SR1002

# Circular RNA Separation by SEC and RP Columns

*Sepax Technologies*

# Overview

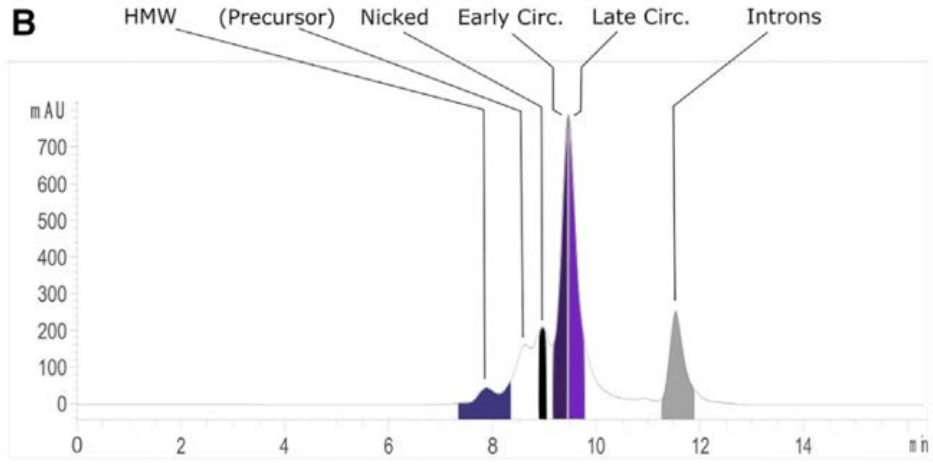
mRNA has continued to gain success as a new therapeutic agent with broad potential for application in biological systems. However, it has fundamental limitations relating to stability and its relatively short half-life. To overcome the shortcomings of linear mRNA, circular RNA is emerging as a new promising alternative modality.

**In this application:**
Size Exclusion and Reversed Phase HPLC methods are discussed for the separation of the Circular RNA sample. Sepax **SRT SEC-2000** column in 2000 Å large pore size was able to separate its aggregates and fragments. And the Sepax **Bio-C18** column in 300 Å can be used to analyze the precursor, nicked and circular RNA.

## Sample information:

- Precursor: Uncircularized RNA, approximately 2800 nt.
- Nicked: Linear RNA, approximately 2500 nt.
- Circular: Circular RNA, approximately 2500 nt.

