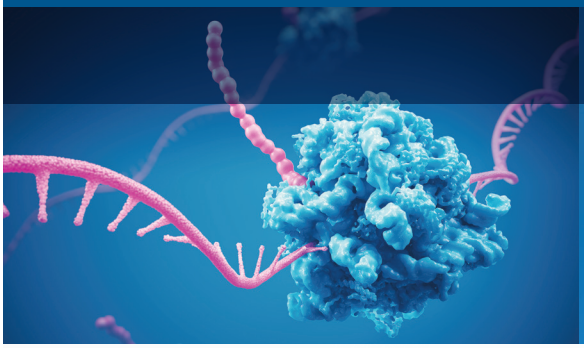
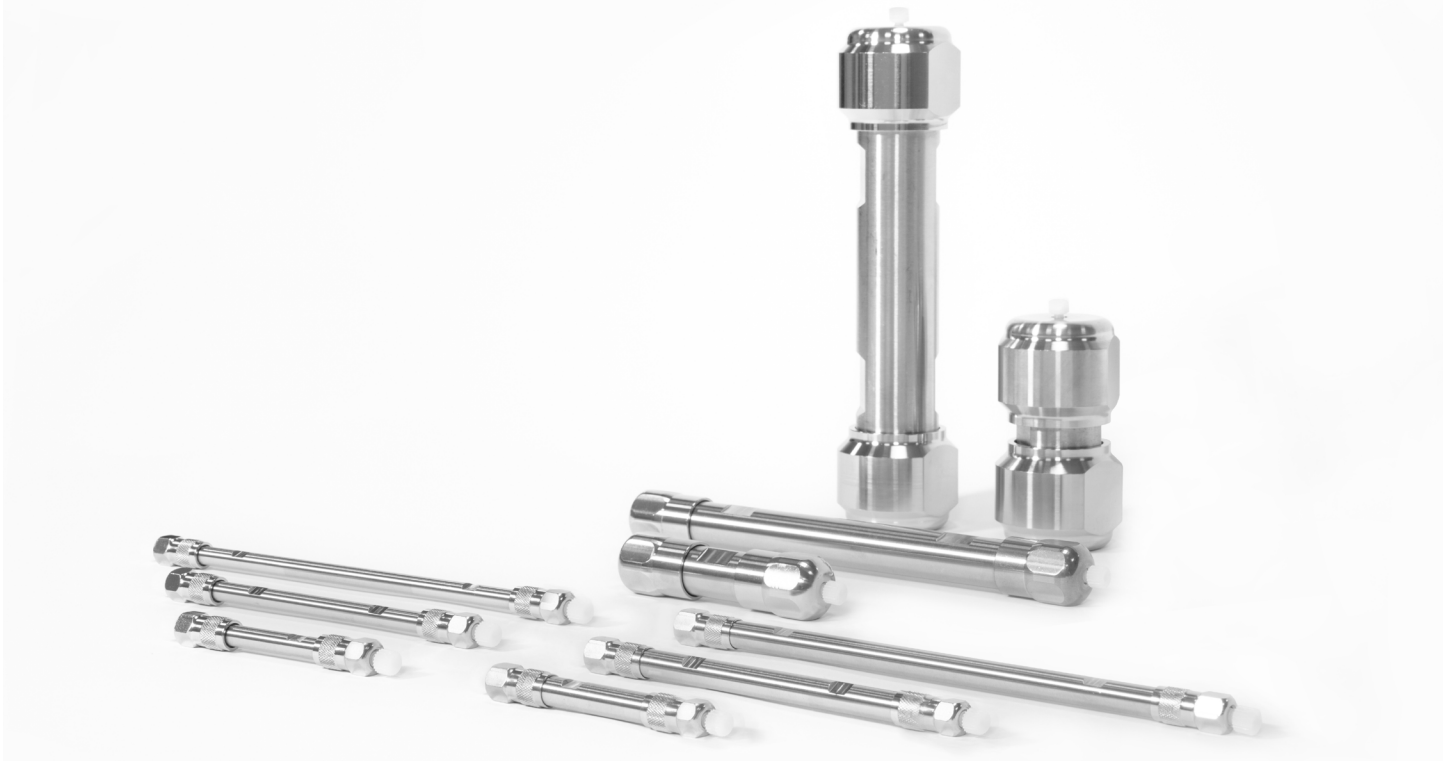
Agilent
Trusted Answers

AdvanceBio Your Oligonucleotide Workflow

From analytical to semi-prep and preparative scale, Agilent AdvanceBio Oligonucleotide columns, software, and instrumentation pair seamlessly to minimize method optimization and downtime.





