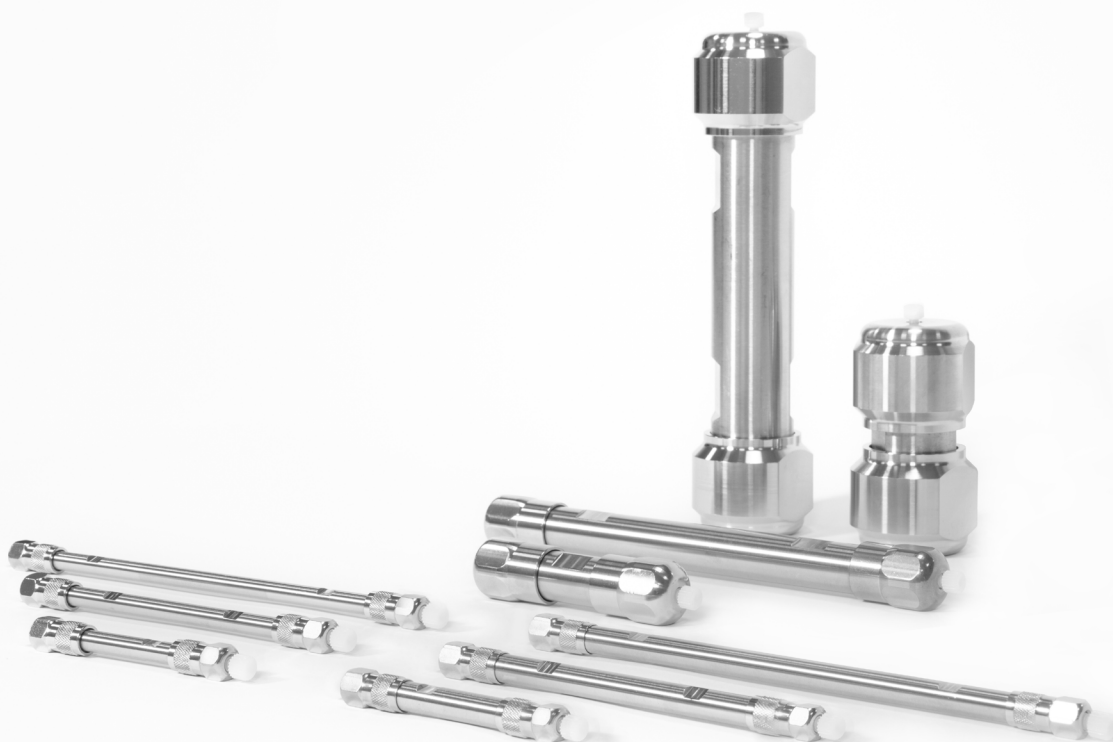
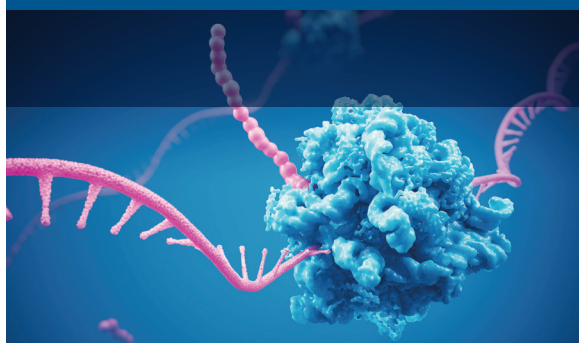


# AdvanceBio Your Oligonucleotide Workflow

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 $\mu\text{m}$ and 4 $\mu\text{m}$
pH stability	3-11
Temperature stability	65 $^{\circ}\text{C}$
Pore size	120 $\text{\AA}$
Internal diameters available	2.1, 4.6, 10 and 21.2 mm
Pressure Stability	600 bar

