

## 8. mRNA and Oligonucleotide Purification Columns

RNA-based therapeutics represent a powerful new tool for gene therapy. In RNA production, impurities such as nucleotide triphosphates, abortive transcripts, and DNA templates can affect downstream applications by triggering immune responses. Gel-based methods of purification, such as preparative denaturing polyacrylamide gel electrophoresis (PAGE) and agarose gels, are limited by poor resolution of RNA size and potential chemical modifications from reagents such as formaldehyde. To date, high performance liquid chromatography (HPLC) remains a staple method for purification of messenger RNA (mRNA) and RNA oligonucleotides. Presented here are two HPLC methods: a size exclusion-based method for the purification of long mRNA, and a reversed-phase method for the purification of oligonucleotides.

### Specifications

| Packing Material      | RNA-SEC-1000                                     | RNA-SEC-2000 | RNA-RP1         |
|-----------------------|--------------------------------------------------|--------------|-----------------|
| Silica Gel            | Silica Gel                                       |              |                 |
| Average Particle Size | 5 $\mu\text{m}$                                  |              |                 |
| Average Pore Size     | 1,000 Å                                          | 2,000 Å      | –               |
| Bonded Phase          | Hydrophilic group                                |              | Octadecyl group |
| Features              | For analysis of nucleic acids longer than 100 nt |              |                 |

