COSMOSIL

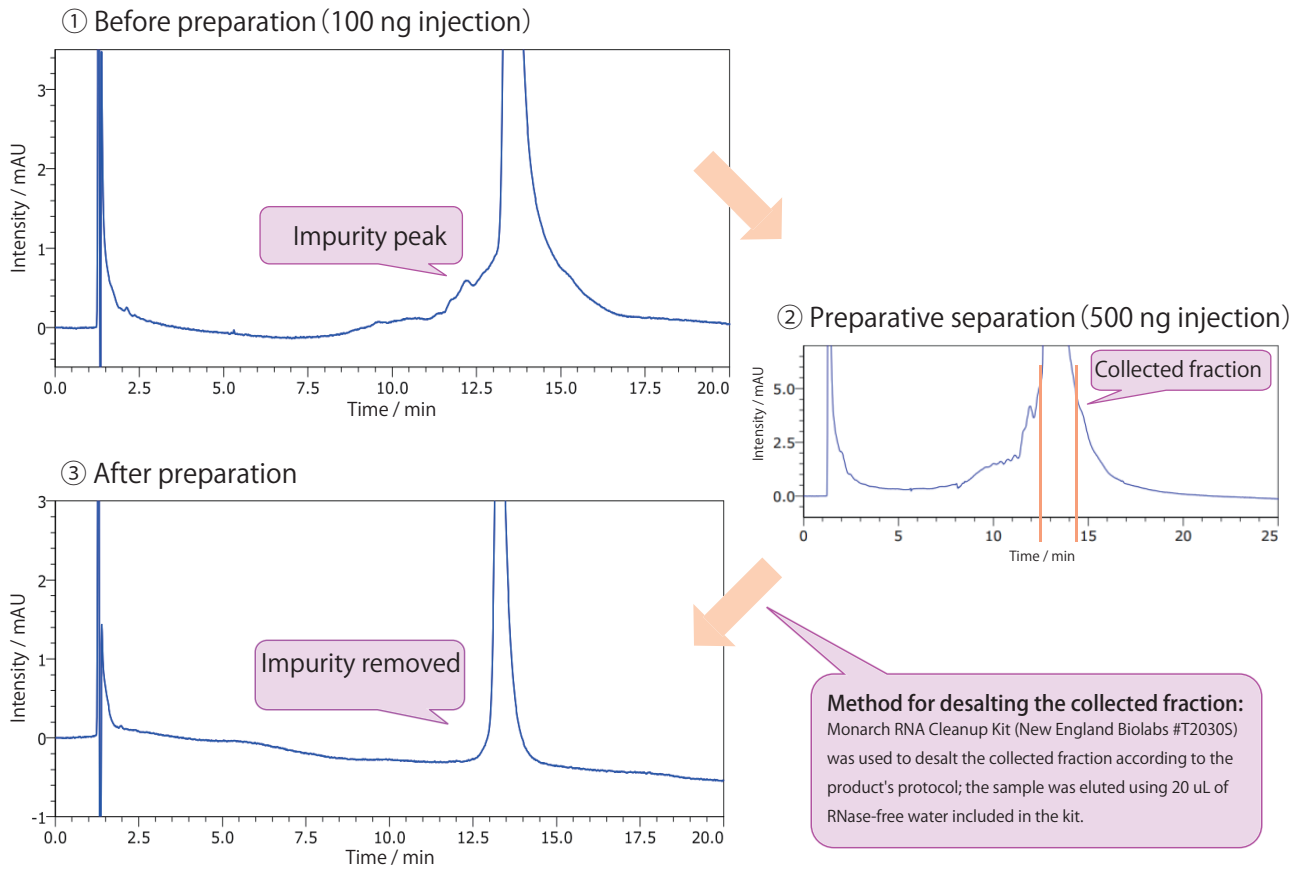
HPLC columns for RNA and Oligonucleotide Separation

COSMOSIL RNA Series

- **Detects impurities not observable with electrophoresis.**
- **Scale up to preparative separations.**
- **Separate long RNA strands from 100 to 5,000 bases.**
- **Two modes available: reversed-phase and size-exclusion.**

Preparative separation of long-stranded ssRNA

Reversed-phase column: COSMOSIL RNA-RP1



Method for desalting the collected fraction:
Monarch RNA Cleanup Kit (New England Biolabs #T2030S) was used to desalt the collected fraction according to the product's protocol; the sample was eluted using 20 uL of RNase-free water included in the kit.

