Supelco®
Analytical Products

MERCK

Unleash the Performance Power for Oligo Analysis

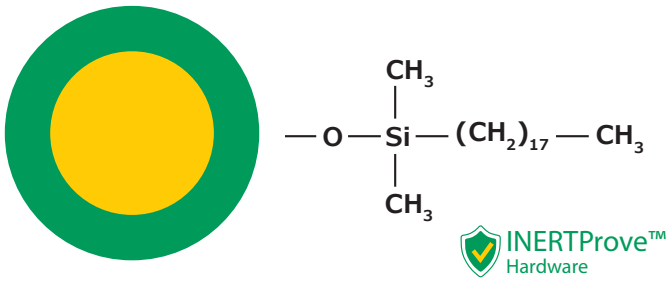
BIOshell™ A120 (2.7 µm) Oligo C18 INERTProve™ HPLC Columns: from 1.5 mm I.D. to 4.6 mm I.D.

Oligonucleotides, which are short sequences of nucleotides, have gained significant attention for their diverse applications in biotherapeutics, including gene therapy, RNA interference, and antisense oligonucleotide therapies. The therapeutic efficacy of these compounds heavily depends on their purity and structural integrity, making robust analytical methods essential for quality control.

High-Performance Liquid Chromatography (HPLC) is a critical technique for monitoring oligonucleotide synthesis, offering valuable insights into their purity and structural integrity. However, traditional HPLC methods often face challenges in separating oligonucleotides due to their complex structures and the presence of impurities. Elevated pH and temperature conditions, required for optimal and efficient separations, can further complicate these separations, as conventional silica materials used in Fully Porous Particle columns are typically unsuitable for such environments.

The new BIOshell™ A120 Oligo C18 INERTProve™ column is specifically designed for high pH stability, in high performance separations of oligonucleotides. Built on the proven Fused-Core® particle technology for speed and efficiency, the BIOshell™ A120 Oligo C18 INERTProve™ column features surface-modified organo-silane technology for enhanced alkaline resistance, resulting in excellent stability under elevated pH operating conditions commonly encountered in oligonucleotide separation methods.

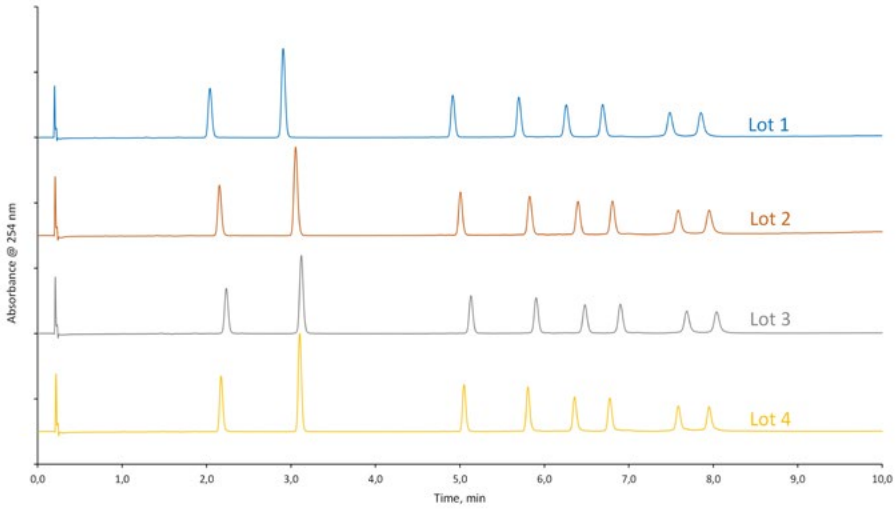
The BIOshell™ A120 Oligo C18 INERTProve™ columns are housed in surface-passivated column hardware (high inertness) to address adsorption concerns, making them directly ready for use with standard or bio-inert instrumentation.