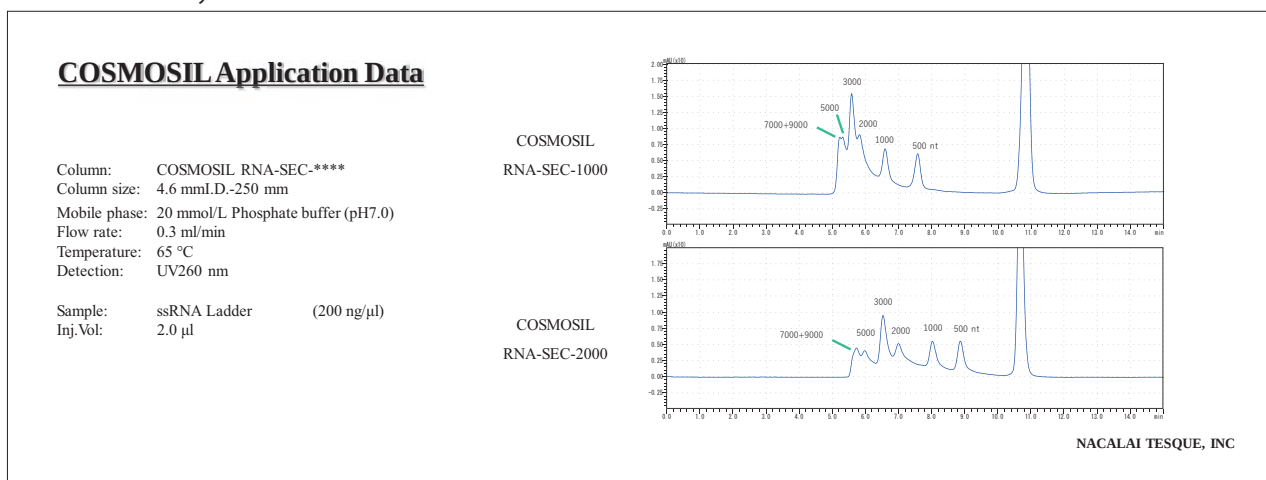


### COSMOSIL RNA-SEC-1000, RNA-SEC-2000

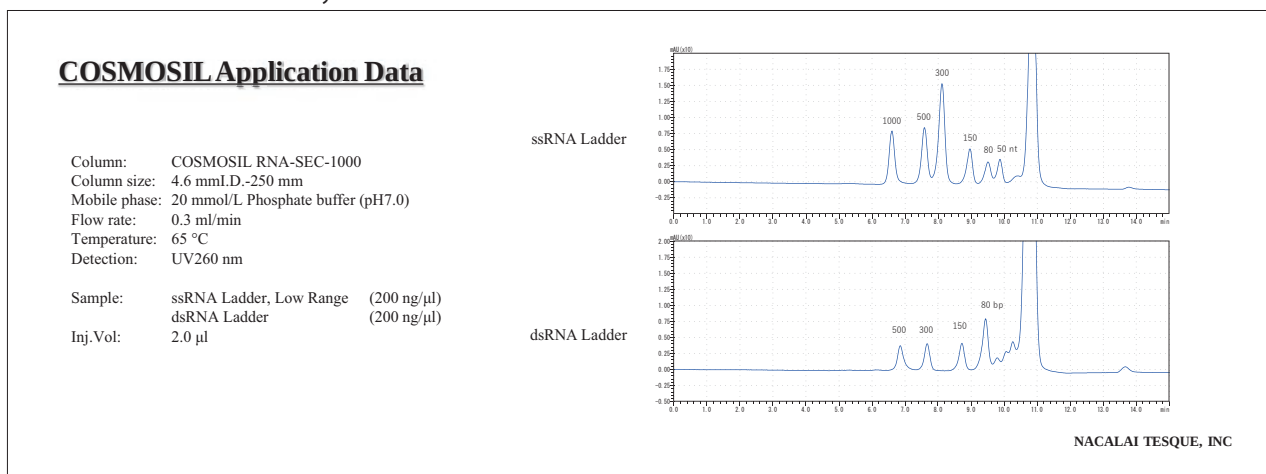
- Analyze a wide range of molecular weights
- Two pore sizes available for different samples

### Applications

- ssRNA ladder by SEC-1000 and SEC-2000



- ssRNA and dsRNA ladder by SEC-1000



mRNA and Oligonucleotide Purification Columns

1

2

3

4

5

6

7

8

9

10

11

12

13

14

# Applications

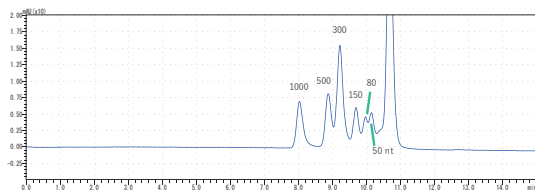
## ● ssRNA and dsRNA ladder by SEC-2000

### COSMOSIL Application Data

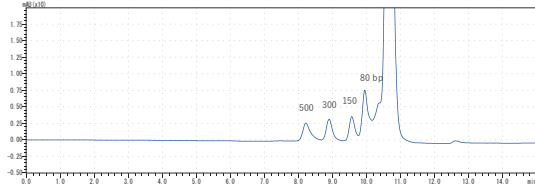
Column: COSMOSIL RNA-SEC-2000  
Column size: 4.6 mmI.D.-250 mm  
Mobile phase: 20 mmol/L Phosphate buffer (pH7.0)  
Flow rate: 0.3 ml/min  
Temperature: 65 °C  
Detection: UV260 nm

Sample: ssRNA Ladder, Low Range (200 ng/μl)  
dsRNA Ladder (200 ng/μl)  
Inj.Vol: 2.0 μl

ssRNA Ladder



dsRNA Ladder

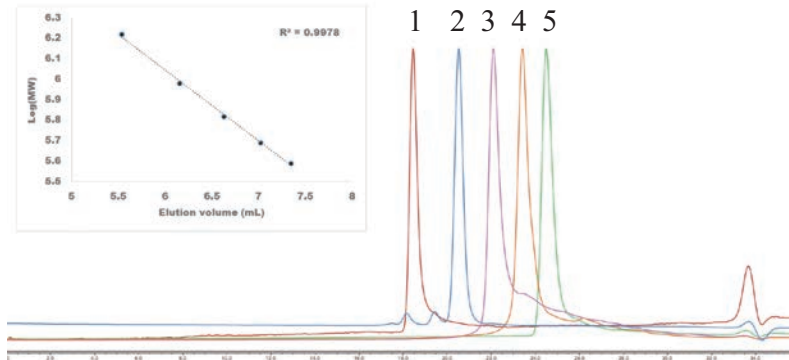
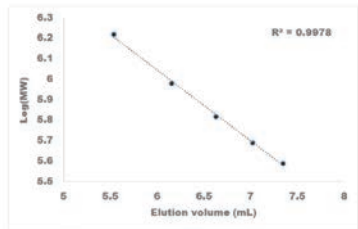