<Conditions>

Column: COSMOSIL RNA-RP1 2.0 mm I.D. × 100 mm

Mobile phase: A: 100 mmol/L Triethylamine Acetate
+ 20 mmol/L Phosphate Buffer (pH 7.0)
B: Methanol / Acetonitrile = 50 / 50
B conc. 11% → 13% (0 → 20 min), 13% (20 → 24 min)

Flow rate: 0.2 mL/min

Temperature: 60℃

Detection: UV 260 nm

Sample: pQBI T7-GFP RNA, digested with *Hin*dIII and *Bam*HI (4.8 knt)

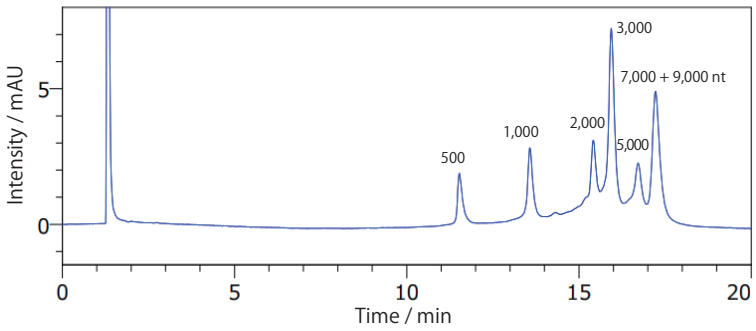
Inj. Vol.: ① Before preparation; 100 ng/µL × 1 µL
② Preparation; 100 ng/µL × 5 µL
③ Preparated sample analysis (After preparation);
desalted solution (undiluted) × 2 µL

For this analysis, in vitro transcribed long-chain ssRNA (4.8 knt) precipitated using lithium chloride was used as the sample. When the long-chain ssRNA was analyzed using electrophoresis, the impurity was not detected (see page 6); however, using COSMOSIL RNA-RP1, a peak of an impurity thought to be of different length was detected (see chromatogram ① above). Using preparative separation by HPLC, the impurity peak was successfully removed (see chromatogram ③ above).

Two modes to choose from

The COSMOSIL RNA series is comprised of RNA-RP1, a reversed-phase column, and RNA-SEC-1000 and RNA-SEC-2000, size-exclusion columns. Chromatograms of these columns using the same ssRNA ladder are compared below.

Reversed-phase: COSMOSIL RNA-RP1



Elution: Strands with low hydrophobicity are eluted first

Separation: High

Analysis conditions: Complex, with many conditions to consider

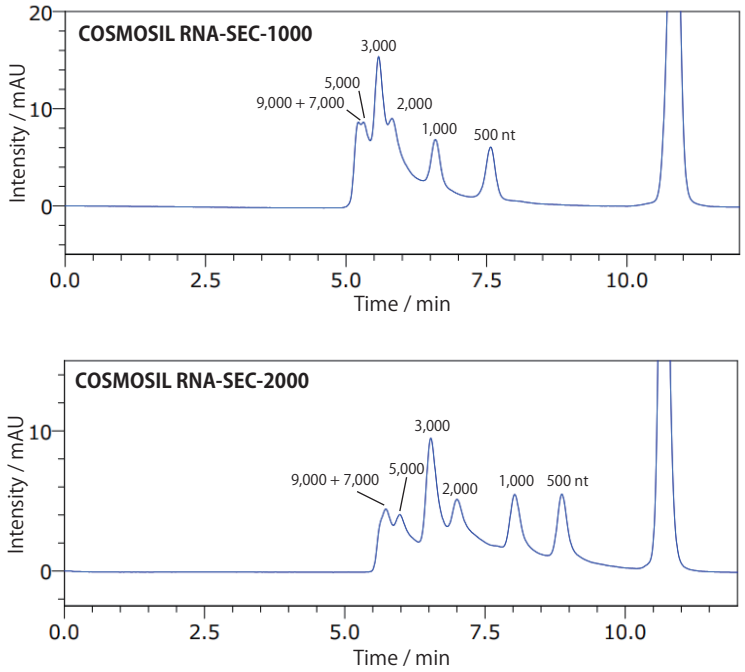
<Conditions>

Column: COSMOSIL RNA-RP1 4.6 mm I.D. × 100 mm
Mobile phase: A: 100 mmol/L Triethylamine Acetate (pH 7.0)
B: Acetonitrile
B conc. 8% → 13% (0 → 20 min), 13% (20 → 24 min)
Flow rate: 1.0 mL/min
Temperature: 65℃

Detection: UV 260 nm
Sample: ssRNA Ladder (New England Biolabs #N0362S)
2.5 times dilution
Inj. Vol.: 5 µL

As the hydrophobicity of RNA strands generally increases with length, long strands are typically eluted after short strands. However, the hydrophobicity also differs by sequence, so it is not necessarily the case that strands are eluted in order of length. By optimizing the analysis conditions, it is possible to improve separation. For example, it is possible to adjust the mobile phase, temperature, and gradient slope. See the references on page 8 for details.

Size-exclusion: COSMOSIL RNA-SEC-1000, RNA-SEC-2000



Elution: Large strands are eluted first

Separation: Rough separation by molecular size

Analysis conditions: Simple

<Conditions>

Column: COSMOSIL RNA-SEC-**** 4.6 mm I.D. × 250 mm
Mobile phase: 20 mmol/L Phosphate Buffer (pH 7.0)
Flow rate: 0.3 mL/min
Temperature: 65℃