BIOshell™ A120 Oligo C18 column features:

- 120 Å Pore Size: Allows for the separation of oligomers up to 60 bases in length.
- High pH and Temperature Stability: Engineered to perform under conditions ideal for oligonucleotide separations.
- Compatible with UHPLC and Mass Spectrometry: The stationary phase is designed for compatibility with advanced analytical techniques.
- Surface-Passivated INERTProve™ Column Hardware: Minimizes the risk of sample components being adsorbed by stainless steel.

BIOshell™ A120 Oligo C18 2.7 µm columns exhibit high lot-to-lot consistency



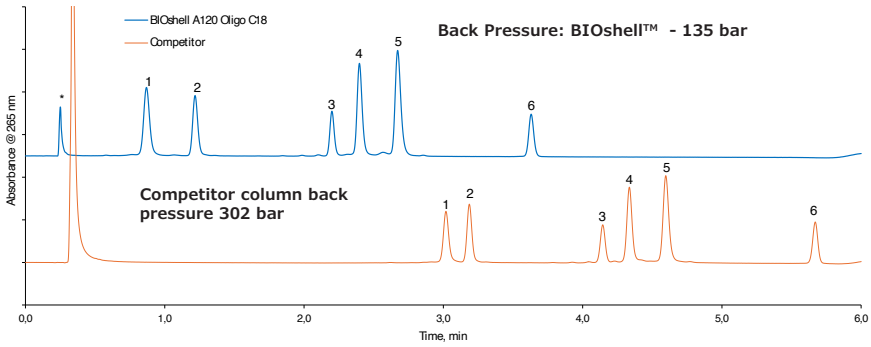
LC Conditions

Instrument:	Shimadzu Nexera X2
Column:	BIOshell™ A120 Oligo C18, 2.7 µm, 5 cm x 2.1 mm I.D. (**70003-U**)
Mobile Phase:	[A] 10 mM TEAA, pH 8.5; [B] acetonitrile
Gradient:	**Time (min) / %B**: 0.0 / 5; 10.0 / 11; 11.0 / 11; 11.5 / 5
Flow Rate:	0.5 mL/min
Pressure:	125 bar
Column Temp.:	60 °C
Detector:	UV/PDA, 254 nm; flow cell: 1 µL; data rate: 100 Hz; response time: 0.025 s
Injection:	1.0 µL
Sample(s):	Oligo mix (10- to 60-mer) in 10 mM Tris HCl/1 mM EDTA
Peak IDs:	1 - 10 mer, 2 - 15 mer, 3 - 20 mer, 4 - 25 mer, 5 - 30 mer, 6 - 40 mer, 7 - 50 mer, 8 - 60 mer.

Faster separations at the lower backpreassure

BIOshell™ A120 Oligo C18 2.7 µm INERTProve™ column outperformed a Fully Porous Particles (FPP) Oligo C18 column in oligonucleotide separations, achieving faster separations while maintaining efficiencies comparable to a 1.9 µm FPP which had higher backpressure.

The BIOshell™ A120 Oligo C18 2.7 µm INERTProve™ column also exhibited higher resolution between critical peak pairs (peaks 1-2 and 3-4).