# Agilent Oligonucleotide Separation and Purification Guide

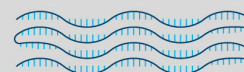
## Oligo therapeutic classes



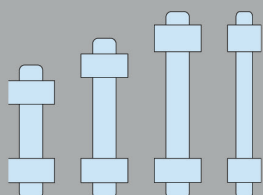
18-30 bases  
siRNA/ASO



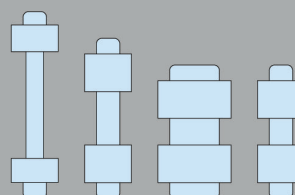
Up to 200 bases  
gRNA



1000s of bases  
mRNA



## Columns for oligonucleotide chromatography



### Ion paired reversed-phase (IP-RP)

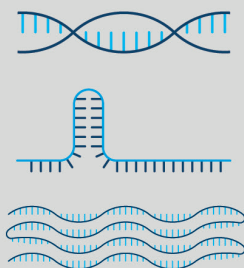
#### Agilent AdvanceBio Oligonucleotide columns

Suitable for siRNA/ASO and gRNA



#### Agilent PLRP-S columns

Suitable for siRNA/ASO, gRNA and mRNA



### Anion exchange (AEX)

#### Agilent Bio SAX columns

Suitable for siRNA/ASO and gRNA



#### Agilent PL-SAX columns

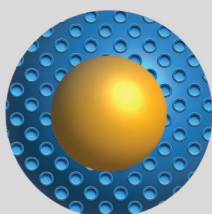
Suitable for siRNA/ASO and gRNA



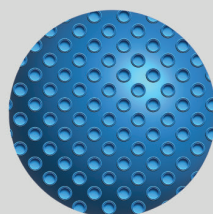
### High pH and temperature stability

#### MS Compatible

- High efficiency particles in 2.7 and 4  $\mu\text{m}$
- Available from analytical to 21.2 mm id columns
- Agilent Poroshell technology
- Optimized 120  $\text{\AA}$  pore size for targeted, high resolution IP-RP separations

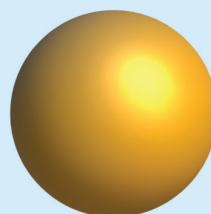


- Particle sizes 3 to 50  $\mu\text{m}$
- Available from analytical to 100 mm id preparative columns and bulk media
- Fully porous polymeric particle
- Available in 4 pore sizes: 100, 300, 1000 & 4000  $\text{\AA}$

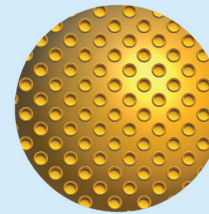


#### MS Compatible with desalting

- Particle sizes from 1.7 to 10  $\mu\text{m}$
- Available in analytical to 21.2 mm id columns
- Nonporous particle for high resolution IEX separations



- Particle sizes 5 to 30  $\mu\text{m}$
- Available from analytical to 100 mm id preparative columns and bulk media
- Fully porous polymeric particle
- Available in 1000 & 4000  $\text{\AA}$  pore sizes



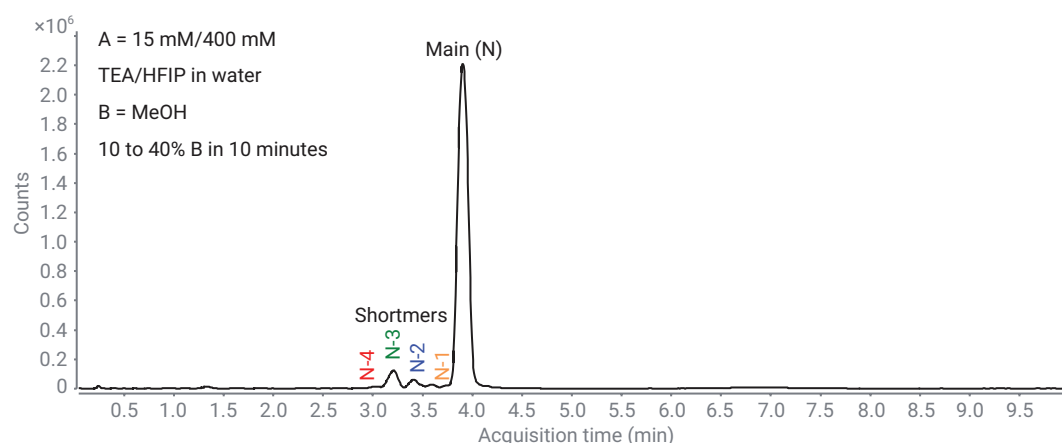
# Simplified Oligonucleotide Analysis and Characterization