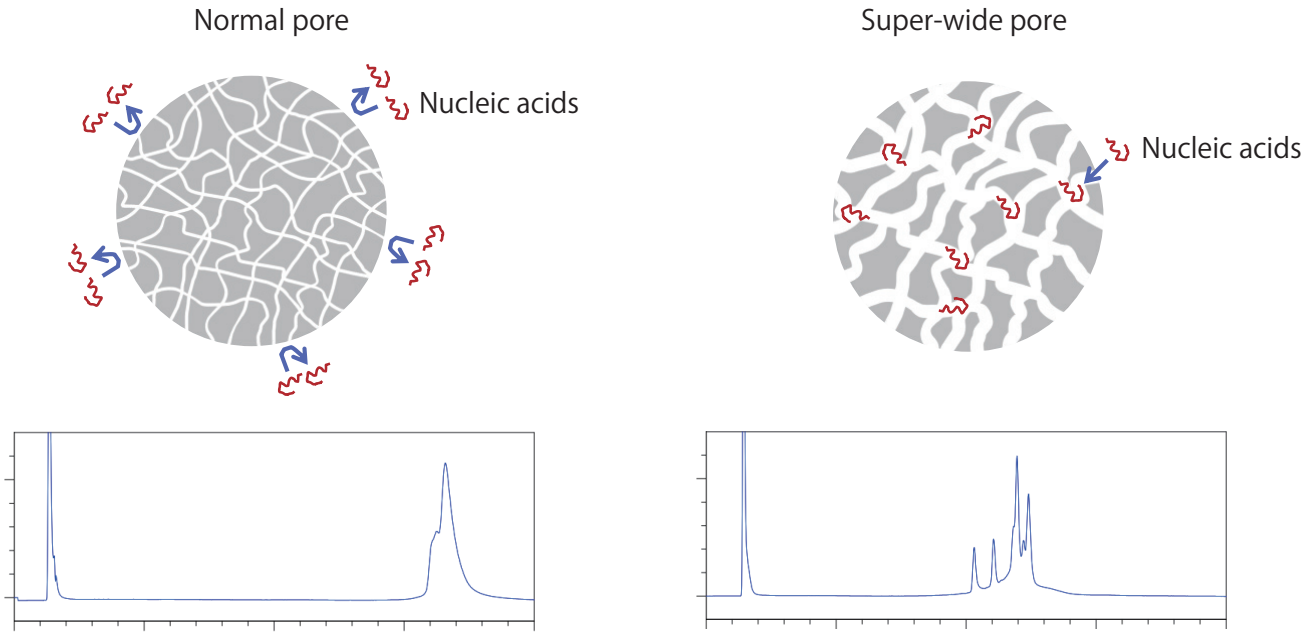
Detection: UV 260 nm
Sample: ssRNA Ladder (New England Biolabs #N0362S)
2.5 times dilution
Inj. Vol.: 2 µL

Compared to COSMOSIL RNA-SEC-1000, which has a pore size of 100 nm, RNA-SEC-2000, with a pore size of 200 nm, is suitable for analysis of large strands. Use different pore sizes depending on the strand sizes you need to separate.

Properties of packing material

Pore size

Long-strand nucleic acids have a very large molecular size, so they do not enter the pores of normal packing material, so the individual peaks do not separate. The super-wide pores allow the nucleic acids to enter, enabling much better separation.

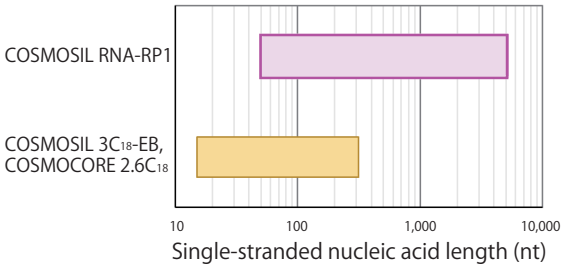
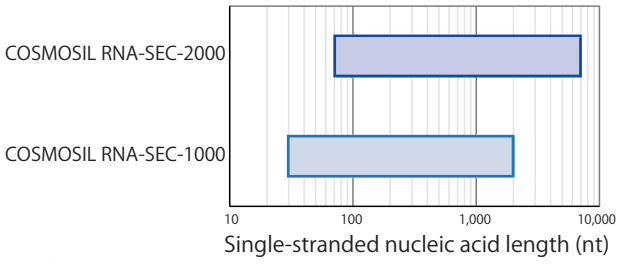


HPLC columns for general use typically have silica gel with an average pore size of 8 to 12 nm, whereas COSMOSIL RNA-RP1 uses silica gel with super-wide pores.
RNA-SEC-1000 and RNA-SEC-2000 use silica gel with 100 and 200 nm pores, respectively, so long-chain nucleic acids can enter the pores and be separated.

Target molecular sizes

Our recommendations for target molecular sizes for reversed-phase and size-exclusion columns are noted below.

* These figures only represent the range of general suitability; it does not indicate that any nucleic acids in the range can be separated.

