

For High Performance Liquid Chromatography

COSMOSIL[®] RNA-SEC and RNA-RP 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 RNA-SEC packed columns are for size-exclusion chromatography (SEC) of RNA and DNA. The COSMOSIL RNA-RP packed columns are for reversed phase chromatography of RNA and DNA.

2. TYPES OF STATIONARY PHASES AND THEIR CHARACTERISTICS

Product name	Particle size	Pore size	Column size	Flow rate
COSMOSIL RNA-SEC-1000	5 μm	1000 Å	7.5 mmI.D.-50 mm	7.5 mmI.D.; 0.5-1.0 ml/min 4.6 mmI.D.; 0.2-0.4 ml/min
COSMOSIL RNA-SEC-2000		2000 Å	7.5 mmI.D.-300 mm 4.6 mmI.D.-250 mm	

Product name	Particle size	Pore size	Column size	Flow rate
COSMOSIL RNA-RP	5 μm	--	2.0 mmI.D.-100 mm 4.6 mmI.D.-100 mm 10 mmI.D.-100 mm	2.0 mmI.D.; 0.1-0.2 ml/min 4.6 mmI.D.; 0.5-1.0 ml/min 10 mmI.D.; 2.0-5.0 ml/min

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. Keep pressure under 15 MPa.
4. Keep the pH of the mobile phase within the range of 2 - 7.5.
5. Use only HPLC grade solvents.
6. Pass mobile phase through membrane filter (less than 0.45μm in pore size) before use.
7. Filter the sample before injection. Avoid precipitation at injection.
8. In order to maximize the column performance, minimize the dead volume in the equipment by shortening and/or narrowing the width of tubing.
9. Maintain constant column and tubing temperature.
10. After analysis, wash the column with acid-and/or salt-free solvent. Then store it tightly plugged.
11. We recommended keeping the chromatography conditions constant, since frequent changes of mobile phase shorten column life.
12. If necessary, perform RNase inactivation treatment before use.

RNase inactivation treatment procedure (example)

Wash the column sequentially with the following solvents.

- 1) Acetonitrile (10 min)
- 2) Chloroform (20 min)
- 3) Acetonitrile (RNase-free) (10 min)
- 4) Acetonitrile/water = 60/40 (RNase-free) (15 min)

■ Advice on handling RNA samples

- RNA is weak to RNase and heat, so when handling wear gloves and work on ice. The same applies to sample dilution and injection into the HPLC instrument.
- RNA easily adsorbs to glass surfaces, so it is recommended to keep the sample solution in a plastic tube.
- RNA has low mechanical strength, so avoid excessive pipetting.

■ Advice on analysis

- To prevent RNA adsorption on new columns, flow phosphate buffer through the column before analysis. Afterwards, analyze a standard sample to make sure separation patterns and peak shape are consistent, then analyze your sample.

4. TROUBLESHOOTING

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

- (1) Disconnect column from the detector. Let mobile phase through the column in reverse direction for 30 min.
- (2) Wash the end filter or replace it with a new one.
- (3) When precipitation of salt in mobile phase, wash the column with deionized water. (SEC)
- (4) Wash the column with high salt concentration solution or buffer (pH3) for 30 min. (SEC)
- (5) Wash the column with 6mol/l urea solution or 6mol/l guanidine hydrochloride for an hour. (SEC)
- (6) Wash the column with 50% organic solvents (methanol, acetonitrile, or 2-propanol) for 30 min. (RP)
- (7) 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 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.