Easy-to-use full workflow solution

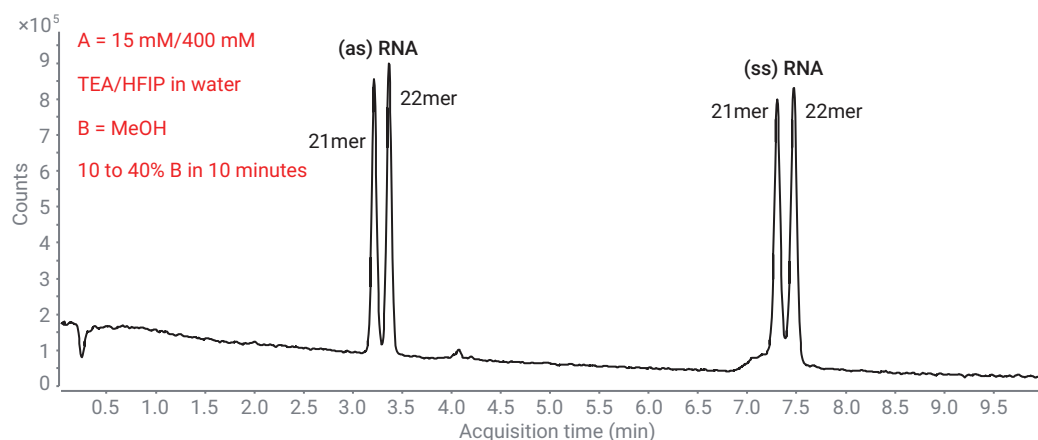


## Optimize resolution with AdvanceBio Oligonucleotide columns

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.

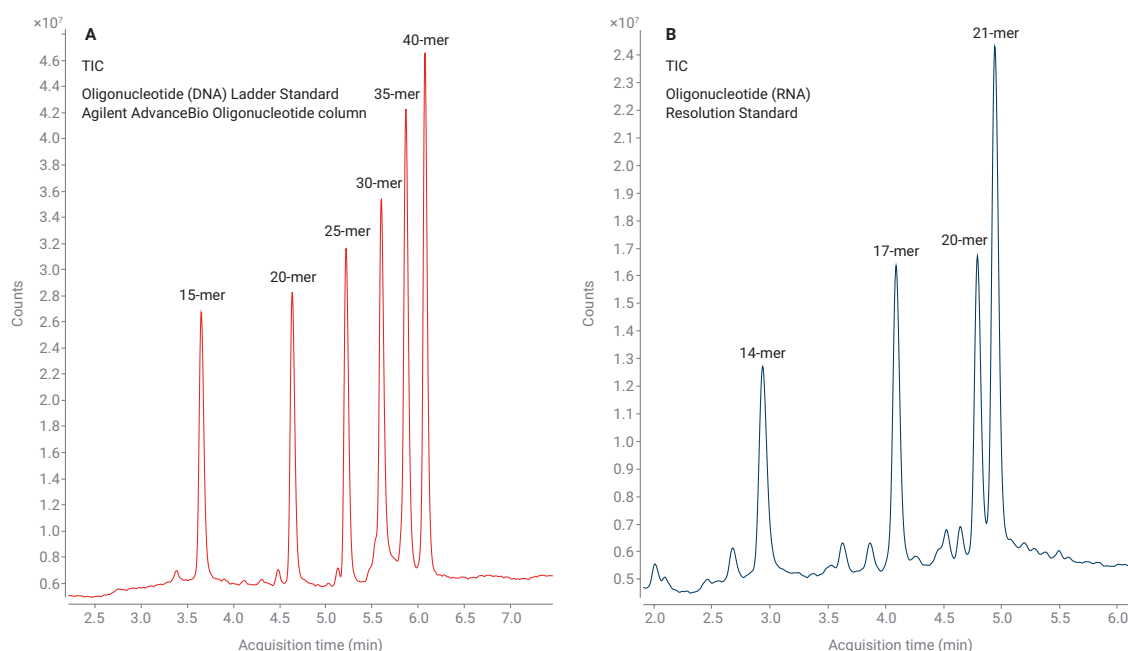
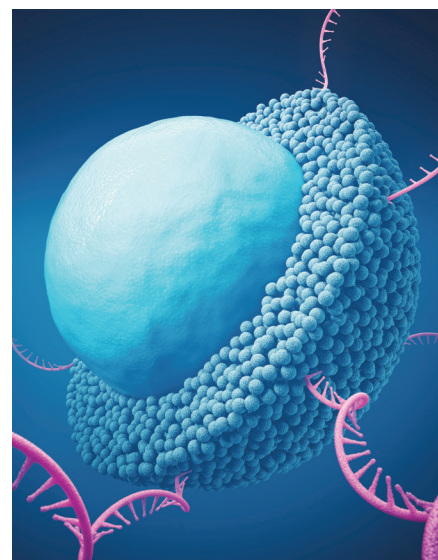
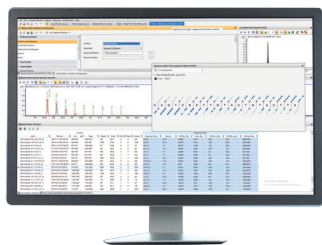