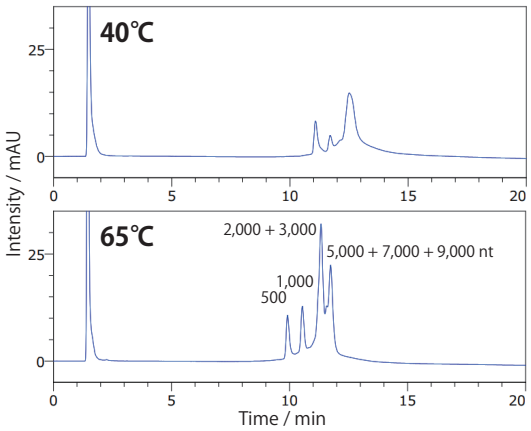
Reversed-phase	Size-exclusion
Suitable range for reversed-phase chromatography  	Suitable range for size-exclusion chromatography 
For mid-length to long-stranded nucleic acids above 100 nt, the super-wide pore silica gel in COSMOSIL RNA-RP1 is recommended. For oligonucleotides, columns with smaller pores, such as 3C18-EB (average pore size 12 nm) or COSMOCORE 2.6C18 (average pore size 9 nm) are recommended.	Using COSMOSIL RNA-SEC-1000, it is possible to separate smaller strands of less than 100 nt, up to long strands of thousands of nucleotides. Using RNA-SEC-2000, even longer strands of greater than 5,000 nt can be separated.

Analysis conditions

Analysis temperature

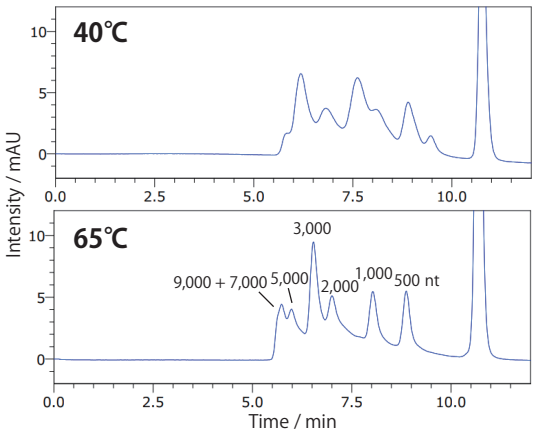
● Single-strand nucleic acids

Reversed-phase: COSMOSIL RNA-RP1



<Conditions>
Column: COSMOSIL RNA-RP1 2.0 mm I.D. × 100 mm
Mobile phase: A: 100 mmol/L Triethylamine Acetate (pH 7.0)
B: Acetonitrile
B conc. 7.5% → 17.5% (0 → 20 min), 17.5% (20 → 24 min)
Flow rate: 0.2 mL/min
Temperature: **℃
Detection: UV 260 nm
Sample: ssRNA Ladder (New England Biolabs #N0362S)
2.5 times dilution × 2 µL

Size-exclusion: COSMOSIL RNA-SEC-2000

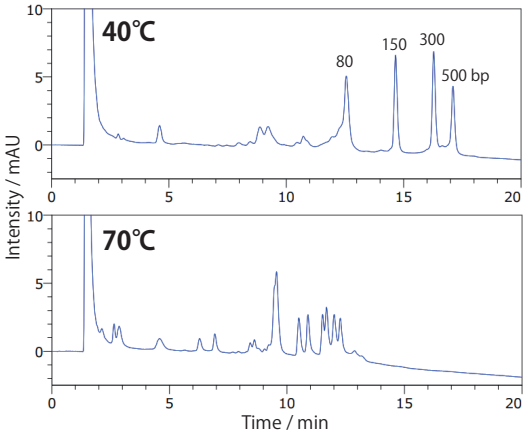


<Conditions>
Column: COSMOSIL RNA-SEC-2000 4.6 mm I.D. × 250 mm
Mobile phase: 20 mmol/L Phosphate Buffer (pH 7.0)
Flow rate: 0.3 mL/min
Temperature: **℃
Detection: UV 260 nm
Sample: ssRNA Ladder (New England Biolabs #N0362S)
2.5 times dilution × 2 µL

At low temperatures, single-strand nucleic acids tend to form higher-order structures, resulting in broad peaks. This may be improved by analyzing at higher temperature.

● Double-strand nucleic acids

Reversed-phase: COSMOSIL RNA-RP1

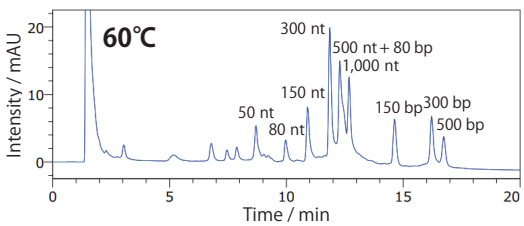


<Conditions>
Column: COSMOSIL RNA-RP1 2.0 mm I.D. × 100 mm
Mobile phase: A: 100 mmol/L Triethylamine Acetate (pH7.0)
B: Acetonitrile
B conc. 5% → 20% (0 → 20 min), 20% (20 → 24 min)
Flow rate: 0.2 mL/min
Temperature: **℃
Detection: UV 260 nm
Sample: dsRNA Ladder (New England Biolabs #N0363S)
2.5 times dilution × 2 µL

For analyzing double-strand nucleic acids in their nondenatured state, care is required when setting analysis temperature, as the strands tend to dissociate at high temperatures. Around the dissociation temperature, small differences in temperature can lead to very different separations. See the references on page 8 for details.

