactured under highly controlled conditions, must pass a series of stringent tests before being accepted for shipment. (Please refer to the column inspection report). To ensure optimal performance and durability of the column, please read these instructions carefully before using this column.

2. Specifications

	Packing material	Particle size (µm)	Pore size (nm)	C%	Usable pH range	Usable temperature range	
						Regular use (recommended)	Upper limit
<i>Pro</i> series	<i>Pro</i> C18	3, 5, 10	12	16	2 – 8	20 – 40 °C	50 °C
	Hydrosphere C18	3, 5	12	12			
	<i>Pro</i> C18 RS	3, 5	8	22	1 – 10		
	<i>Pro</i> C8	3, 5	12	10	2 – 7.5		
	<i>Pro</i> C4	3, 5	12	7			

The “WT” or “PTH” at the end of the product code indicates the style of column endfittings.

WT = Waters style / PTH= Parker style

3. Shipping solvent

Indicated in the COLUMN INSPECTION REPORT. Replace with this solvent for storage. When replacing a mobile phase containing buffer salts/additives, extra care must be taken to prevent salt precipitation.

4. Mobile phase

- The correct direction of the solvent flow is indicated by an arrow on the column identification label.
- Aqueous or non-aqueous solvent can be used as a mobile phase. Repetitive replacement among solvents with large difference in polarities might degrade the column performance. In general, organic solvents are acetonitrile, methanol and tetrahydrofuran (THF) are recommended for regular use. When using THF as a mobile phase, be mindful of the solvent resistance of your system or tubing (PEEK parts are especially unsuitable for use with THF).
- Recommendations of pH for column use are shown in the specifications table in section 2. When using the column at pH near the upper or lower limit, the column lifetime will shorten under certain conditions by temperature and mobile phase composition. In addition, please note the following.
 - For all products: When using the column at pH near the limit, mobile phase containing 10% concentration of organic solvent should be used.
 - For Hydrosphere C18: Although Hydrosphere C18 is compatible with 100% aqueous mobile phase, 10% concentration of organic solvent should be contained when using the column at pH near the limit.
 - For *Pro* C18 RS: When using the column at pH more than 9, mobile phase containing 50% concentration of organic solvent should be used.
- When using *Pro* C18 RS column, its extremely high hydrophobicity might cause difficulties of replacement or equilibration with mobile phase containing low concentration of organic solvent. The mobile phase should be contained more than 10% of methanol or 5% of other lower polar organic solvent. When replacing methanol aqueous solution with acetonitrile aqueous solution, mobile phase containing less than 20% of acetonitrile may result in irregularities in retention time or peak shapes. In these cases, first replace with 60% acetonitrile aqueous solution, and then replace with the mobile phase.

5. Column cleaning (general method)

- Flush the column with solution containing a higher ratio of organic solvent for washing out the compounds that have a great capacity for retention in the column after using mobile phases not containing buffer salts/additives. Usable concentration of organic solvent is up to 100%. A cleaning solution containing THF might be effective when removing highly hydrophobic (lipid-soluble) substances that are adsorbed onto the gel.
- When using mobile phase containing buffer salts/additives, first replace with a water/organic solution containing no buffer salts/additives (A ratio of water to organic solvent should be set at the same proportions as a mobile phase). Then flush the column in accordance with the method described above. Mobile phase containing about 50 mM or less in buffer salts/additives can be replaced directly with 60% acetonitrile aqueous solution.
- Flushing with 100% water after using the column around the pH limit might shorten the column lifetime. Flush the column with water/organic solution as described above, such as 60% acetonitrile aqueous solution.
- Once macromolecules such as proteins or polysaccharides are adsorbed onto the gel, they are hardly removed, even if solvents with high eluting capability are used. To avoid contamination of the column by them, conduct sample pretreatment carefully before introduction into the column. Alternatively use a guard column.

6. Other environments

- The operating pressure should be kept under 20 MPa (2900 psi) for 150 mm length column or less, under 25 MPa (3625 psi) for 250 mm length column, under 10 MPa (1450 psi) for 10 mm I.D. column or more. For 3 μ m particle size, the operating pressure should be kept under 25 MPa (3625 psi) with no dependence on column length.
- To prevent exposure of the column to excessive pressure, the sample solution should be filtered through a 0.2 μ m membrane or smaller to remove particulates. We recommend using a pre-column filter to prevent the column frit from being clogged with samples.
- Avoid using a column repeatedly near the pressure limit or abrupt change in pressure to prevent shortening of the column life.
- Adjust the flow rate appropriately because the pressure changes depending on the column length, temperature, types of organic solvent etc.
- Recommendations of temperature for column use are shown in the specifications table in section 2. When using the preparative columns at a higher temperature than ambient temperature, the thermal mismatch between mobile phase and column temperature might cause peak distortion. To avoid those phenomena, we recommend preheating the mobile phase.