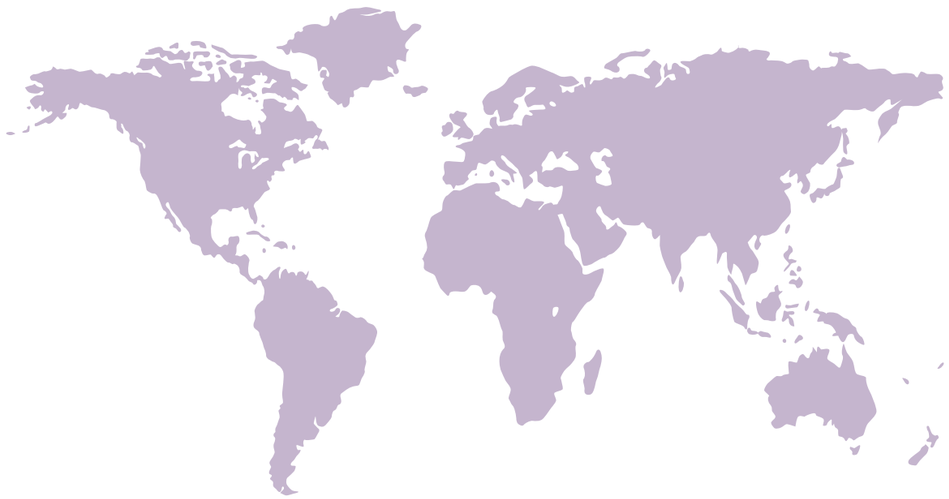

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NANOCHROM

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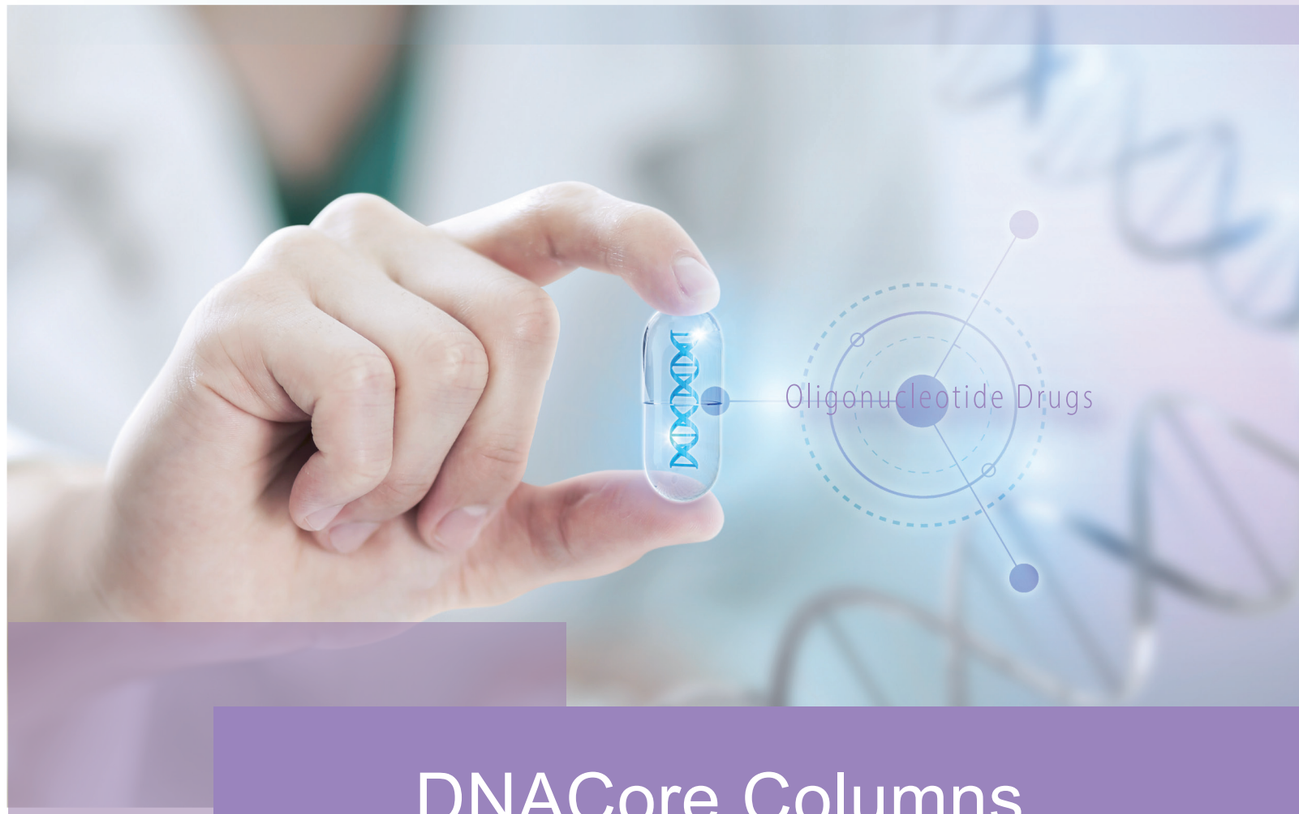
NanoChrom Technologies