● Mix of single- and double-strand nucleic acids

Reversed-phase: COSMOSIL RNA-RP1

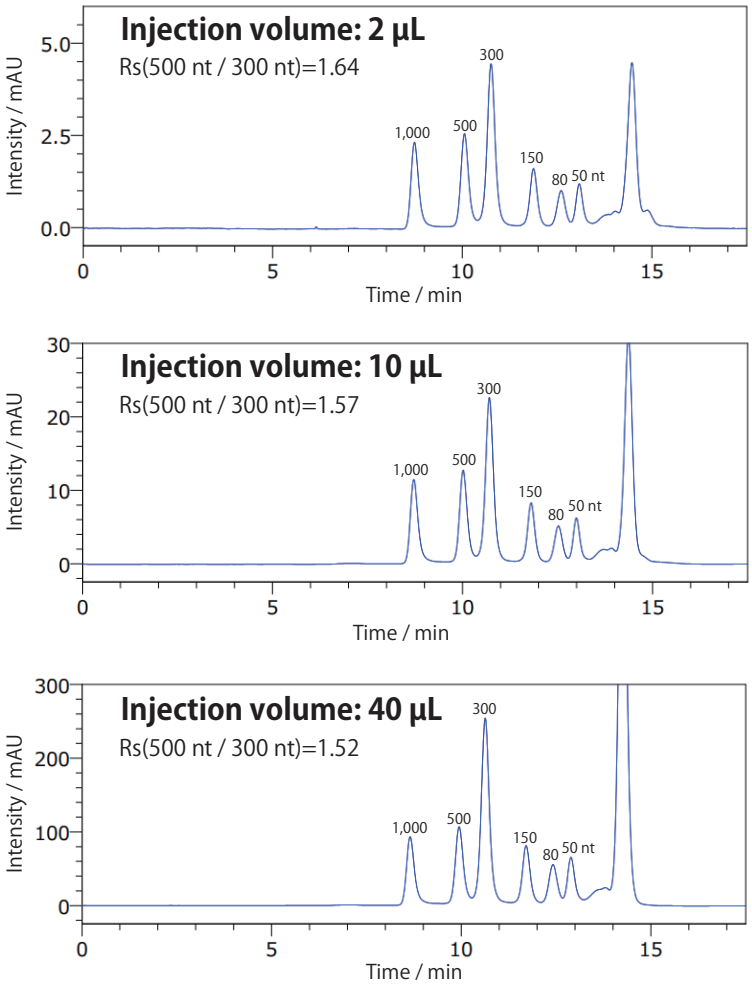


<Conditions>
Column: COSMOSIL RNA-RP1 2.0 mm I.D. × 100 mm
Mobile phase: A: 100 mmol/L Triethylamine Acetate (pH7.0)
B: Acetonitrile
B conc. 5% → 20% (0 → 20 min), 20% (20 → 24 min)
Flow rate: 0.2 mL/min
Temperature: 60℃
Detection: UV 260 nm
Sample: dsRNA Ladder (New England Biolabs #N0363S)
2.5 times dilution × 2 µL
+ Low Range ssRNA Ladder (New England Biolabs #N0364S) 2.5 times dilution × 2 µL

By analyzing at a temperature at which single strands do not form higher-order structures and double strands do not dissociate, it is possible to simultaneously analyze both.

Considering loading capacity

Size-exclusion: COSMOSIL RNA-SEC-1000



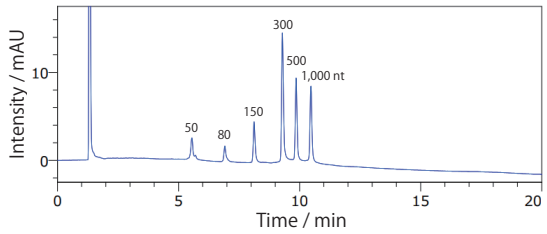
<Conditions>
Column: COSMOSIL RNA-SEC-1000
7.5 mm I.D. × 300 mm
Mobile phase: 20 mmol/L Phosphate Buffer (pH7.0)
Flow rate: 1.0 mL/min
Temperature: 65℃
Detection: UV 260 nm
Sample: Low Range ssRNA Ladder
(New England Biolabs #N0364S)

Even injecting 40 uL of sample, a similar chromatogram to a 2 uL injection was obtained (vertical axis scale differs). Loading capacity differs by sample, so determine it experimentally using an analysis column before scaling to preparative size.

Application data

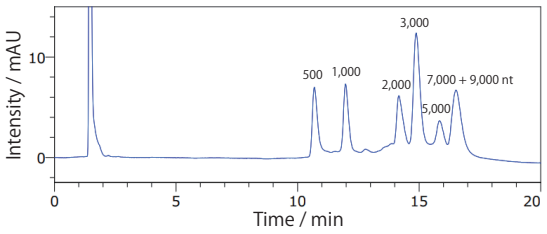
COSMOSIL RNA-RP1 (RNA ladder)

