

For High Performance Liquid Chromatography

COSMOSIL[®] 5Diol-II PACKED COLUMNS

1. INTRODUCTION

Thank you for purchasing our COSMOSIL Packed Column. Ensuring maximum efficiency and long column life, we ask you to read this manual carefully.

COSMOSIL Packed Columns are made of stainless steel and packed with specially bonded high purity spherical porous silica. The COSMOSIL 5Diol-II packed columns are for size-exclusion chromatography (SEC) of proteins and water-soluble polymers.

2. TYPES OF PACKING MATERIALS AND THEIR CHARACTERISTICS

Product name	Particle size	Pore size	MW range		Column size (mm I.D.-mm)	Flow rate (ml/min)
			Protein	Water soluble polymer		
COSMOSIL 5Diol-120-II	5μm	120 Å	5,000-100,000	1,000-20,000	7.5-50 7.5-300 7.5-600	0.5-1.0
COSMOSIL 5Diol-300-II	5μm	300 Å	10,000-700,000	5,000-100,000	7.5-50 7.5-300 7.5-600	0.5-1.0
COSMOSIL 5Diol-1000-II	5μm	1000 Å	—	50,000-500,000	7.5-50 7.5-300	0.5-1.0

3. CARE AND USE

1. Avoid mechanical shocks to the column.
2. Connect the column according to the flow direction indicated on the label.
3. For 5Diol-120-II and 5Diol-300-II, keep pressure under 20MPa. For 5Diol-1000-II, keep pressure under 15MPa.
4. Let through the column 20-30ml mobile phase before connecting to the detector.
5. Keep the pH of the mobile phase within the range of 2-7.
6. Use the buffer concentration less than 0.5mol/l.
7. Use the salt concentration less than 0.5mol/l.
8. If you use water-soluble organic solvents, be careful not causing salt precipitation.
9. Pass mobile phase through membrane filter (less than 0.45μm in pore size) before use.
10. Filter the sample before injection. Avoid precipitation at injection.
11. Depending on the sample, gelation might be happen on the end filter or in the column. Dissolve the sample to the mobile phase before the injection, and check that there is no gel or no precipitation.
12. Insoluble matters from the pumping system, mobile phase or samples will be trapped in the filter (2μm) at the inlet of the column, and may increase the pressure.
13. We recommend using guard column to protect main column from irreversible adsorption on the packing material, clogging of the end filter by insoluble matters, or rapid increase of the pressure.
14. To keep packing condition, avoid injecting air and changing flow rate rapidly. Change mobile phase at less than 0.5ml/min flow rate.
15. We recommend flow rate under 1.0ml/min.
16. In order to maximize the column performance, minimize the dead volume in the equipment by shortening the length and/or narrowing the bore of tubing.
17. Maintain column and tubing at constant temperature.
18. After analysis, wash the column with deionized water. Then store it tightly plugged. If you don't use beyond a month, replace mobile phase with deionized water containing 0.05% sodium azide or methanol / deionized water = 30 / 70.
19. Do not tighten the plugs and tubings too much. The ferrules would be broken.
20. We recommend keeping the chromatography conditions constant, since frequent changes of mobile phase shorten column life.

4. TROUBLESHOOTING

Trouble	Cause	Solution
Increase of pressure	Clogging of the end filter Clogging of the packing material Gelation of the sample in the column Precipitation in the column	(1)(2) (1) (3) (4)
Poor resolution	Contamination of packing material Disorder of packing material	(4)(5)(6)(7) Unregenerable
Split peak	Void in the column	Unregenerable
Unstable baseline	Contamination of packing material Contamination of mobile phase	(4)(5)(6)(7) (8)

- (1) Disconnect column from the detector. Let mobile phase through the column in reverse direction at 0.5ml/min flow rate for 30 min.
- (2) Wash the end filter or replace it with a new one.
- (3) Wash the column by the mobile phase which solves the gel at 0.3ml/min flow rate for an hour.
- (4) When precipitation of salt in mobile phase, wash the column with deionized water.
- (5) Wash the column with high salt concentration solution or buffer (pH3) at 0.5ml/min flow rate for 30 min.
- (6) Wash the column with 6mol/l urea solution or 6mol/l guanidine hydrochloride at 0.3ml/min flow rate for an hour.
- (7) Wash the column with 20% organic solvents (methanol, acetonitrile, or 2-propanol) at 0.5ml/min flow rate for 30 min.
- (8) Use the deionized water or HPLC grade solvents.

5. WARRANTY

Nacalai Tesque will change defective columns reported within 2 weeks of receipt. Nacalai Tesque approves return in case of:

- (1) Damage during the transportation caused by our incomplete packing.
- (2) Theoretical plate number measured according to the test method specified in the Inspection Report is significantly lower than guaranteed.
(Please note that the plate number decreases when using an apparatus with large dead volume or injecting a big amount of sample.)

We cannot accept claims for deterioration of column performance caused by taking off the end filters or end-fittings, or long shelf life. Return shipment is unacceptable unless we have given prior permission and shipping instructions.