NACALAI TESQUE, INC

## ● Molecular weight determination for mRNA up to 5,000 bases

### COSMOSIL Application Data

Column: RNA-SEC-2000  
Column size: 7.5x300 mm  
Mobile phase: A- 30mM TEAA in water  
B-Acetonitrile  
Gradient: 30% B isocratic  
Flow rate: 0.3 mL/min  
Temperature: 60 Celsius  
Detection: uv 260nm  
Inj.Vol: 20 μL  
Sample: mRNA [nucleotides (nt)]  
1- 1,656 kDa (4870 nt)  
2- 952 kDa (2500 nt + modification)  
3- 657 kDa (1931 nt)  
4- 489 kDa (1437 nt)  
5- 388 kDa (1140 nt)



NACALAI TESQUE, INC

# COSMOSIL RNA-RP1



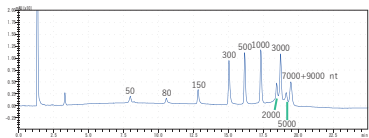
## ● High resolution for oligonucleotides

### ● ssRNA ladder

#### COSMOSIL Application Data

Column: COSMOSIL RNA-RP1  
Column size: 4.6 mmI.D.-100 mm  
Mobile phase: A; 100 mmol/L Triethylamine, 100 mmol/L Acetic Acid (pH7)  
B; Acetonitrile  
B conc. 5→13 % (0→20 min), 13 % (20-24 min)  
Flow rate: 1.0 ml/min  
Temperature: 65 °C  
Detection: UV260 nm

Sample: ssRNA Ladder, mixture  
Inj.Vol: 4.0 μl



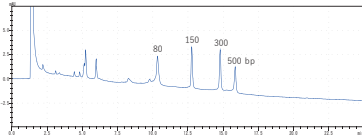
NACALAI TESQUE, INC

### ● dsRNA ladder

#### COSMOSIL Application Data

Column: COSMOSIL RNA-RP1  
Column size: 4.6 mmI.D.-100 mm  
Mobile phase: A; 100 mmol/L Triethylamine, 100 mmol/L Acetic Acid (pH7)  
B; Acetonitrile  
B conc. 5→20 % (0→20 min), 20 % (20-24 min)  
Flow rate: 1.0 ml/min  
Temperature: 40 °C  
Detection: UV260 nm

Sample: dsRNA Ladder (200 ng/μl)  
Inj.Vol: 5.0 μl

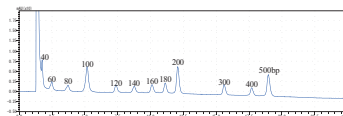


NACALAI TESQUE, INC

● 20 bp DNA ladder

### **COSMOSIL Application Data**

Column: COSMOSIL RNA-RP1  
 Column size: 4.6 mm I.D. × 100 mm  
 Mobile phase: A; 100 mmol/L Triethylamine, 100 mmol/L Acetic Acid (pH7)  
 B; Acetonitrile  
 B conc. 11 → 16 % (0 → 20 min), 16 % (20-25 min)  
 Flow rate: 1.0 ml/min  
 Temperature: 40 °C  
 Detection: UV260 nm  
 Sample: 20 bp DNA Ladder  
 (200 ng/μl)  
 Inj. Vol: 5.0 μl



NACALAI TESQUE, INC

## Ordering Information

● COSMOSIL RNA-SEC-1000 Analytical Columns

Packed Column

| I.D. x Length (mm) | Product Number | I.D. x Length (mm) | Product Number |
|--------------------|----------------|--------------------|----------------|
| 4.6 × 250          | 21088-01       | 7.5 × 300          | 19380-21       |
| 7.5 × 50           | 20785-91       |                    |                |

● COSMOSIL RNA-SEC-2000 Analytical Columns

Packed Column

| I.D. x Length (mm) | Product Number | I.D. x Length (mm) | Product Number |
|--------------------|----------------|--------------------|----------------|
| 4.6 × 250          | 21095-01       | 7.5 × 300          | 19381-11       |
| 7.5 × 50           | 21096-91       |                    |                |

● COSMOSIL RNA-RP1 Analytical Columns

Packed Column

| I.D. x Length (mm) | Product Number | I.D. x Length (mm) | Product Number |
|--------------------|----------------|--------------------|----------------|
| 2.0 × 100          | 21078-31       | 10 × 100           | 21080-81       |
| 4.6 × 100          | 21079-21       |                    |                |