● Low Range ssRNA Ladder



<Conditions>
Column: COSMOSIL RNA-RP1 4.6 mm I.D. × 100 mm
Mobile phase: A: 100 mmol/L Triethylamine Acetate (pH 7.0)
B: Acetonitrile
B conc. 5% → 20% (0 → 20 min), 20% (20 → 24 min)
Flow rate: 1.0 mL/min
Temperature: 65℃
Detection: UV 260 nm
Sample: Low range ssRNA Ladder (New England Biolabs #N0364S)
2.5 times dilution × 5 µL

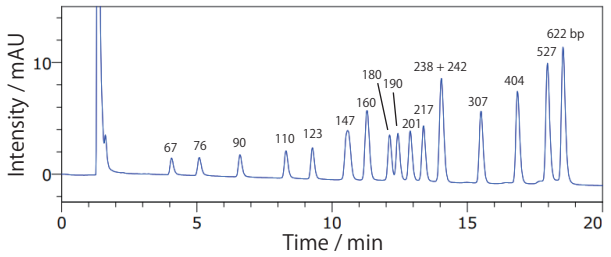
● ssRNA Ladder



<Conditions>
Column: COSMOSIL RNA-RP1 2.0 mm I.D. × 100 mm
Mobile phase: A: 100 mmol/L Triethylamine Acetate (pH 7.0)
B: Acetonitrile
B conc. 7% → 10% (0 → 20 min), 10% (20 → 24 min)
Flow rate: 0.2 mL/min
Temperature: 65℃
Detection: UV 260 nm
Sample: ssRNA Ladder (New England Biolabs #N0362S)
2.5 times dilution × 2 µL

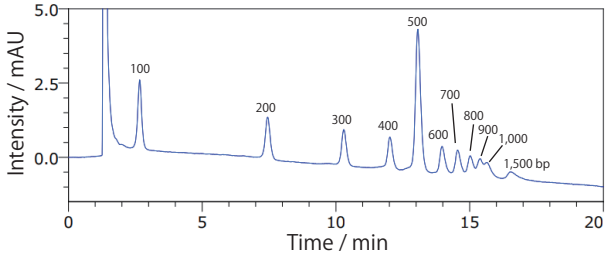
COSMOSIL RNA-RP1 (DNA ladder)

● pBR322 / *Msp*I digest



<Conditions>
Column: COSMOSIL RNA-RP1 4.6 mm I.D. × 100 mm
Mobile phase: A: 100 mmol/L Triethylamine Acetate (pH 7.0)
B: Acetonitrile
B conc. 10% → 17% (0 → 20 min), 17% (20 → 25 min)
Flow rate: 1.0 mL/min
Temperature: 40℃
Detection: UV 260 nm
Sample: pBR322 / *Msp*I digest (Nippongene #317-01501)
200 ng/µL × 5 µL

● 100 bp DNA Ladder

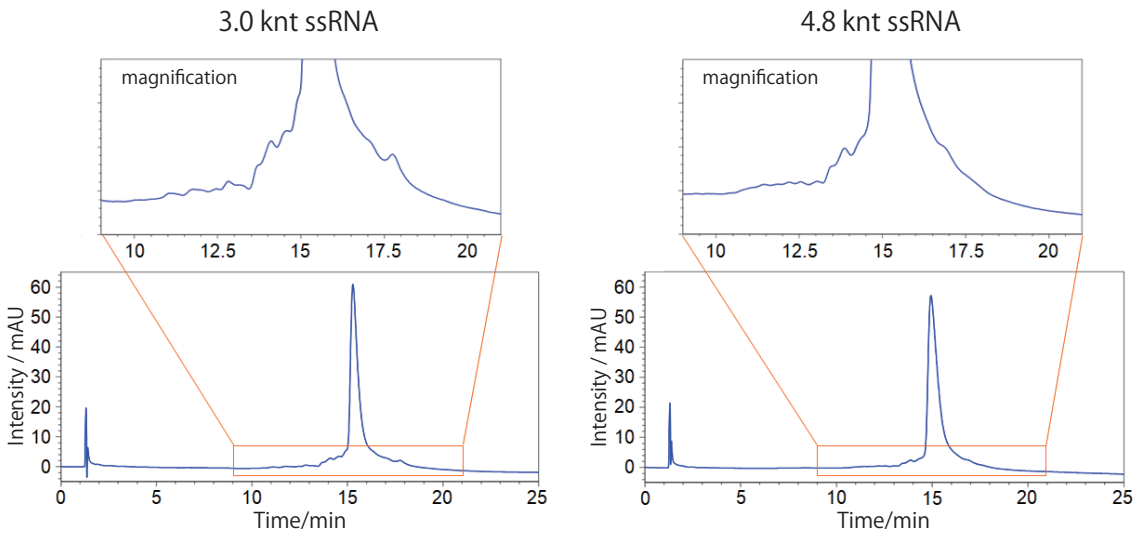


<Conditions>
Column: COSMOSIL RNA-RP1 4.6 mm I.D. × 100 mm
Mobile phase: A: 100 mmol/L Triethylamine acetate (pH 7.0)
B: Acetonitrile
B conc. 12% → 18% (0 → 20 min), 18% (20 → 25 min)
Flow rate: 1.0 mL/min
Temperature: 40℃
Detection: UV 260 nm
Sample: 100bp DNA Ladder (Takara Bio #3407A)
130 ng/µL × 5 µL

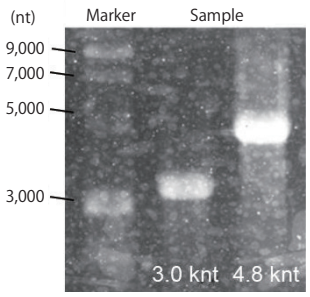
Fragments that are difficult to separate using agarose gel electrophoresis can be separated using COSMOSIL RNA-RP1. See the references on page 8 for details.