LC Conditions

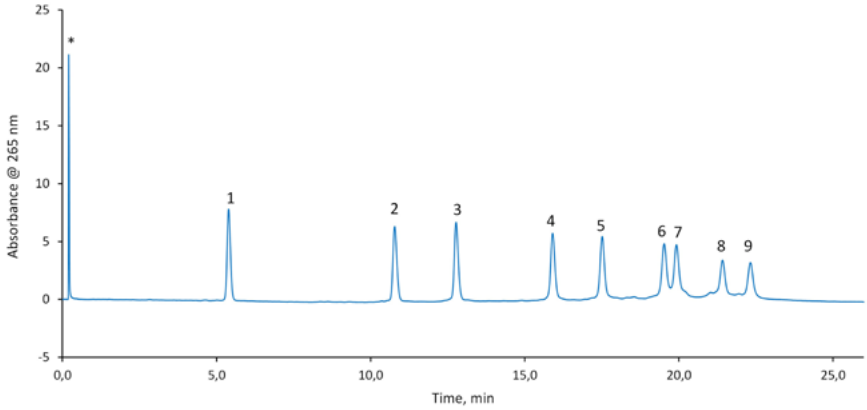
Instrument:	Shimadzu Nexera X2
Columns:	BIOshell™ A120 Oligo C18, 2.7 µm, 5 cm x 2.1 mm I.D. (**70003-U**) Competitor FPP 120 Å C18, 1.9 µm, 2.1 x 50 mm
Mobile phase:	[A] 100 mM TEAA, pH 7; [B] methanol
Gradient:	**Time / %B**: 0.0 / 17; 5.0 / 30; 5.3 / 60; 5.6 / 60; 5.8 / 17
Flow Rate:	0.4 mL/min
Pressure:	135 bar (BIOshell™), 302 bar (competitor FPP)
Column temp.:	50 °C
Detector:	PDA, 265 nm; flow cell: 1 µL; data rate: 40 Hz; response time: 0.05 s
Injection:	1.0 µL
Sample(s):	Performance Standard Mix in 10 mM Tris HCl/1 mM EDTA pH 8.0
Peak IDs:	1 - 20 mer, 2 - 15 mer, 3 - 12 mer, 4 - 25 mer, 5 - 33 mer, 6 - 12 mer

On average, the signal-to-noise ratio in the chromatogram for BIOshell™ columns (superficially porous particles) is significantly higher compared to fully porous particle-packed columns, with values of 176 versus 114. This higher signal-to-noise ratio leads to better detectability and improved detection/quantification limits, primarily due to greater column efficiency and reduced sample loss. The superficially porous particle (SPP) design minimizes the diffusion pathway that biomolecules must traverse through the particles, resulting in sharper peaks and more accurate quantification.

Separation of long sequence oligomers

Even separations of large, single-stranded DNA (ssDNA) oligomers are achievable with BIOshell™ A120 Oligo C18 INERTProve™ columns. As demonstrated in the example on the next page, these columns successfully separate up to 100-mer oligomers, showcasing their capability to handle complex and lengthy sequences with exceptional precision and efficiency.

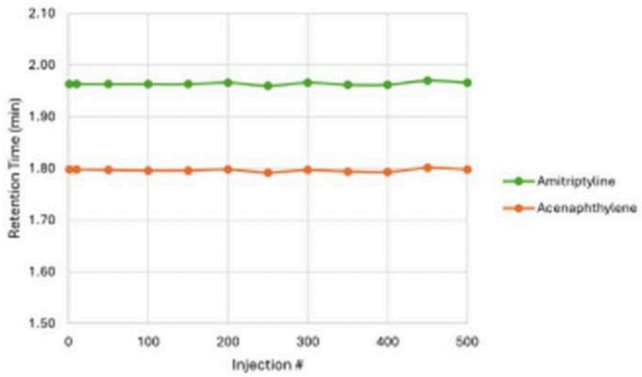
Separation of long oligomers: from 20- to 100- mer



LC Conditions

Instrument:	Shimadzu Nexera X2
Column:	BIOshell™ A120 Oligo C18, 2.7 µm, 5 cm x 2.1 mm I.D.(**70003-U**)
Mobile phase:	[A] 10 mM TEAA, pH 7; [B] acetonitrile
Gradient:	**Time** / **%B**: 0.0 / 6.5; 30.0 / 11.0; 31.0 / 11.0; 31.1 / 6.5; 35.0 / 6.5
Flow Rate:	0.5 mL/min
Pressure:	144 bar
Column temp.:	60 °C
Detector:	PDA, 265 nm; flow cell: 1 µL; data rate: 40 Hz; response time: 0.5 s
Injection:	2.0 µL (10 µg)
Sample:	Oligo mix (ssDNA 20- to 100-mer) in 10 mM Tris HCl/1 mM EDTA, pH 8.0
Peak IDs:	1 - 20 mer, 2 - 30 mer, 3 - 40 mer, 4 - 50 mer, 5 - 60 mer, 6 - 70 mer, 7 - 80 mer, 8 - 90 mer, 9 - 100 mer

