Column Care and Use Instructions YMC-Pack Diol

1. Introduction

Thank you for purchasing a YMC high-performance liquid chromatography (HPLC) column. "YMC-Pack Diol" is a HPLC column for gel filtration chromatography column that the diol group was chemically bonded to the porous spherical silica gel. The functional group is chemically bonded with low nonspecific adsorption even in the separation of hydrophilic proteins.

YMC HPLC columns, 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.

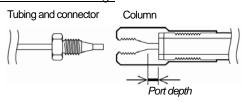
2. Specifications

Column	Particle size (µm)	Pore size (nm)	Functional group	Usable pH range	Temperature (Upper limit)
Diol-60	2.5	6	Dihydroxypropyl	5.0 – 7.5	40 °C
Diol-120	3, 5	12			
Diol-200	2.2.5	20			
Diol-300	2, 3, 5	30			

3. Recommendations for column connections

• The "PTH" or "WT" at the end of the product code indicates the style of column endfittings (see below for details).

Consideration of connector and endfittings



Particle size	The end of the product code	Port depth	Style of endfittings	
2 µm	PTH	ca. 2 mm / 0.09 inch	Parker style	
3 µm, 5 µm	WT	ca. 3 mm / 0.13 inch	Waters style	

Tubing must have flat ends and must bottom out in the column endfitting. Tubing must be connected to the column correctly to
avoid creating a void between the column frit and tubing, which can cause a leak and result in poor column performance (e.g.
peak tailing, loss of theoretical plate number).

4. Shipping solvent

The column is shipped in the 0.05% sodium azide aqueous solution. Flush with water sufficiently before replacing with the mobile phase.

5. Precautions for use

- · The correct direction of the solvent flow is indicated by an arrow on the column identification label.
- The column pressure limit and recommended flow rate are the following.

Particle size	The end of the product code	Pressure limit ^{*1}
2 µm	PTH	45 MPa ^{*2} 30 MPa or less for regular use
3 µm, 5 µm	WT	20 MPa ^{*2}

^{*1} Avoid using a column repeatedly near the pressure limit or abrupt change in pressure to prevent shortening of the column life.

- Aqueous mobile phase are basically used. Total salt concentration of mobile phase should be lesser than 0.7 M. Tris-HCl, citrate, etc.
 are applicable as buffer solution. These buffer solutions are available with solutions containing buffer salt/additives such as sodium
 chloride, sodium sulfate, and ammonium sulfate.
- Aqueous solutions of urea and guanidine hydrochloride which are used for a denaturant of the protein can be used. Moreover, 0.1%
 or less concentration of surface-active agents such as Tween80, SDS is also usable. When using these mobile phases, the
 equilibration of the column needs long time as compared to the general mobile phase.
- Methanol or acetonitrile can be added to mobile phase. When using a mobile phase containing methanol or acetonitrile, be mindful of the operation pressure rising by increasing viscosity and the precipitation of buffer salts/additives.
- · Recommendations of pH and temperature for column use are shown in the specifications table in section 2.
- Column lifetime varies depending on conditions of use such as pH, temperature and mobile phase composition. In general, usage at higher temperatures and higher concentrations of buffer salts/additives can shorten the column lifetime.
- For storage except daily use, the column should be flushed thoroughly with water, replaced in aqueous solution of 0.05% of sodium azide, which should be sealed the both ends tightly and stored in a location with minimal temperature change. In next time, flush with water sufficiency before replacing with the mobile phase.

6. Column cleaning (general method)

In the case that some hydrophobic proteins or the hydrophobic materials are adsorbed or retained, flush the column with solvent containing high salt concentration (approx. 0.5 M). At that time, be careful about usable pH.

7. Other environments

- The operating pressure should be kept under 5 MPa (725 psi) for Glass column.
- To prevent exposure of the column to excessive pressure, the sample solution should be filtered through a 0.2 µm membrane or smaller to remove particulates. We recommend using a pre-column filter to prevent the column frit from being clogged with samples.

^{*2} Adjust the flow rate appropriately because the pressure changes depending on the column length, temperature, types of mobile phase etc.