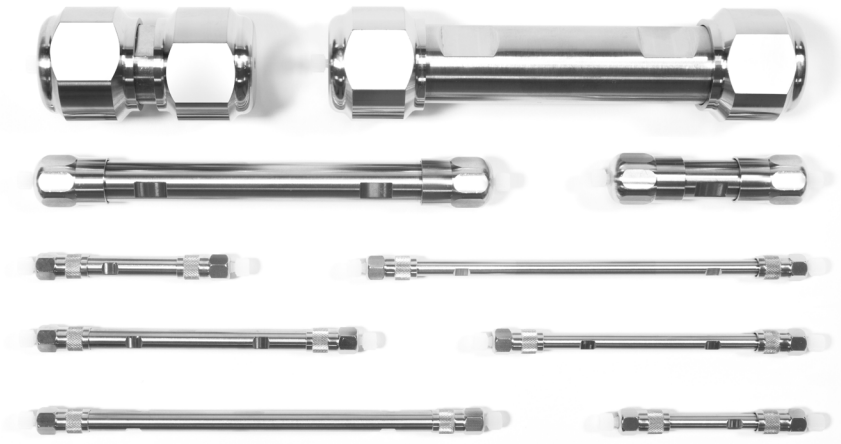
Maximize your resources - from analytical to semi-prep to preparative purification

Whether you are using LC/MS or UV analysis, the AdvanceBio Oligonucleotide family of scalable chemistries and accompanying instrumentation and software platforms will advance your oligonucleotide analysis workflow into the future for characterization and purification.

Let your technology work for you

In recent years, synthetic oligonucleotides such as aptamers, guide RNA, small interfering RNA, and antisense oligos have moved into the focus of life science and diagnostics research with many showing promise in disease treatment from viral infections, cancers, and a variety of alternate therapeutic applications. However, impurities arising from incomplete capping of coupling reactions, product-related impurities, impurities in the starting materials, and impurities from post-synthesis processing must be monitored, identified, and removed. Key challenges in the development and manufacture of nucleic acid based therapeutics are the need for analytical methods to separate and identify impurities, and purification methods that result in high quality and high yield target sequences.

These molecules typically exhibit chain lengths of fewer than 100 nucleotides and can thus be analyzed using ion pair-reversed phase (IP-RP) HPLC. This technique has been successfully applied for the characterization and purification of oligonucleotides. Scaling up methods to preparative conditions, however, requires large amounts of costly hexafluoroisopropanol (HFIP), which can be a limiting factor. While mass directed purification can be accomplished using DBA and TRIS as a less costly alternative, AdvanceBio Oligonucleotide analytical, semi-prep, and preparative columns are suitable for both LC/MS and UV analysis, exceptionally well suited to RP-IP analysis and purification, and benefit from the manufacturing optimization of the superficially porous particle technology.



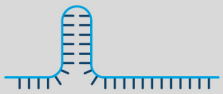
AdvanceBio Oligonucleotide column specifications

Particle sizes	2.7 µm and 4 µm
pH stability	3-11
Temperature stability	65 °C
Pore size	120 Å
Internal diameters available	2.1, 4.6, 10 and 21.2 mm
Pressure Stability	600 bar

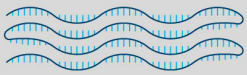
Agilent Oligonucleotide Separation and Purification Guide

Oligo therapeutic classes

18-30 bases
siRNA/ASO



Up to 200 bases
gRNA



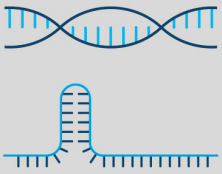
1000s of bases
mRNA

Columns for oligonucleotide chromatography

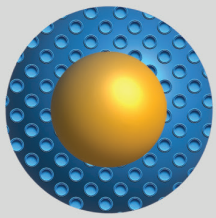
Ion paired reversed-phase (IP-RP)

Agilent AdvanceBio Oligonucleotide columns

Suitable for siRNA/ASO and gRNA

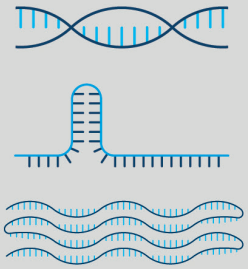


- High efficiency particles in 2.7 and 4 µm
- Available from analytical to 21.2 mm id columns
- Agilent Poroshell technology
- Optimized 120 Å pore size for targeted, high resolution IP-RP separations

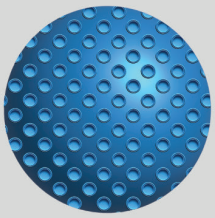


Agilent PLRP-S columns

Suitable for siRNA/ASO, gRNA and mRNA



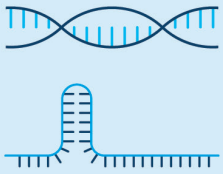
- Particle sizes 3 to 50 µm
- Available from analytical to 100 mm id preparative columns and bulk media
- Fully porous polymeric particle
- Available in 4 pore sizes: 100, 300, 1000 & 4000 Å



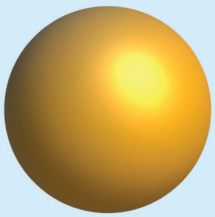
Anion exchange (AEX)

Agilent Bio SAX columns

Suitable for siRNA/ASO and gRNA

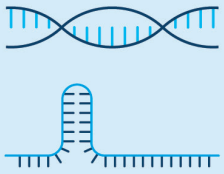


- Particle sizes from 1.7 to 10 µm
- Available in analytical to 21.2 mm id columns
- Nonporous particle for high resolution IEX separations