DNACore Columns

for oligonucleotide separation

Website

WeChat Official Account

Introduction

Oligonucleotide therapeutics can treat diseases at genetic level and have become a new generation of medicines. Due to COVID-19 pandemic, the demand for oligonucleotides in clinical diagnostics has increased dramatically. More importantly, mRNA-based vaccines have attracted much attention worldwide for their efficacy against COVID-19 and stimulated fanatical booming in developing oligonucleotides for gene therapeutics.
Plasmid (pDNA) production is an important step in manufacturing mRNA, as well as other gene therapeutics. Plasmids have several topologies, including open circular, supercoiled, linear, etc. Among them, supercoiled plasmids have strong impact on cell transfection efficiency and safety of the drug.
Small oligonucleotides, such as ASO, siRNA, miRNA and Aptamer, can also potentially used as gene therapeutics.

DNA

mRNA

Plasmid

For critical quality attribute analysis of short oligonucleotides, mRNA and plasmids, HPLC is undoubtedly one of important techniques for oligonucleotide analysis with advantages in throughput, sensitivity, precision and detectability, to ensure drug efficacy and safety.

Oligonucleotides, including mRNA and plasmids, contain different number of nucleic acid monomers whose structure consists of a nucleic acid base, a ribose and a phosphate group. They are highly polar molecules with large quantity of negative charges. Ion-pairing reversed-phase, anion-exchange and size exclusion chromatography are commonly used for characterizing oligonucleotides for determining purity, plasmid topology content and aggregates in mRNA/plasmids, respectively.

DNACore HPLC Columns

DNACore is a column family, designed for high-resolution separation of oligonucleotides, including mRNAs, plasmids and short oligos, by liquid chromatography. These columns are based on advanced mono-dispersed particle technology and specially designed column chemistry, resulting in high resolution, ideal selectivity, good stability and decent consistency. DNACore column offering consists of three separation modes: IP-RP, SAX and SEC, providing a complete tool-set and a broad application coverage for oligonucleotide separation in bio-tech, biopharmaceutical and academic research.

Main Features

- Ideal selectivity for oligonucleotides of different sizes
- Multiple separation modes (IP-RP, IEX and SEC) for broad application coverage
- High stability for long column life
- Good column-to-column consistency

DNACore HPLC Columns

Specification

Product Name	DNACore 1000 C18	DNACore NP-Q	DNACore SEC-1000
Functional Group	Octadecyl	Quatenary Ammonium	Diol
Substrate	Monodispersed, spherical silica particles	Monodispersed, spherical PS/DVB particles	Monodispersed, high pore-volume, spherical silica particles
Particle Size	5 µm	5 µm	3 µm
Pore Size	1000 Å	Nonporous	1000 Å
Pressure Limit	5000 psi	5000 psi	2500 psi
Temperature Limit	60 °C	80 °C	40 °C
pH Range	2-11	2-12	2-8
Column Dimension	4.6×150 mm 4.6×250 mm	4.6×150 mm 4.6×250 mm	4.6×300 mm 7.8×300 mm
Application	Purity analysis of oligonucleotides, including short-chain oligos, DNA, mRNA, plasmids, etc.	Content analysis of plasmid topology	Aggregates and fragment analysis in mRNAs and plasmids

Quality Assurance

Each batch of separation media is produced in accordance with a strict quality management system and tested with relevant biological molecules to ensure separation performance and batch-to-batch consistency.

Each DNACore column is produced by reliable packing methods and tested individually using well-designed chromatography tests, to ensure quality and consistency. A certificate of assurance of the separation media and a column quality assurance report are shipped with every DNACore column.