Figure 3. DNA ladder and RNA resolution standards on an AdvanceBio Oligonucleotide analytical column



## Agilent MassHunter BioConfirm 12.0 software

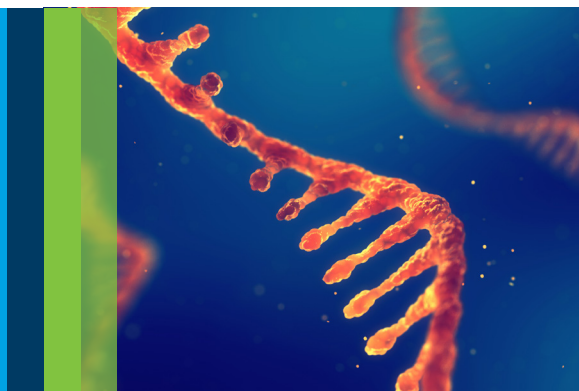
Novel, automated and integrated data analysis for the characterization of oligonucleotides and their impurities demonstrate high accuracy and reproducibility.

MassHunter BioConfirm 12.0 enables automated TPI data processing in a high-throughput manner and significantly reduce data analysis time.



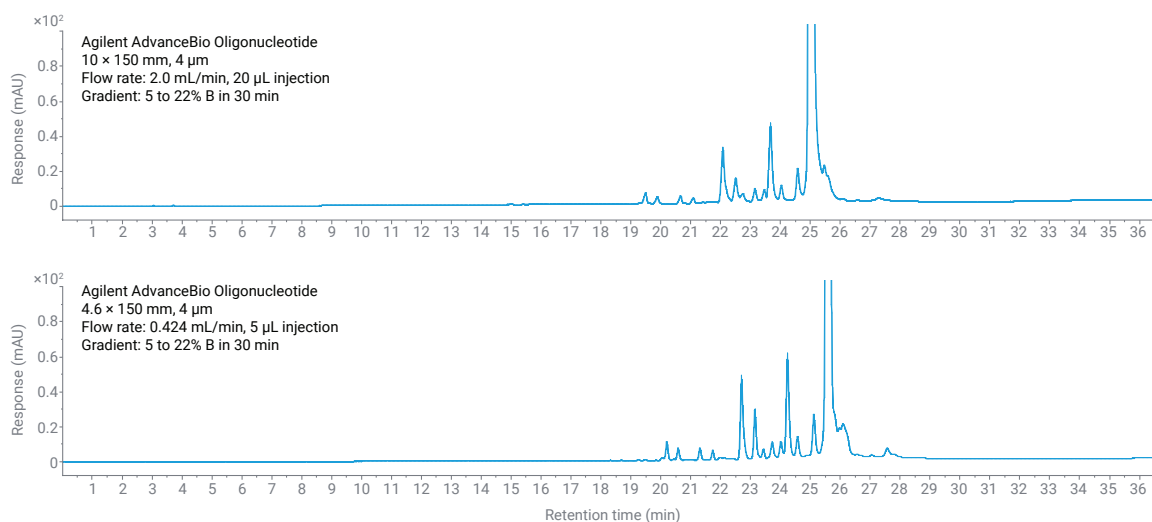
# Optimal Oligonucleotide Resolution and Simplified Scale-Up

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  $\mu\text{m}$  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

This calculator can help you:

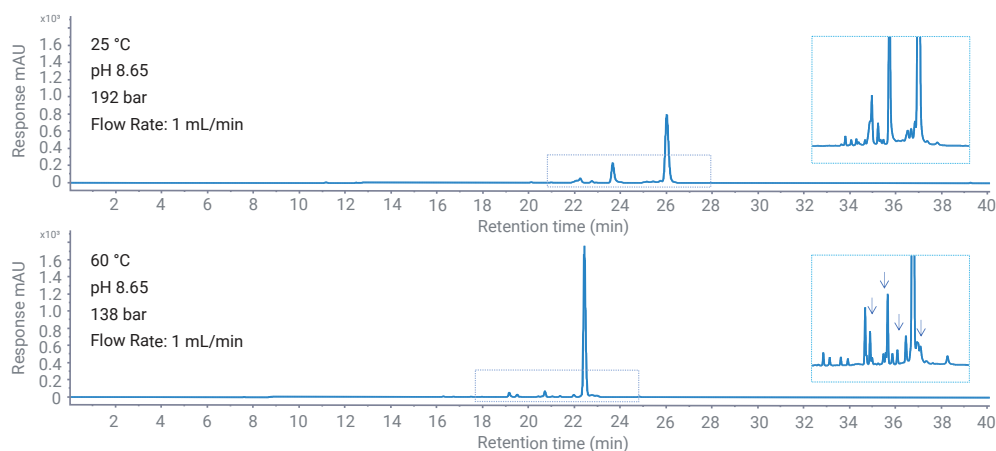
- Scale analytical method parameters to your preparative column. Calculate key method parameters (flow rate, injection volume, or gradient) for your preparative column. The calculator also accounts for differences in particle size, column lengths and system dead volumes to ensure seamless scale up.
- Scale preparative method conditions to your analytical column. Calculate analytical method conditions based on an established preparative method. This can be helpful when investigating analytical columns with alternate dimensions and particle sizes to improve screening throughput.
- Calculate column capacity and total number of injections. Use the column capacity and injection number to determine the perfect size preparative column for your purification campaign.
- Calculate time and mobile phase consumption. Use these values to manage solvent inventory and instrumentation scheduling.

Using the prep scaling calculator is simple

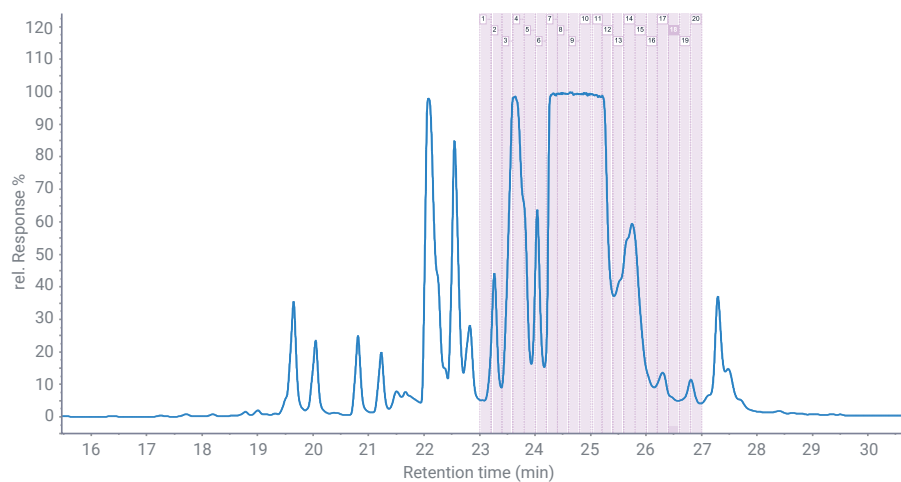
- Use the "batch method" button to set the scaling direction for analytical to preparative versus preparative to analytical.
- Enter the required fields (indicated by \*). Enter your scaled method.
- Click on the [i](#) for more information.