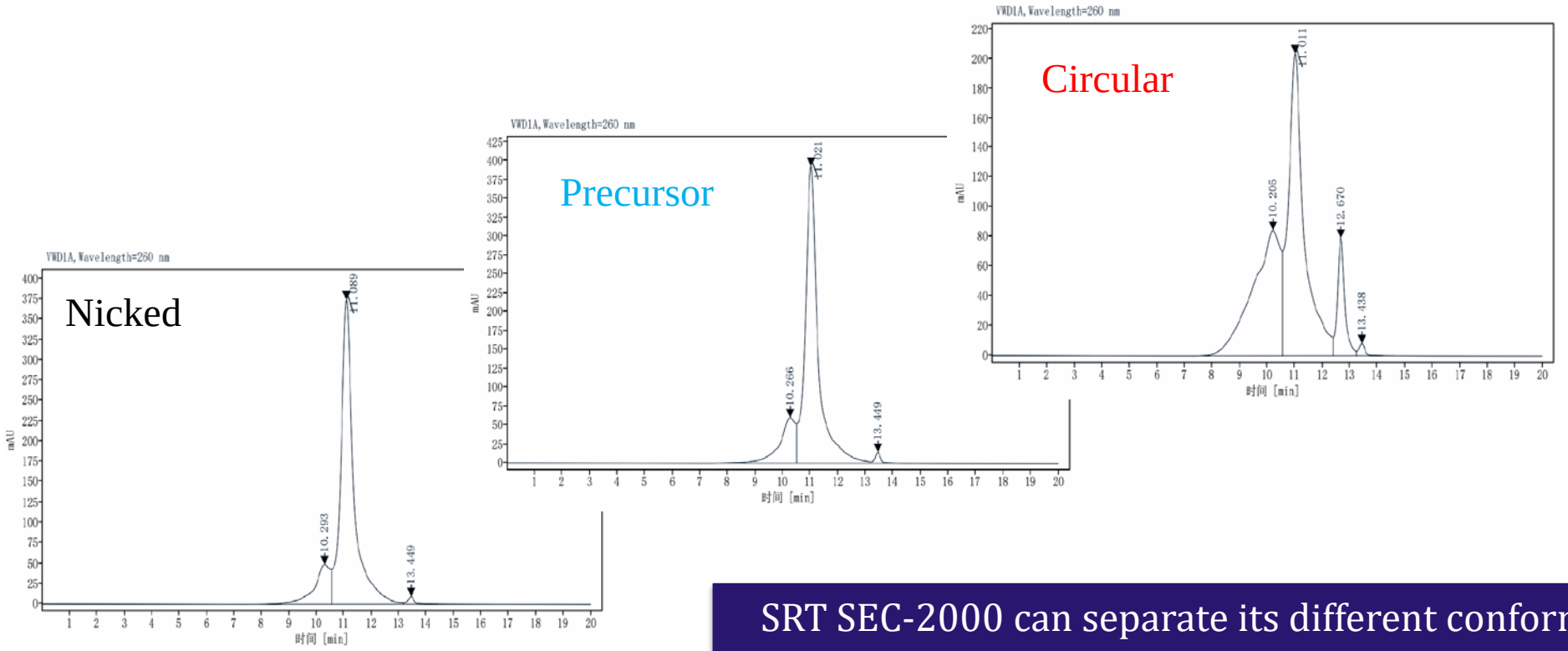
*Ensure the solvent used for sample prep is RNase-free water




**Picture Source: Alexander, et al. "RNA circularization diminishes immunogenicity and can extend translation duration in vivo." Molecular cell 74.3 (2019): 508-520

# circRNAs – SEC method

Column: SRT SEC-2000, 5 µm, 2000 Å, 7.8 × 300 mm; Part Number: 215980-7830;  Mobile Phase: 150 mM PB pH 7.0; Flow Rate: 0.8 mL/min; Detector: UV 260 nm; Column Temperature: RT; Injection Volume: 10 µL, 1 mg/mL; Column Pressure: 58 bar