COSMOSIL RNA-RP1 (separation of impurities)

● ssRNA synthesized by in vitro transcription



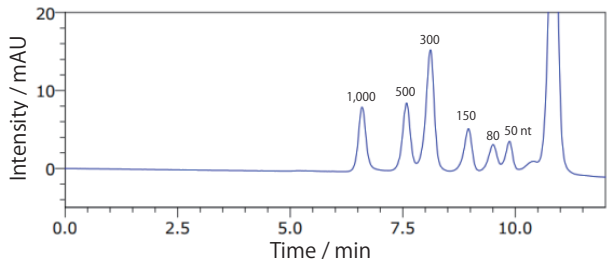
<Conditions>
Column: COSMOSIL RNA-RP1 2.0 mm I.D. × 100 mm
Mobile phase: A: 100 mmol/L Triethylamine Acetate + 20 mmol/L Phosphate Buffer (pH 7.0)
B: Methanol / Acetonitrile = 50 / 50
B conc. 11% → 13% (0 → 20 min), 13% (20 → 24 min)
Flow rate: 0.2 mL/min
Temperature: 60℃
Detection: UV 260 nm
Sample: pQBI T7-GFP RNA, digested with *Bgl*II and *Eag*I (3.0 knt), 100 ng/µL × 5 µL
pQBI T7-GFP RNA, digested with *Hin*dIII and *Bam*HI (4.8 knt), 100 ng/µL × 5 µL



Upon analyzing long-chain ssRNA purified using lithium chloride precipitation, impurity peaks not detected with agarose gel electrophoresis were confirmed. This shows that it is possible to separate impurities in vitro transcribed ssRNA using reversed-phase HPLC. Also see page 1 for information about purification of long-chain ssRNA.

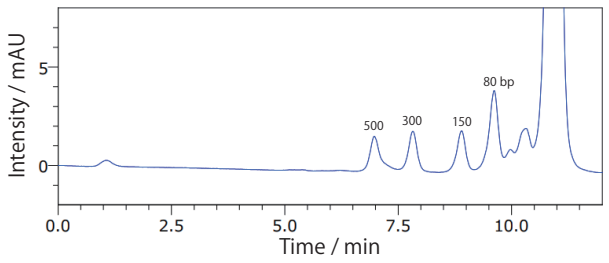
COSMOSIL RNA-SEC-1000• RNA-SEC-2000 (RNA ladder)

● Low Range ssRNA Ladder (65℃)



<Conditions>
Column: COSMOSIL RNA-SEC-1000 4.6 mm I.D. × 250 mm
Mobile phase: 20 mmol/L Phosphate Buffer (pH 7.0)
Flow rate: 0.3 mL/min
Temperature: 65℃
Detection: UV 260 nm
Sample: Low range ssRNA Ladder (New England Biolabs #N0364S)
2.5 times dilution × 2 µL

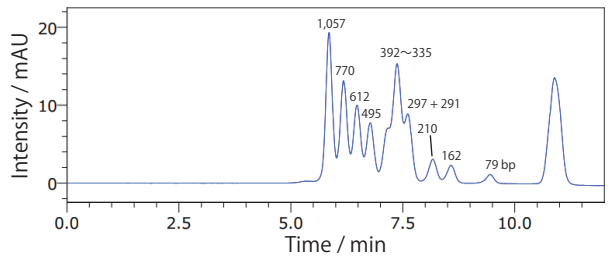
● dsRNA Ladder (40℃)



<Conditions>
Column: COSMOSIL RNA-SEC-1000 4.6 mm I.D. × 250 mm
Mobile phase: 20 mmol/L Phosphate Buffer (pH 7.0)
Flow rate: 0.3 mL/min
Temperature: 40℃
Detection: UV 260 nm
Sample: dsRNA Ladder (New England Biolabs #N0363S)
2.5 times dilution × 2 µL

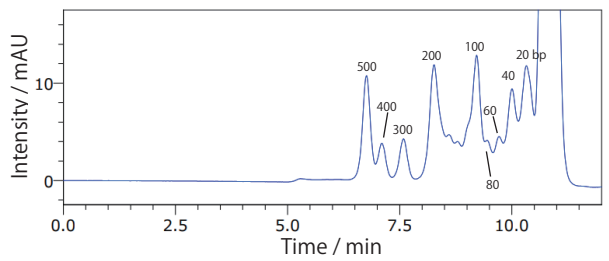
COSMOSIL RNA-SEC-1000• RNA-SEC-2000 (DNA ladder)