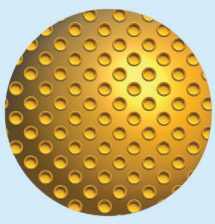


Agilent PL-SAX columns

Suitable for siRNA/ASO and gRNA



- Particle sizes 5 to 30 µm
- Available from analytical to 100 mm id preparative columns and bulk media
- Fully porous polymeric particle
- Available in 1000 & 4000 Å pore sizes



High pH and temperature stability

MS Compatible

MS Compatible with desalting

Simplified Oligonucleotide Analysis and Characterization

Easy-to-use full workflow solution

Optimize resolution with AdvanceBio Oligonucleotide columns

AdvanceBio Oligonucleotide stationary phase chemistry is a high-quality, high resolution ion-pair reversed-phase (IP-RP) column chemistry that delivers excellent selectivity for oligonucleotides and supports analytical characterization through purification with seamless method scale-up. Each batch is qualified with a fit for purpose standard to ensure it meets the highest quality specifications and delivers consistent performance. The novel surface chemistry modification of the AdvanceBio Oligonucleotide columns ensure stability at elevated pH and temperature which provides better separation and denaturing conditions for optimal resolution and oligonucleotide separation for characterization workflows.

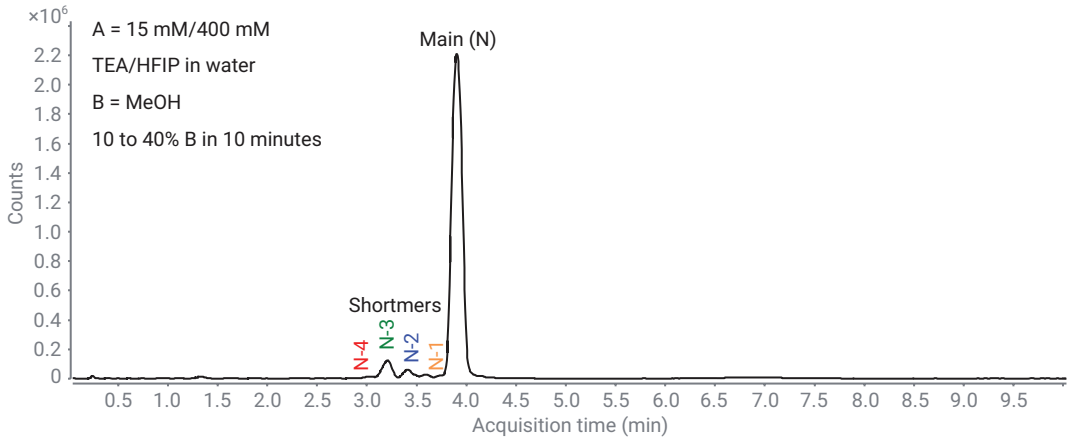


Figure 1. LC/MS results for sample A analyzed with 15 mM TEA/400 mM HFIP reference condition.

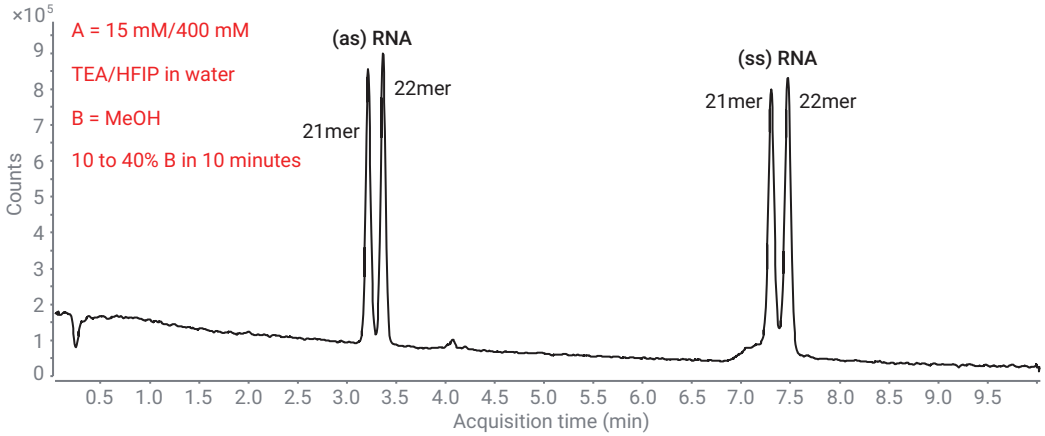
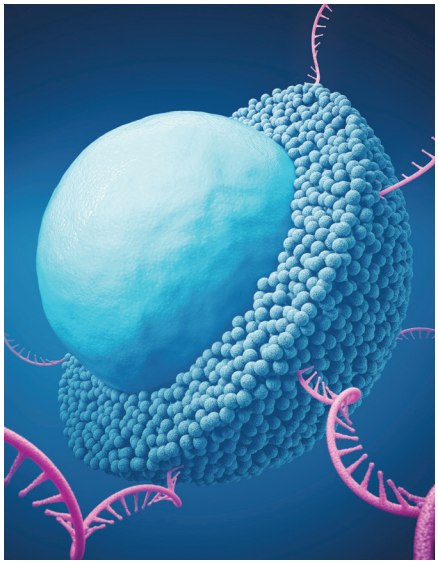


Figure 2. LC/MS results for sample B analyzed with 15 mM TEA/400 mM HFIP reference condition.