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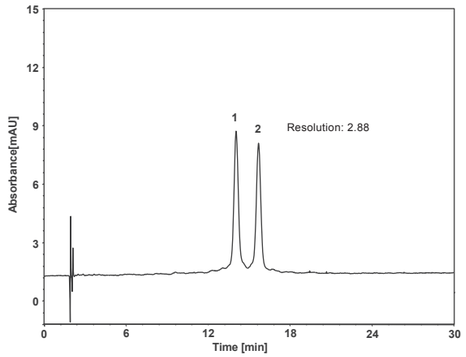
Application

Ion-Pairing Reversed-Phase Mode

During oligonucleotide synthesis, it is unavoidable to produce impurities with sizes close to target molecules such as N-1 and N+1. To ensure drug quality, purity analysis is important. In this application, two short-chain oligonucleotides (20 mer and 21 mer) with one unit difference are separated with baseline resolution (Rs=2.88) on the DNACore 1000 C18 column under IP-RP mode.

Short-Chain Oligonucleotides



Column: **DNACore 1000 C18**, 5 μm
Dimension: 4.6×150 mm
Mobile Phase: A) 100 mM TEAA*, pH7.0
B) MeCN

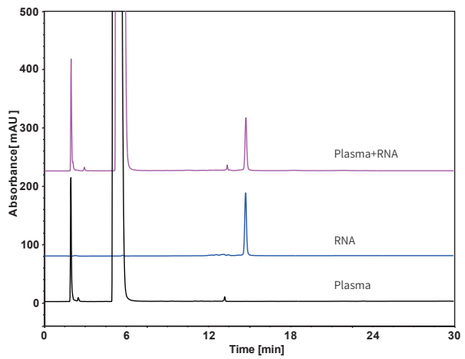
Gradient:	t (min)	%A	%B
	0	92	8
	30	90	10
	30.1	0	100
	32	0	100
	32.1	92	8
	45	92	8

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 5 μL
Detection: UV 260 nm
Sample: 20 mer+21 mer (2 nmol/mL each)
Peaks: 1. 20 mer
2. 21 mer

TEAA*: Triethylamine acetate

Pharmacokinetic research is an important part in drug preclinical research and clinical research. A RNA drug (180 nt) can be successfully separated from other components in plasma under optimized chromatographic condition on a DNACore 1000 C18 column, demonstrating its suitability for monitoring metabolism of RNA drug in human body.

RNA



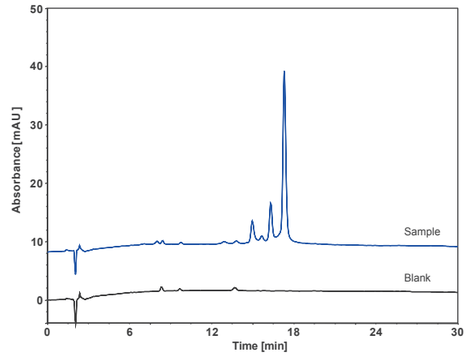
Column: **DNACore 1000 C18**, 5 μm
Dimension: 4.6×150 mm
Mobile Phase: A) 100 mM TEAA, pH7.0
B) 25/75 v/v MeCN/ 100 mM TEAA, pH7.0

Gradient:	t(min)	%A	%B
	0	75	25
	30	25	75
	31	50	50
	40	50	50

Flow Rate: 1.0 mL/min
Temperature: 50 ℃
Injection: 10 μL
Detection: UV 260 nm
Sample: RNA (180 nt, 100 μg/mL)

Purity is critical for efficacy and safety of mRNA vaccines. The DNACore 1000 C18 column provides satisfactory separation for purity analysis of a mRNA vaccine using IP-RP chromatography, meeting customer needs.

mRNA-Based Vaccine



Column: **DNACore 1000 C18**, 5 μm
Dimension: 4.6×250 mm
Mobile Phase: A) 100 mM TEAA, pH7.0
B) 25/75 v/v MeCN/100 mM TEAA, pH7.0