● ΦX174 / *Hin*cII digest



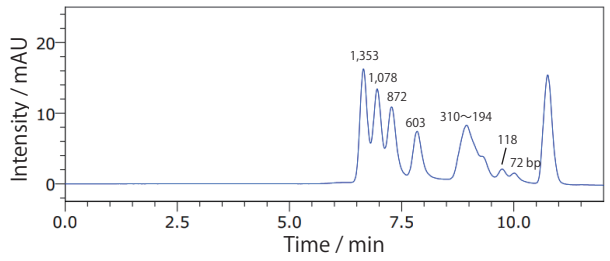
<Conditions>
Column: COSMOSIL RNA-SEC-1000 4.6 mm I.D. × 250 mm
Mobile phase: 20 mmol/L Phosphate Buffer (pH 7.0)
Flow rate: 0.3 mL/min
Temperature: 40℃
Detection: UV 260 nm
Sample: ΦX174 / *Hin*cII digest (Takara Bio #3406A)
200 ng/µL × 1.25 µL

● 20 bp DNA Ladder



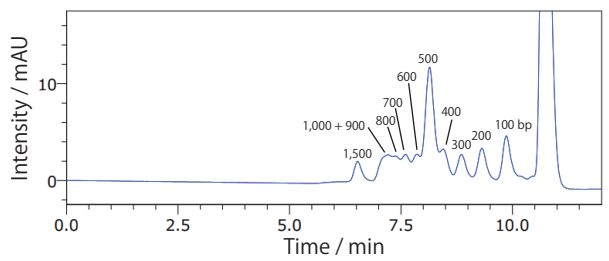
<Conditions>
Column: COSMOSIL RNA-SEC-1000 4.6 mm I.D. × 250 mm
Mobile phase: 20 mmol/L Phosphate Buffer (pH 7.0)
Flow rate: 0.3 mL/min
Temperature: 40℃
Detection: UV 260 nm
Sample: 20bp DNA Ladder (Takara Bio #3409A)
200 ng/µL × 2.5 µL

● ΦX174 / *Hae*III digest



<Conditions>
Column: COSMOSIL RNA-SEC-2000 4.6 mm I.D. × 250 mm
Mobile phase: 20 mmol/L Phosphate Buffer (pH 7.0)
Flow rate: 0.3 mL/min
Temperature: 40℃
Detection: UV 260 nm
Sample: ΦX174 / *Hae*III digest (Takara Bio #3405A)
200 ng/µL × 1.25 µL

● 100 bp DNA Ladder



<Conditions>
Column: COSMOSIL RNA-SEC-2000 4.6 mm I.D. × 250 mm
Mobile phase: 20 mmol/L Phosphate Buffer (pH 7.0)
Flow rate: 0.3 mL/min
Temperature: 40℃
Detection: UV 260 nm
Sample: 100bp DNA Ladder (Takara Bio #3407A)
130 ng/µL × 4 µL

Product specifications

Packing material	RNA-RP1	RNA-SEC-1000	RNA-SEC-2000
Separation mode	Reversed-phase	Size-exclusion	
Silica gel	Fully-porous high-purity spherical silica gel		
Average particle size (μm)	5		
Average pore size (nm, approx.)	—	100 (1,000 Å)	200 (2,000 Å)
Bonded phase	Octadecyl group	Hydrophilic group	
Suitable pH range	2 ~ 7.5		
Maximum pressure (MPa)	15		

Our article on the separation of nucleic acids using COSMOSIL RNA-RP1 has been published in Analytical Sciences

Our article on an experimental method for analyzing long-stranded RNA of about the same length as that using in COVID-19 vaccines using COSMOSIL RNA-RP1 has been published in Analytical Sciences and was later selected for inclusion in the "Hot Articles 2023" collection.

Article name：Separation of long-stranded RNAs by RP-HPLC using an octadecyl-based column with super-wide pores
Authors：Tomomi Kuwayama, Makoto Ozaki, Motoshi Shimotsuma, Tsunehisa Hirose
Journal：Analytical Sciences, 39, 417-425 (2023)

The article has information on setting various conditions when analyzing long-chain RNA using reversed-phase HPLC, including mobile phase composition, temperature, and gradient settings. We hope it will be useful.

Ordering Information

Product Name	Size	Catalog Number
COSMOSIL RNA-RP1		
COSMOSIL RNA-RP1 Packed Column	2.0 mm I.D. × 100 mm	21078-31
	4.6 mm I.D. × 100 mm	21079-21
	10 mm I.D. × 100 mm	21080-81
COSMOSIL RNA-SEC-1000		
COSMOSIL RNA-SEC-1000 Guard Column	7.5 mm I.D. × 50 mm	20785-91
COSMOSIL RNA-SEC-1000 Packed Column	4.6 mm I.D. × 250 mm	21088-01
	7.5 mm I.D. × 300 mm	19380-21
COSMOSIL RNA-SEC-2000		
COSMOSIL RNA-SEC-2000 Guard Column	7.5 mm I.D. × 50 mm	21096-91
COSMOSIL RNA-SEC-2000 Packed Column	4.6 mm I.D. × 250 mm	21095-01
	7.5 mm I.D. × 300 mm	19381-11

Other sizes may be available. Please contact us.

nacalai tesque
The quality for certainty.

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