Agilent MassHunter BioConfirm 12.0 enables sequence confirmation for precise characterization

Advanced analytical methods, such as LC/MS analysis, are indispensable for the characterization of target oligonucleotides and their impurities, which are often numerous, present at very low abundances, and found in combination with one another. As such, software that supports and automates these profiling efforts can be of great value.

When seamlessly paired with MassHunter BioConfirm 12.0 software and the 1290 Infinity II UHPLC System, AdvanceBio Oligonucleotide analytical columns provide a solution to these challenges for RNA or DNA based targets and their impurities. AdvanceBio Oligonucleotide columns also benefit from the advances of superficially porous particle technology, allowing for sub-two micron efficiencies at less than half the backpressure of a sub-two micron fully porous particle.



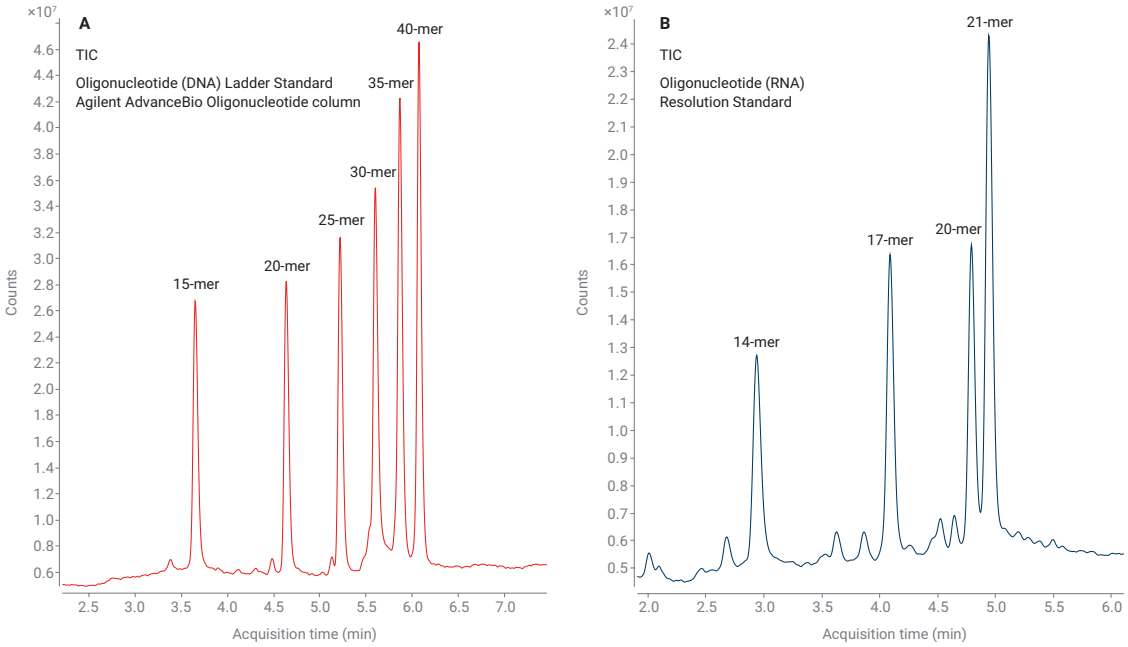
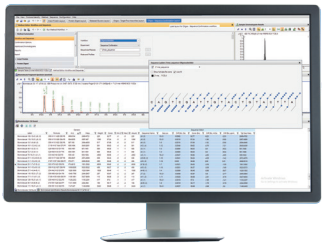


Figure 3. DNA ladder and RNA resolution standards on an AdvanceBio Oligonucleotide analytical column

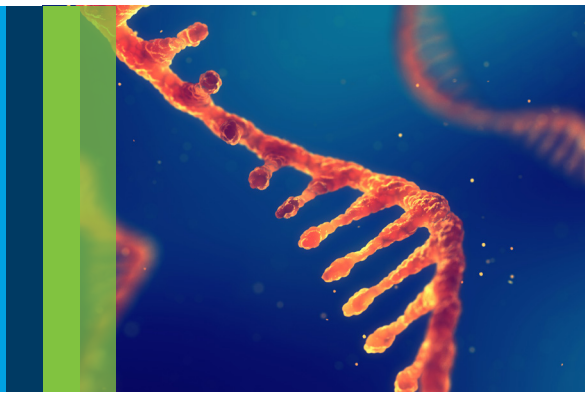


Agilent MassHunter BioConfirm 12.0 software

Novel, automated and integrated data analysis for the characterization of oligonucleotides and their impurities demonstrate high accuracy and reproducibility. MassHunter BioConfirm 12.0 enables automated TPI data processing in a high-throughput manner and significantly reduce data analysis time.

Optimal Oligonucleotide Resolution and Simplified Scale-Up

Agilent AdvanceBio Oligonucleotide scalar, semipreparative, and preparative columns



Leverage the flexibility of the AdvanceBio Oligonucleotide semipreparative dimensions

It is often advantageous during the oligonucleotide therapeutic development process to do small scale purification of your crude sample without the need to switch to large scale purification columns and instrumentation. The AdvanceBio Oligonucleotide 10 mm id semipreparative columns available in 2.7 and 4 µm particle sizes, unlock the ability to utilize a single analytical LC instrument and software for bench-top semi-preparative purification and fraction analysis. Enabling one to utilize the column heating capability of the analytical instrument promotes denaturing conditions and reduces secondary interactions that greatly improves resolution of the target product to achieve higher purity and product recovery.