## Agilent Preparative LC Scaling Calculator

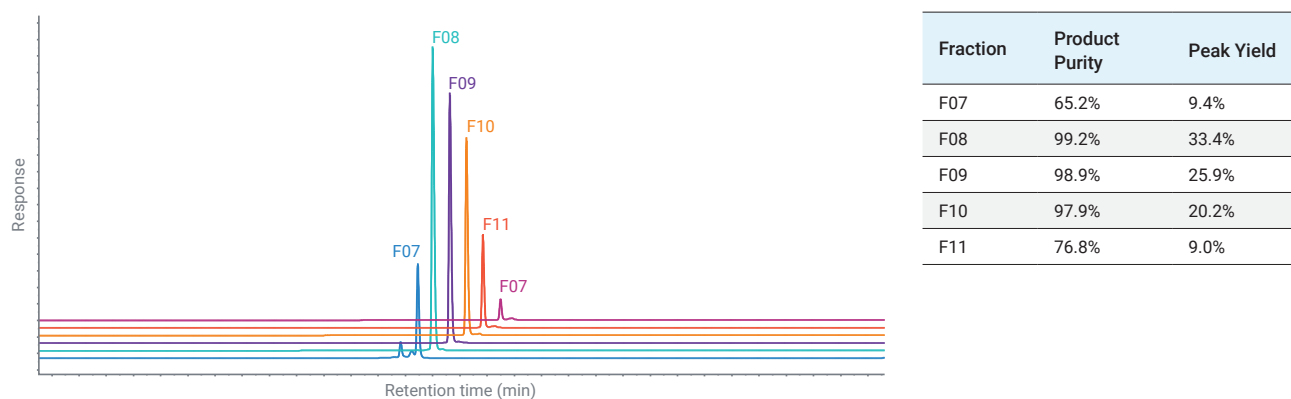
To learn how this calculator can help you, click on the link: [Scale up calculator](#)



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



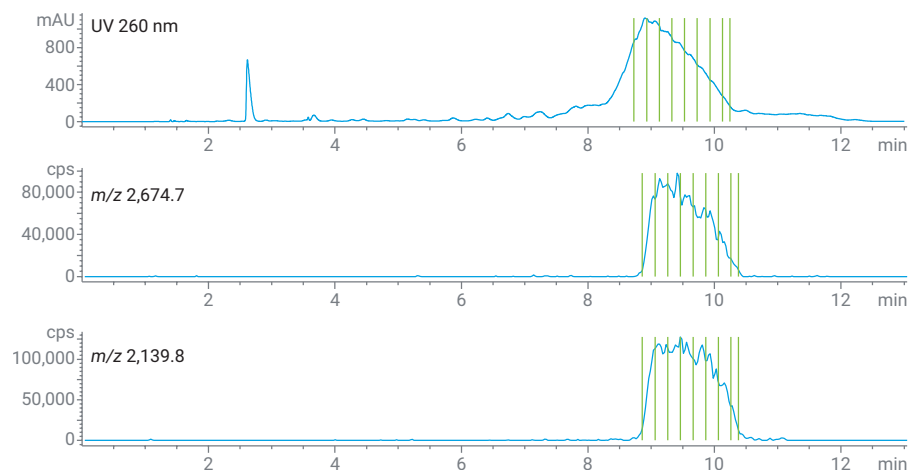
**Figure 6.** Chromatogram (UV 260 nm) of a 160  $\mu$ L (3.2 mg on column) injection on the semi-preparative column. Purple bars represent fraction collection of 0.2-minute time slices.



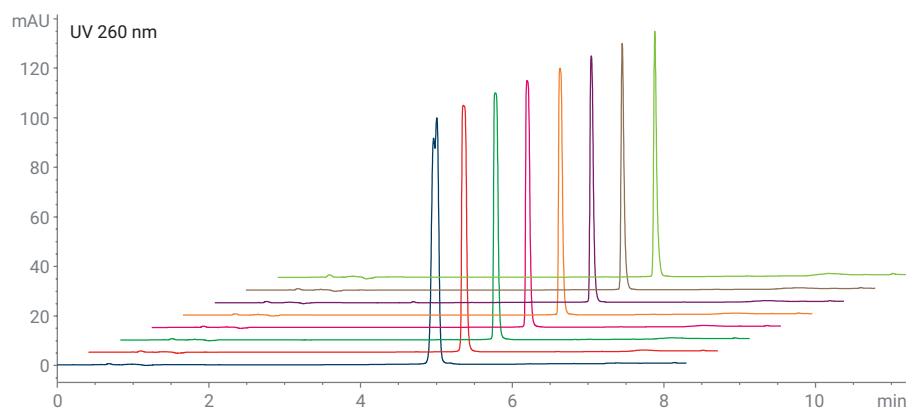
**Figure 7.** Chromatogram overlay (UV 260 nm) of the reanalysis of six fractions collected from the fraction collector. The table (inset) indicates the purity and yield of the main peak.