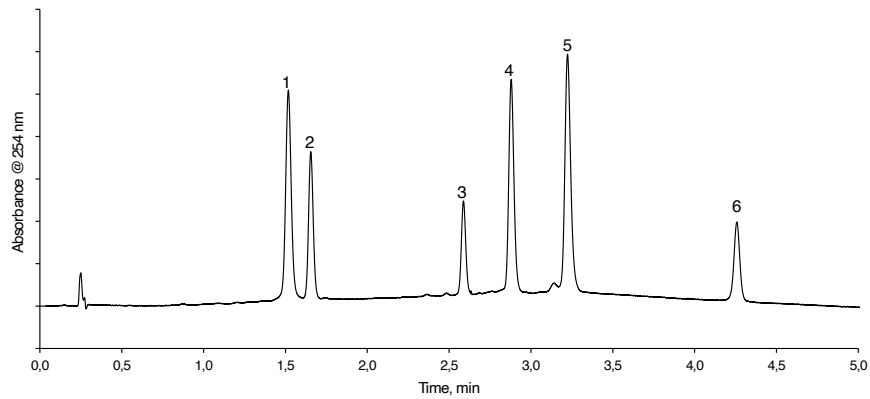
You can confidently rely on the long-term performance of the BIOshell™ A120 Oligo C18 INERTProve™ column. Extensive testing of the packing material stability demonstrated that there is less than a 1% change in retention over 20,000 column volumes, indicating exceptional durability and consistency. This stability assessment was conducted under challenging conditions, including high pH (10) and elevated temperature (60 °C), which are commonly encountered in oligonucleotide separations. Such resilience ensures that the BIOshell™ A120 Oligo C18 INERTProve™ column maintains its performance over extended use, making it a reliable choice for your analytical needs.



Long term column stability under challenging conditions (pH 10 at 60 °C)

Rapid separation example using short BIOshell™ Oligo column

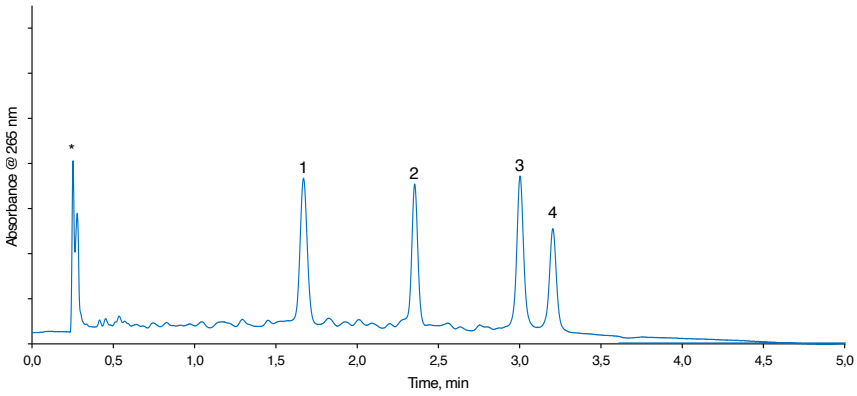
Save time and solvent with rapid separations using BIOshell™ Oligo columns, designed for high efficiency and exceptional stability. These columns enable quick and reliable analysis, allowing for faster throughput in your oligonucleotide workflows while maintaining excellent separation performance. By using a 5 cm x 2.1 mm I.D. BIOshell™ A120 Oligo C18 INERTProve™ column under high pH conditions, a sample of six different oligonucleotides can be separated in under five minutes. Using the Supelco® Oligonucleotide Performance Standard Mix (**PHR8667**), the utility of the column can be explored. The sample has a range of oligomers from 12 to 33 in base length with two of the six oligomers having the same base length. The two 12 base length oligomers are separated with ease on this short BIOshell™ A120 Oligo INERTProve™ C18 column.