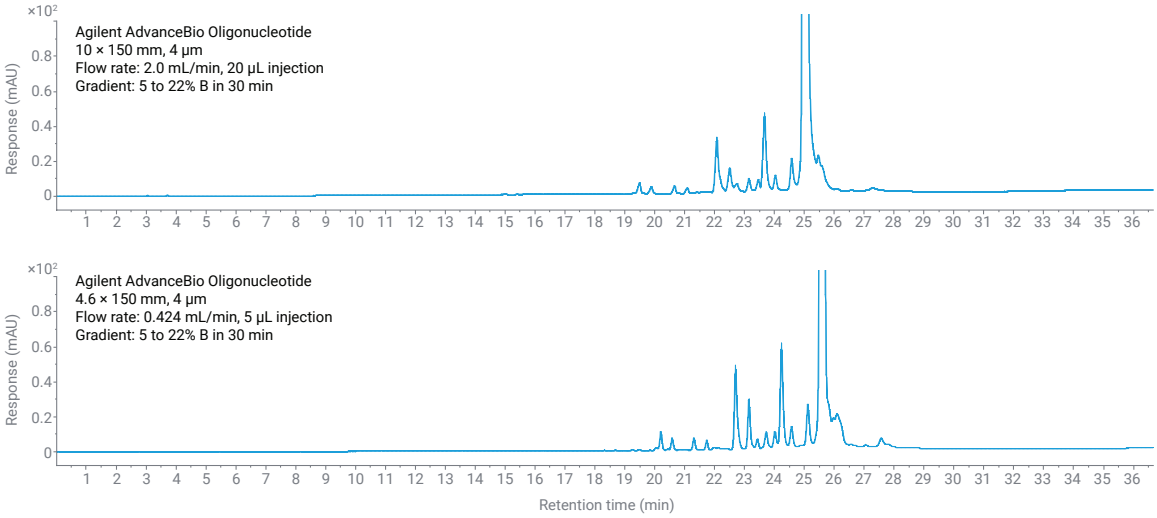
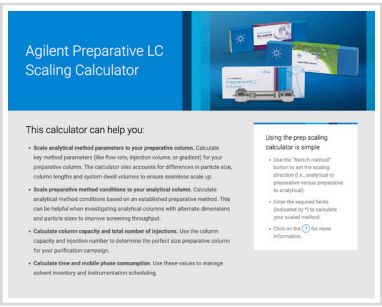


Figure 4. Analytical and semi-preparative LC-UV chromatograms showing the same linear velocity separation for the RNA oligonucleotide sample.



Agilent Preparative LC Scaling Calculator

To learn how this calculator can help you, click on the link: Scale up calculator

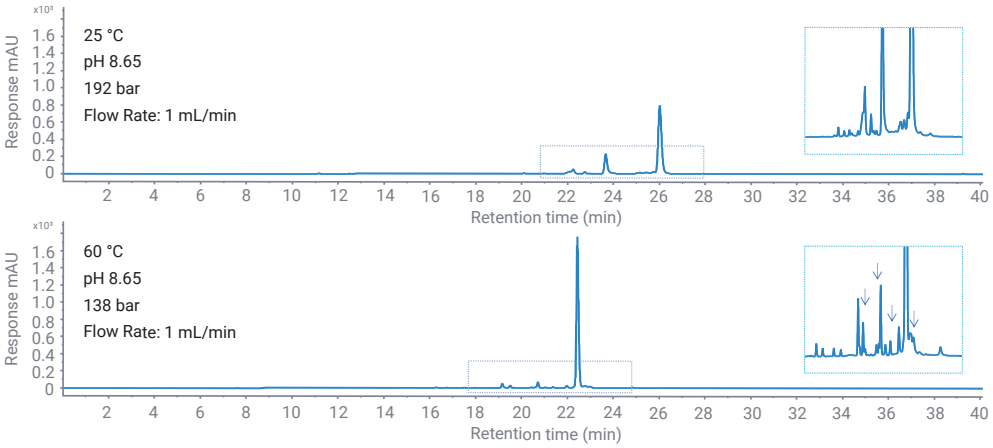


Figure 5. Analytical LC-UV chromatograms (260 nm) showing the improved resolution with increased temperature for a RNA oligonucleotide sample on a 4 µm 4.6 x 150 mm, AdvanceBio Oligonucleotide column.