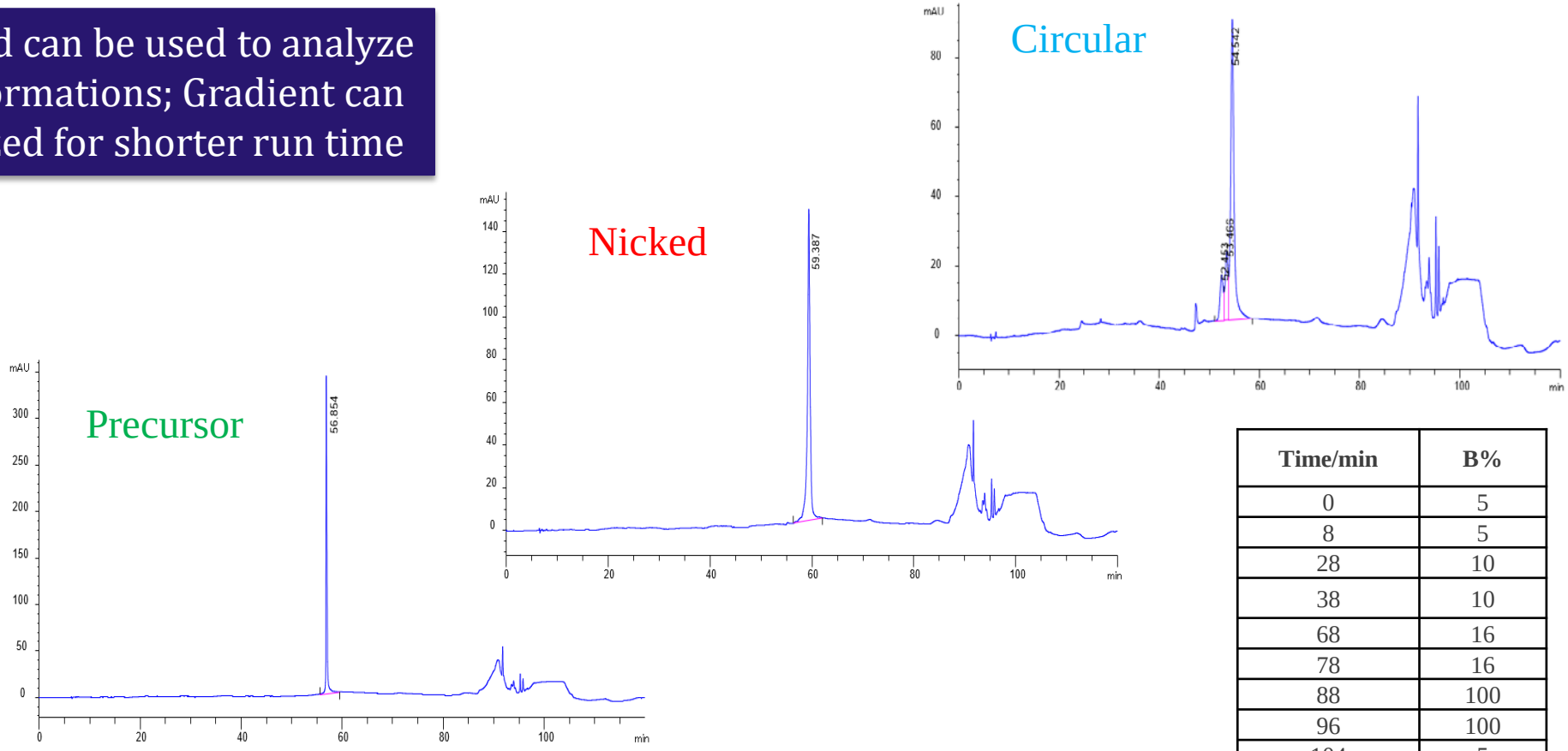



SRT SEC-2000 can separate its different conformations and effectively separate its aggregates and fragments

