Column Care and Use Instructions
YMC-SEC MAB

1. Introduction

Thank you for purchasing a YMC high-performance liquid chromatography (HPLC) column. YMC-SEC MAB is a HPLC column for size exclusion chromatography packed with the porous spherical silica gel that the diol group is chemically bonded to. The functional group with low nonspecific adsorption even in the separation of hydrophilic proteins is chemically bonded. Especially, YMC-SEC MAB enables to achieve better resolution of the peaks derived from monoclonal antibody including monomers, aggregates, and fragments.

YMC-SEC MAB columns, which are manufactured under highly controlled conditions, must pass a series of strict 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

	YMC-SEC MAB
Particle size (μm)	3
Pore size (nm)	25
Functional group	Dihydroxypropyl
Temperature (Upper limit)	40 °C
Usable pH range	5 – 7.5

3. Recommendations for column connections

- The column endfitting is Waters style connection.
- 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).
- The shortest possible length of tubing with narrow inner diameters should be used for the connection from the injector to the column and from the column to the detector. Make sure not to have a void in the connection.

4. Shipping solvent

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.
- Do not disconnect a column from the LC system before the pressure drops to zero.
- The column pressure limit and recommended flow rate are the following.

Column I.D.	Pressure limit*1	Recommended flow rate*2
4.6 mmI.D.	14 MPa	0.15 - 0.4 mL/min (Max. flow rate : 0.5 mL/min)
8.0 mmI.D.	12 MPa	0.45 - 1.2 mL/min (Max. flow rate : 1.5 mL/min)

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

*2 Adjust flow rate as recommended in the table seen above. When repeatedly using a flow rate at or near the upper limit, the column durability will shorten. When using column dimensions other than listed, adjust flow rate according to the cross-section area of the column.

*1,2 Adjust the flow rate appropriately because the pressure changes depending on the temperature, types of mobile phase etc.

- Aqueous mobile phase are basically used. Total salt concentration of mobile phase should be lesser than 0.7 M. Phosphate, 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.
- Alcohol or acetonitrile can be added to mobile phase. When using a mobile phase containing alcohol 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 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.
- To preventing 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.

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.

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