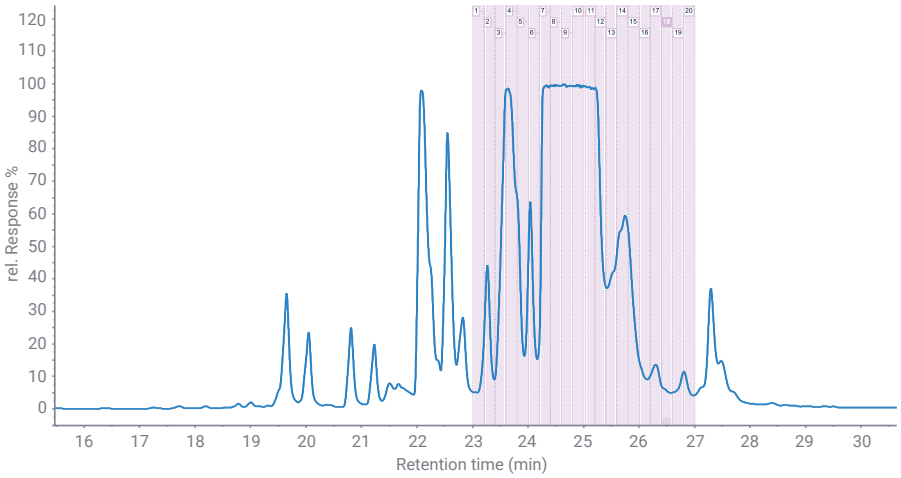
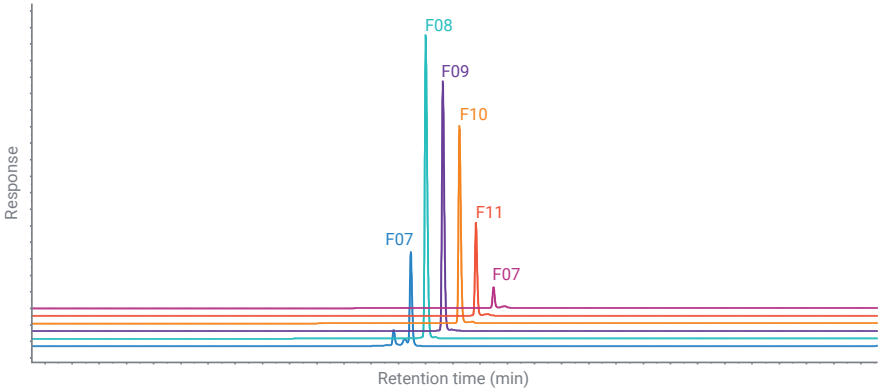


Figure 6. Chromatogram (UV 260 nm) of a 160 µL (3.2 mg on column) injection on the semi-preparative column. Purple bars represent fraction collection of 0.2-minute time slices.



Fraction	Product Purity	Peak Yield
F07	65.2%	9.4%
F08	99.2%	33.4%
F09	98.9%	25.9%
F10	97.9%	20.2%
F11	76.8%	9.0%

Figure 7. Chromatogram overlay (UV 260 nm) of the reanalysis of six fractions collected from the fraction collector. The table (inset) indicates the purity and yield of the main peak.

Simplified method scale up for optimum purity and yield

AdvanceBio Oligonucleotide 21.2 mm id preparative and analytical scalar columns allow for no fuss method transfer while using the same trusted stationary phase for your analytical characterization, and a new 4 µm particle with the same bonded chemistry across the AdvanceBio Oligonucleotide family of products, creating the best balance between loading capacity and resolution. This combination of superficially porous particle technology and n(n-1) batch qualified HPH-C18 in the 21.2 mm id is particularly well suited to purification, whether UV or mass selected detections, or mass directed prep.

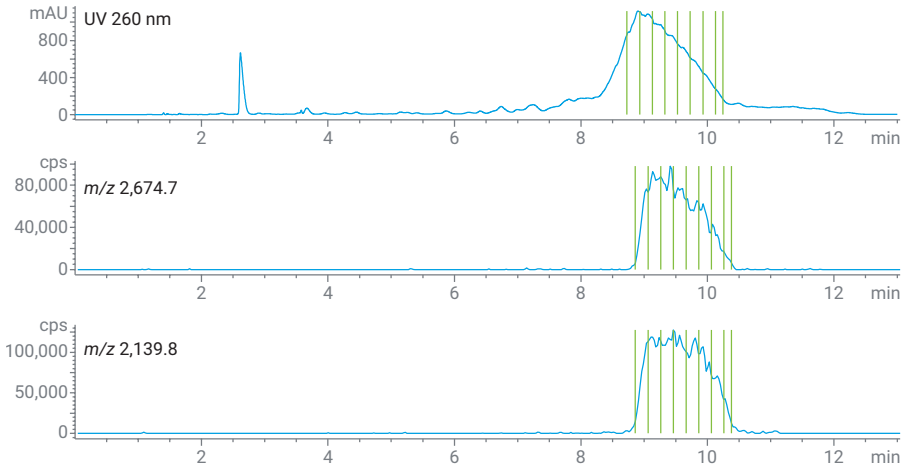


Figure 8. Preparative purification run of the short ON, using DBA/TRIS and a focused gradient. Green bars represent time slices of fraction collection.