Test Conditions:

Instrument:	Shimadzu Nexera X2
Column:	BIOshell™ A120 Oligo C18, 2.7 µm, 5 cm x 2.1 mm I.D. (**70003-U**)
Mobile phase:	[A] 10 mM TEAA, pH 8.5; [B] acetonitrile
Gradient:	**Time** / **%B**: 0.0 / 7.5; 5.0 / 15.0; 5.3 / 60.0; 5.6 / 60.0; 8.0 / 7.5
Flow Rate:	0.4 mL/min
Pressure:	142 bar
Column temp.:	50 °C
Detector:	PDA, 254 nm; flow cell: 1 µL; data rate: 100 Hz; response time: 0.5 s
Injection:	1 µL
Sample:	Oligonucleotide Performance Standard Mix, 12-33 NT in 10 mM Tris HCl/1 mM EDTA, pH 8.0
Peak IDs:	1 - 20 mer, 2 - 15 mer, 3 - 12 mer, 4 - 25 mer, 5 - 33 mer, 6 - 12 mer

An ssRNA ladder of mixed sequence and length is separated on the BIOshell™ A120 Oligo C18 INERTProve™ column. The RNA, ranging from 17 - 29 mer in length, show less retention compared to similar length ssDNA samples, but are still baseline separated.



LC Conditions:

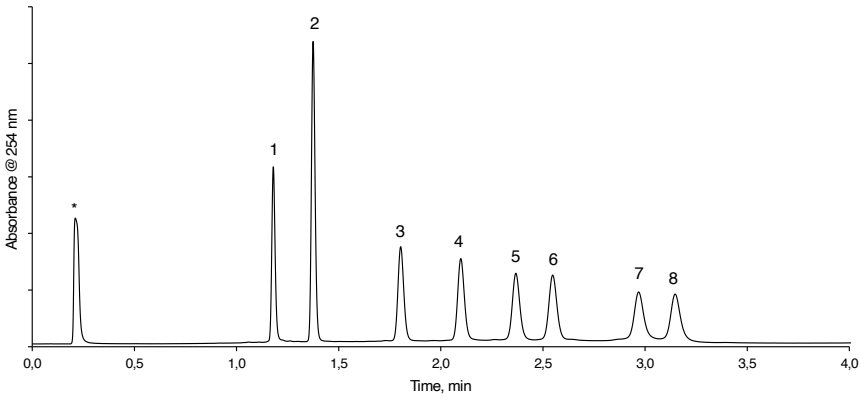
Instrument:	Shimadzu Nexera X2
Column:	BIOshell™ A120 Oligo C18, 2.7 µm, 5 cm x 2.1 mm I.D. (**70003-U**)
Mobile phase:	[A] 10 mM TEAA adjusted to pH 8.56; [B] acetonitrile

Gradient:	Time	%B
	0.0	5
	5.0	10
	5.3	60
	5.6	60
	5.7	5
	9.0	5

Flow Rate:	0.4 mL/min
Pressure:	116 bar
Column temp.:	60 °C
Detector:	PDA, 265 nm;flow cell: 1 µL; data rate: 40 Hz; response time: 0.05 s
Injection:	3.0 µL, 30 ng on column
Sample:	ssRNA ladder (17- to 29-mer) in 10 mM Tris HCl/1 mM EDTA, pH 8.0
Peak IDs:	1 - 17 mer, 2 - 21 mer, 3 - 25 mer, 4 - 29 mer

Rapid separation of oligonucleotides up to 60-mer using a 5 cm x 2.1 mm I.D. BIOshell™ A120 Oligo C18 column

This example illustrates the resolution of an oligonucleotide standard mixture of mixed sequence and length (10 - to 60 - mer ssDNA) utilizing triethylammonium acetate (TEAA) with absorbance detection and gradient elution with acetonitrile. This rapid separation, completed in less than 3.5 minutes, showcases the excellent peak shape and high resolution of oligonucleotides up to 60 bases in length, achieved by using a 5 cm x 2.1 mm I.D. BIOshell™ A120 Oligo C18 INERTProve™ column.