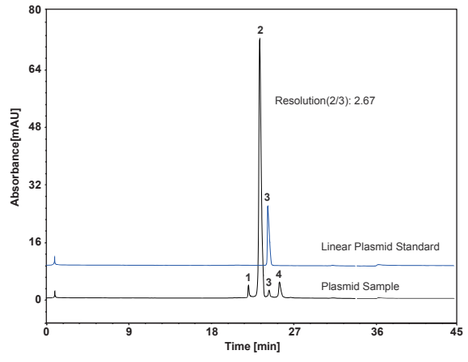
Gradient:	t(min)	%A	%B
	0	60	40
	30	35	65
	31	60	40
	40	60	40

Flow Rate: 1.0 mL/min
Temperature: 60 ℃
Injection: 10 μL
Detection: UV 254 nm
Sample: mRNA Vaccine (1000-2000 nt)

Anion Exchange Mode

In plasmid production process, the supercoiled plasmid is the target and main product, while plasmids with different topology are also present. DNACore NP-Q column can be used to determine the purity of supercoiled plasmid: under strong anion exchange mode, baseline separation can be achieved between supercoiled plasmid (target) and open-circular and linear impurity plasmids.

Plasmid



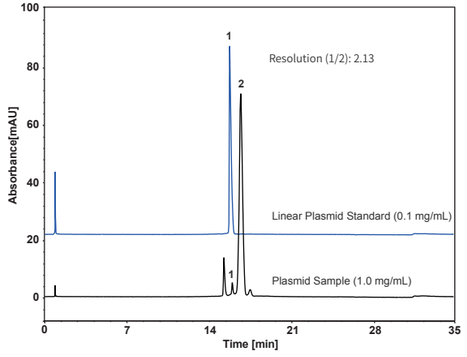
Column: **DNACore NP-Q**, 5 μm
Dimension: 4.6×150 mm
Mobile Phase: A) 20 mM Tris, pH8.5
B) 500 mM NaCl in 20 mM Tris, pH8.5

Gradient:

t (min)	%A	%B
0	50	50
30	15	85
30.1	0	100
35	0	100
35.1	50	50
45	50	50

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 3 μL
Detection: UV 260 nm
Peaks: 1. Open-Circular Plasmid
2. Supercoiled Plasmid
3. Linear Plasmid
4. Unknown Plasmid

Plasmid



Column: **DNACore NP-Q**, 5 μm
Dimension: 4.6×150 mm
Mobile Phase: A) 20 mM Tris, pH8.5
B) 1 M NaCl in 20 mM Tris, pH8.5

Gradient:

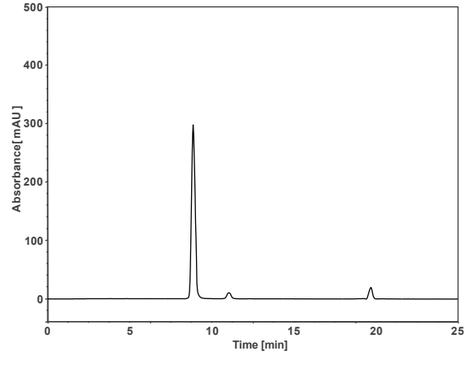
t (min)	%A	%B
-12	40	60
0	40	60
25	15	85
25.1	0	100
30	0	100
30.1	40	60
35	40	60

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection:
Black: 2 μL
Blue: 5 μL
Detection: UV 260 nm
Peaks: 1. Linear Plasmid
2. Supercoiled Plasmid

Size Exclusion Chromatography Mode

SEC is another important separation mode for purity analysis of large size oligonucleotides. Taking plasmids as an example, considering their large size, wide-pore SEC columns are required to separate target supercoiled plasmid from impurities such as aggregates and fragments. In this application, the desired plasmid can be well separated from impurities in a real-life sample on a DNACore SEC-1000 column.

Plasmid



Column: **DNACore SEC-1000**, 3 μm
Dimension: 4.6×300 mm
Mobile Phase: 300 mM NaCl in 50 mM phosphate buffer, pH6.8
Flow Rate: 0.21 mL/min
Temperature: 30 ℃
Injection: 10 μL
Detection: UV 260 nm (2.5 μL flow cell)
Sample: Plasmid

Ordering Information

Product Name	Particle Size (μm)	Column Dimension L x ID (mm)	Part Number
DNACore 1000 C18	5	250×4.6	D003-050100-04625S
		150×4.6	D003-050100-04615S
DNACore NP-Q	5	250×4.6	D301-050000-04625P
		150×4.6	D301-050000-04615P
DNACore SEC-1000	3	300×4.6	D203-030100-04630S
		300×7.8	D203-030100-07830S

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