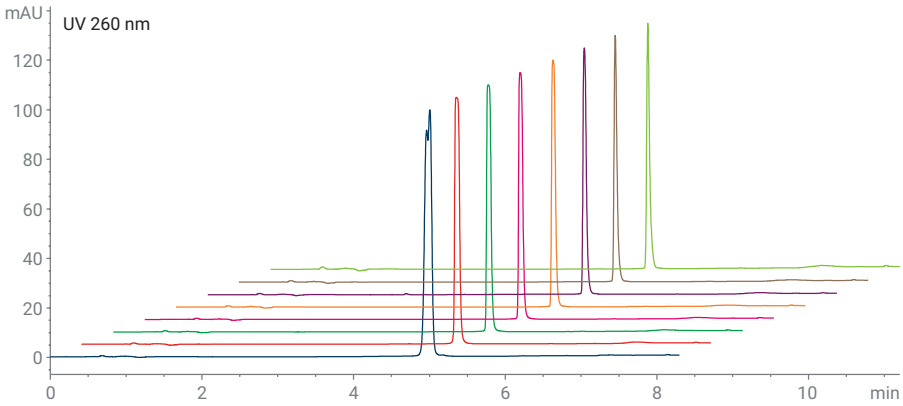


Figure 9. Chromatogram overlay of analyses of the eight fractions collected in the short ON purification run.


Fast and Selective Purification of Oligonucleotides Using Preparative HPLC/MS and Software Support

Purification of Oligonucleotides

To learn more about reducing IP-RP preparative costs using TRIS and DBA instead of HFIP and TEA check out this app note:

Fast and Selective Purification of Oligonucleotides Using Preparative HPLC/MS and Software Support

1290 Infinity II Preparative LC Systems

Purify with the benchmark in efficiency

Aspire to high efficiency in your oligonucleotide purification workflows. Get superior flexibility to solve all your purification challenges with high purity, recovery, and speed.

1260 Infinity II Preparative LC Systems

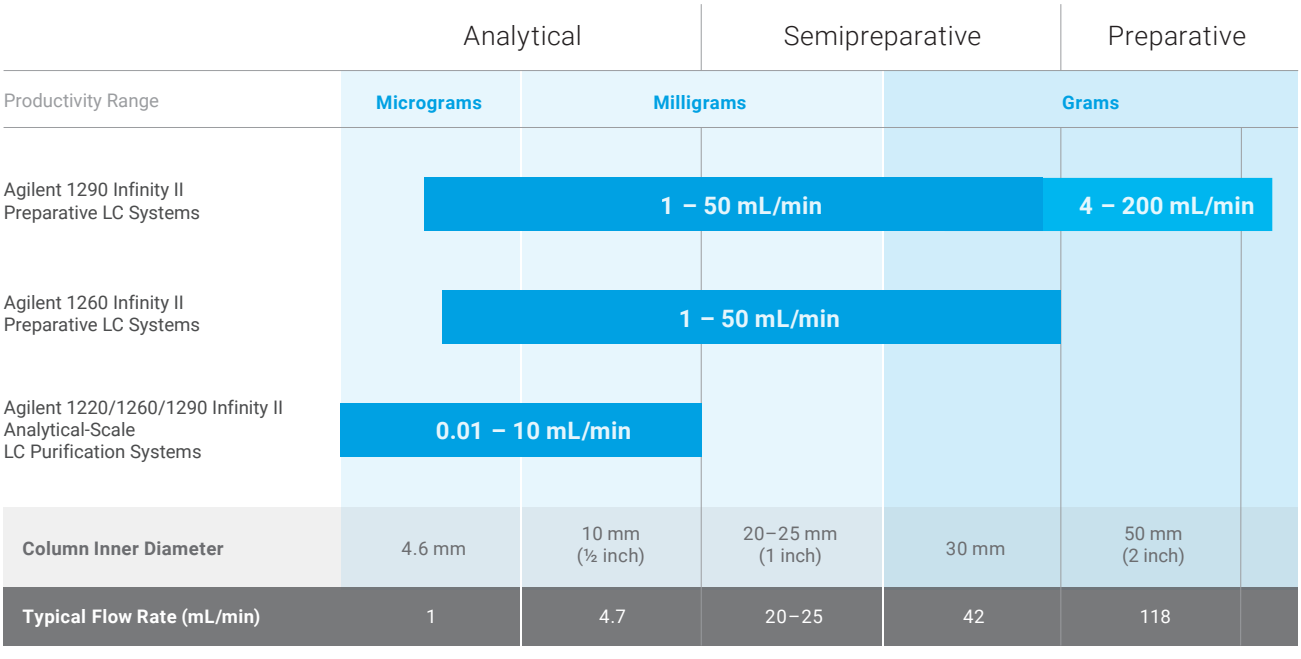
Benefit from efficient purification—every day

Achieve highest purity and recovery of your crude oligonucleotide samples with the most affordable fraction collector. Benefit from high flexibility in fraction size while maintaining high-precision recovery when paired with the AdvanceBio Oligonucleotide preparative columns.

1220/1260/1290 Infinity II Analytical-Scale LC Purification Systems

Experience the power of analytical compound isolation

Isolate your compounds of interest through high-performance separation and low dispersion fraction collection. Ideal for oligonucleotide purity analysis, sequence confirmation, and flow rate ranges that support benchtop semi-prep purification workflows.



Ordering Information

One-click ordering

You can always count on Agilent to support your entire workflow—including sample preparation, columns, supplies, standards, and instruments. To add items to your shopping cart at the Agilent online store, simply click the part number links. Then, enter the quantities for the products you need.