LC Conditions:

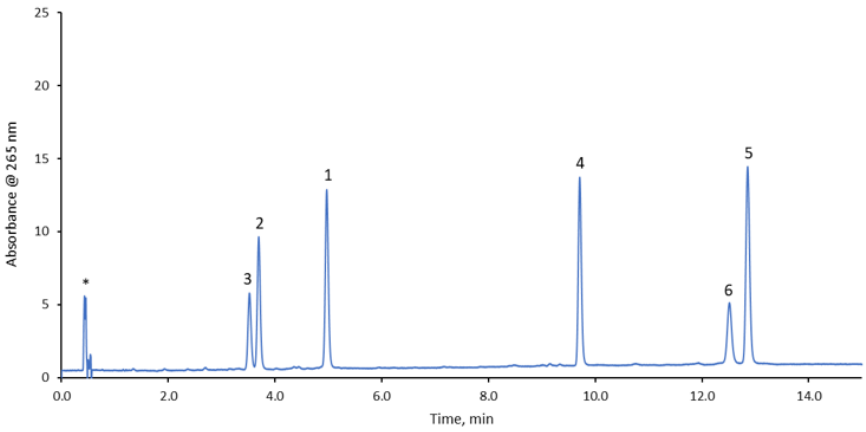
Instrument:	Shimadzu Nexera X2
Column:	BIOshell™ A120 Oligo C18, 2.7 µm, 5 cm x 2.1 mm I.D. (**70003-U**)
Mobile phase:	[A] 10 mM TEAA, pH 8.5; [B] acetonitrile

Gradient	Time	%B
	0.0	5.0
	0.5	7.4
	3.5	10.7
	3.6	20.0
	4.1	20.0
	4.2	5.0

Flow Rate	0.5 mL/min
Pressure	137 bar
Column temp.:	60 °C
Detector:	PDA, 254 nm; flow cell: 1 µL; data rate: 100 Hz; response time: 0.025 s
Injection:	2.0 µL (10 µg)
Sample:	Oligonucleotide standard mixture (10- to 60-mer ssDNA) in 10 mM Tris HCl/1 mM EDTA, pH 8.0
Peak IDs:	1 - 10 mer, 2 - 15 mer, 3 - 20 mer, 4 - 25 mer, 5 - 30 mer, 6 - 40 mer, 7 - 50 mer, 8 - 60 mer

The Oligonucleotide HPLC Performance Mix (**PHR8667**), consisting of six, mixed-sequence oligonucleotides, serves as a reliable benchmark for evaluating new methods, assisting with method development, and assessing the performance of HPLC columns. This versatile reference material can be utilized in ion-pairing reversed phase, HILIC, and ion exchange modes of chromatography, offering a valuable tool for characterizing oligonucleotides. Additionally, this mixture allows for method optimization, such as substituting acetonitrile with methanol, which is considered less hazardous[1-4], enhancing both safety and environmental sustainability in analytical processes.

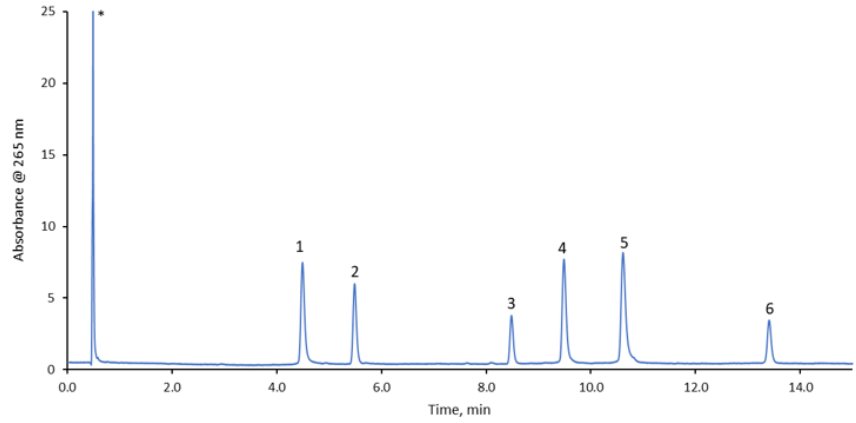
Oligonucleotide separation using acetonitrile