# circRNAs – RP method

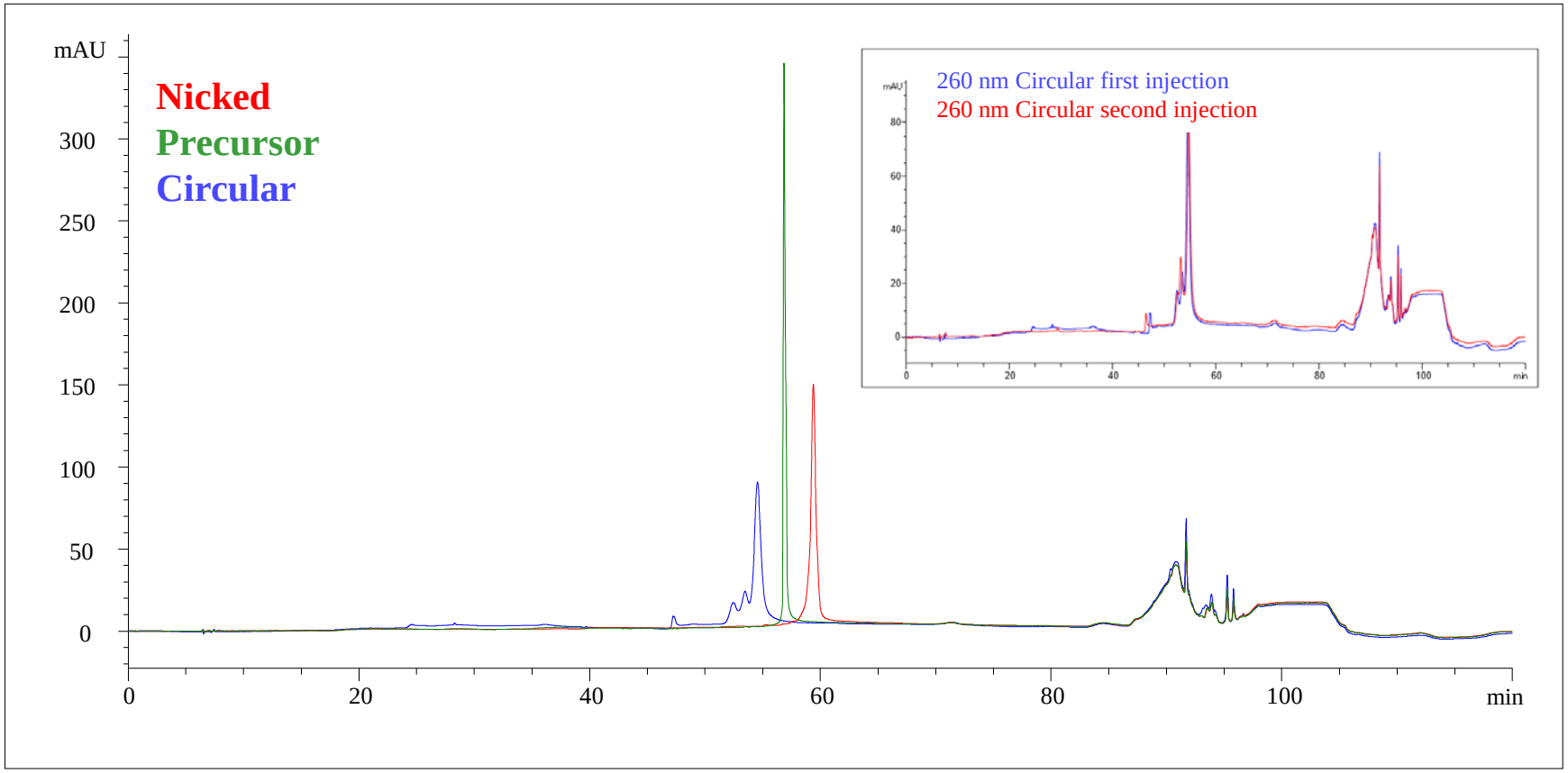
Column: circRNA-specific column Bio-C18, 5 µm, 300 Å, 4.6 × 250 mm; Part Number: 106185-4625;
Mobile Phase: *A:* 100 mM TEAA, *B:* 95% ACN + 5% A; Flow Rate: 0.5 mL/min; Detector: UV at 260 nm;
Column Temperature: 50 °C; Injection Volume: 2 µL, 1 mg/mL; Column Pressure: 57 bar

Bio-C18 RP method can be used to analyze the different conformations; Gradient can be further optimized for shorter run time




| Time/min | B% |
|---|---|
| 0 | 5 |
| 8 | 5 |
| 28 | 10 |
| 38 | 10 |
| 68 | 16 |
| 78 | 16 |
| 88 | 100 |
| 96 | 100 |
| 104 | 5 |
| 120 | 5 |

# circRNAs – RP method overlays



# Literature Review – circRNA Separation

| App ID | Paper Title |
|---|---|
| LR202202962 | RNA Circularization Diminishes Immunogenicity and Can Extend Translation Duration In Vivo |
| LR2022021136 | Design and application of circular RNAs with protein-sponge function |
| LR2022021128 | Engineered circular ADAR-recruiting RNAs increase the efficiency and fidelity of RNA editing in vitro and in vivo |
| LR2022021104 | Intratumoral Delivered Novel Circular mRNA Encoding Cytokines for Immune Modulation and Cancer Therapy |
| LR2022021101 | RNA circles with minimized immunogenicity as potent PKR inhibitors |
| LR2022021052 | Engineering circular RNA for potent and stable translation in eukaryotic cells |

# Order Info

| Product | Particle Size | Pore Size | Column Size | PN |
|---|---|---|---|---|
| SRT SEC-2000 | 5 µm | 2000 Å | 7.8 × 300 mm | 215980-7830 |
| Bio-C18 | 5 µm | 300 Å | 4.6 × 250 mm | 106185-4625 |

Check our website for more products and application data
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SEC | Affinity | IEX | HIC | RP | Service



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