Agilent columns

Description	Part Number
Analytical	
AdvanceBio Oligonucleotide, 2.1 x 100 mm, 2.7 µm	655750-702
AdvanceBio Oligonucleotide, 2.1 x 150 mm, 2.7 µm	653750-702
AdvanceBio Oligonucleotide, 2.1 x 50 mm, 2.7 µm	659750-702
AdvanceBio Oligonucleotide, 4.6 x 100 mm, 2.7 µm	655950-702
AdvanceBio Oligonucleotide, 4.6 x 150 mm, 2.7 µm	653950-702
AdvanceBio Oligonucleotide, 4.6 x 50 mm, 2.7 µm	659950-702
Scalar	
AdvanceBio Oligonucleotide, 4.6 mm, guard, 4 µm	820750-941
AdvanceBio Oligonucleotide, 4.6 x 100 mm, 4 µm	695971-702
AdvanceBio Oligonucleotide, 4.6 x 150 mm, 4 µm	693971-702
AdvanceBio Oligonucleotide, 4.6 x 50 mm, 4 µm	699971-702
Semi-Prep	
AdvanceBio Oligonucleotide, 10 x 50 mm, 2.7 µm	639950-702
AdvanceBio Oligonucleotide, 10 x 100 mm, 2.7 µm	635950-702
AdvanceBio Oligonucleotide, 10 x 150 mm, 2.7 µm	633950-702
AdvanceBio Oligonucleotide, 10 x 50 mm, 4 µm	639750-702
AdvanceBio Oligonucleotide, 10 x 100 mm, 4 µm	635750-702
AdvanceBio Oligonucleotide, 10 x 150 mm, 4 µm	633750-702
Preparative	
AdvanceBio Oligonucleotide, 21.2 x 150 mm, 4 µm	671150-702
AdvanceBio Oligonucleotide, 21.2 x 50 mm, 4 µm	671050-702
Fast Guard	
AdvanceBio Oligonucleotide, 2.1 mm, fast guard	821725-921
AdvanceBio Oligonucleotide, 4.6 mm, fast guard	820750-921

AdvanceBio Oligonucleotide analytical column
p/n: 659750-702

Oligonucleotide standards

Description	Part Number
DNA ladder standard, oligos at 15, 20, 25, 30, 35, and 40 mer, 1 mL	5190-9029
RNA resolution standard, oligos at 14, 17, 20, and 21 mer, 1 mL	5190-9028

AdvanceBio Oligonucleotide standards
p/n: 5190-9029 / 5190-9028

Learn more: Agilent Oligonucleotide Chromatography Solutions

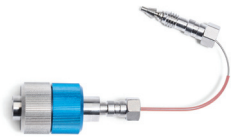
InfinityLab LC supplies

A perfect fit for your biomolecule analysis

Agilent InfinityLab supplies are innovative consumables designed to work seamlessly together with Agilent LC instruments and columns for maximum efficiency and performance for your bio HPLC analysis..

Protect your column against particles

Particulates can lead to column clogging, poor chromatographic results and increased downtime. To safeguard against these issues, employing effective filtration techniques is vital. LC filtration assemblies can be used to filter mobile phases, especially when using water-based buffer solutions, to remove residue of undissolved salt crystals and microbes.



Inline filters can be installed to capture any particles in the flow path that come from solvents, samples or worn system parts. InfinityLab Quick Change inline filter offer tool-free replacement of filter discs and “click and seal” feedback to ensure ultimate ease of use.

Perfect connection for your bio application

Within the InfinityLab family, Agilent offers HPLC capillaries in a variety of materials to meet your needs. Capillaries made of MP35N, PEEK-lined stainless steel and titanium are inert and corrosion resistant and are particularly suitable for bio-applications. In combination with InfinityLab Quick Connect fittings, you can create a perfect finger-tight connection up to 1300 bar. For the best analytical performance.

Reduce chemical vapor in the lab

Acetonitrile and methanol are just two of the many toxic compounds you may be exposed to every day. The InfinityLab Stay Safe caps stop solvents from leaching into the air. Combined with the innovative InfinityLab Stay Safe purging bottle the purging of an HPLC with up to four solvent lines becomes a safe task.

Your sample’s journey starts in a vial – make it the right one!

Agilent offers a comprehensive line of vials, caps and inserts; whether a standard borosilicate glass vial will suffice, a surface-deactivated glass vial or polypropylene vial we have the containment solution for you. Beyond vial composition we offer vials in various designs reflecting the nature and sample volume available.

Tips and tools

Download our vials catalog (5994-4803EN) to help guide you in making the final decision on vials, supported by our vial selection tool.

Agilent CrossLab services

CrossLab is an Agilent capability that integrates services and consumables to support workflow success and important outcomes like improved productivity and operational efficiency. Through CrossLab, Agilent strives to provide insight in every interaction to help you achieve your goals. CrossLab offers method optimization, flexible service plans, and training for all skill levels. We have many other products and services to help you manage your instruments and your lab for best performance.

Learn more about Agilent CrossLab, and see examples of insight that leads to great outcomes, at www.agilent.com/crosslab

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From Insight to Outcome

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