LC Conditions:

Instrument:	Shimadzu Nexera X2
Column:	BIOshell™ A120 Oligo C18, 2.7 µm, 10 cm x 2.1 mm I.D. (**70004-U**)
Mobile phase:	[A] 5 mM TEAA/ 50 mM HFIP, adjusted to pH 8.3; [B] acetonitrile
Gradient:	**Time** / **%B**: 0.0 / 3.5; 9.5 / 5.5; 10.5 / 5.5; 20.0 / 7.5; 20.3 / 60.0; 20.6 / 60.0
Flow Rate:	0.4 mL/min
Pressure:	142 bar
Column temp.:	50 °C
Detector:	PDA, 265 nm; flow cell: 1 µL; data rate: 40 Hz; response time: 0.05 s
Injection:	2.0 µL
Sample:	Performance Standard Mix in 10 mM Tris HCl/1 mM EDTA pH 8.0
Peak IDs:	1 - 20 mer, 2 - 15 mer, 3 - 12 mer, 4 - 25 mer, 5 - 33 mer, 6 - 12 mer

Oligonucleotide separation using less hazardous solvent - methanol



LC Conditions:

Instrument:	Shimadzu Nexera X2
Column:	BIOshell™ A120 Oligo C18, 2.7 µm, 10 cm x 2.1 mm I.D. (**70004-U**)
Mobile phase:	[A] 10 mM TEAA pH 7; [B] methanol
Gradient:	**Time** / **%B**: 0.0 / 15; 20.0 / 30; 20.3 / 60; 20.6 / 60; 20.8 / 15
Flow Rate:	0.4 mL/min
Pressure:	242 bar
Column temp.:	50 °C
Detector:	PDA, 265 nm; flow cell: 1 µL; data rate: 40 Hz; response time: 0.05 s
Injection:	2.0 µL
Sample:	Performance Standard Mix in 10 mM Tris HCl/1 mM EDTA pH 8.0
Peak IDs:	1 - 20 mer, 2 - 15 mer, 3 - 12 mer, 4 - 25 mer, 5 - 33 mer, 6 - 12 mer

References

1. The CHEM21 solvent selection guide. Acsgcipr.org. [accessed 2026 Jan 21]. https://learning.acsgcipr.org/guides-and-metrics/solvent-selection-guides/the-chem21-solvent-selection-guide/
2. Prat D et al. CHEM21 selection guide of classical- and less classical-solvents. Green Chemistry: An International Journal and Green Chemistry Resource: GC. 2016;18(1):288–296. https://doi.org/10.1039/c5gc01008j
3. PubChem. Methanol. Nih.gov. [accessed 2026 Jan 21]. https://pubchem.ncbi.nlm.nih.gov/compound/887
4. PubChem. Acetonitrile. Nih.gov. [accessed 2026 Jan 21]. https://pubchem.ncbi.nlm.nih.gov/compound/6342

Ordering information:

ID (mm)	Length (cm)	Description	Cat.No.
1.5	5	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70000-U
1.5	10	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70001-U
1.5	15	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70002-U
2.1	5	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70003-U
2.1	10	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70004-U
2.1	15	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70005-U
3.0	5	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70006-U
3.0	10	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70007-U
3.0	15	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70008-U
4.6	5	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70009-U
4.6	10	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70010-U
4.6	15	BIOshell™ A120 (2.7 µm) Oligo C18 HPLC INERTProve™ Column, Pk.1	70011-U

Related Products

Description	Cat.No.
Oligonucleotide HPLC Performance Standard Mix, 12-33 NT	PHR8667

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