## 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  $\mu\text{m}$  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.



## 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](#)

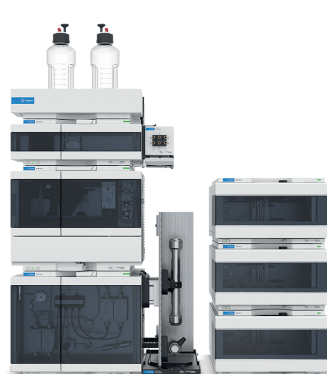




## 1290 Infinity II Preparative LC Systems

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.

	Analytical		Semipreparative		Preparative	
Productivity Range	Micrograms	Milligrams		Grams		
Agilent 1290 Infinity II Preparative LC Systems	1 – 50 mL/min			4 – 200 mL/min		
Agilent 1260 Infinity II Preparative LC Systems	1 – 50 mL/min					
Agilent 1220/1260/1290 Infinity II Analytical-Scale LC Purification Systems	0.01 – 10 mL/min					
Column Inner Diameter	4.6 mm	10 mm (½ inch)	20–25 mm (1 inch)	30 mm	50 mm (2 inch)	
Typical Flow Rate (mL/min)	1	4.7	20–25	42	118	

Flow range extensions made possible by exchangeable pump heads

# 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
<b>Analytical</b>	
AdvanceBio Oligonucleotide, 2.1 x 100 mm, 2.7 µm	<a href="#">655750-702</a>
AdvanceBio Oligonucleotide, 2.1 x 150 mm, 2.7 µm	<a href="#">653750-702</a>
AdvanceBio Oligonucleotide, 2.1 x 50 mm, 2.7 µm	<a href="#">659750-702</a>
AdvanceBio Oligonucleotide, 4.6 x 100 mm, 2.7 µm	<a href="#">655950-702</a>
AdvanceBio Oligonucleotide, 4.6 x 150 mm, 2.7 µm	<a href="#">653950-702</a>
AdvanceBio Oligonucleotide, 4.6 x 50 mm, 2.7 µm	<a href="#">659950-702</a>
<b>Scalar</b>	
AdvanceBio Oligonucleotide, 4.6 mm, guard, 4 µm	<a href="#">820750-941</a>
AdvanceBio Oligonucleotide, 4.6 x 100 mm, 4 µm	<a href="#">695971-702</a>
AdvanceBio Oligonucleotide, 4.6 x 150 mm, 4 µm	<a href="#">693971-702</a>
AdvanceBio Oligonucleotide, 4.6 x 50 mm, 4 µm	<a href="#">699971-702</a>
<b>Semi-Prep</b>	
AdvanceBio Oligonucleotide, 10 x 50 mm, 2.7 µm	<a href="#">639950-702</a>
AdvanceBio Oligonucleotide, 10 x 100 mm, 2.7 µm	<a href="#">635950-702</a>
AdvanceBio Oligonucleotide, 10 x 150 mm, 2.7 µm	<a href="#">633950-702</a>
AdvanceBio Oligonucleotide, 10 x 50 mm, 4 µm	<a href="#">639750-702</a>
AdvanceBio Oligonucleotide, 10 x 100 mm, 4 µm	<a href="#">635750-702</a>
AdvanceBio Oligonucleotide, 10 x 150 mm, 4 µm	<a href="#">633750-702</a>
<b>Preparative</b>	
AdvanceBio Oligonucleotide, 21.2 x 150 mm, 4 µm	<a href="#">671150-702</a>
AdvanceBio Oligonucleotide, 21.2 x 50 mm, 4 µm	<a href="#">671050-702</a>
<b>Fast Guard</b>	
AdvanceBio Oligonucleotide, 2.1 mm, fast guard	<a href="#">821725-921</a>
AdvanceBio Oligonucleotide, 4.6 mm, fast guard	<a href="#">820750-921</a>



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	<a href="#">5190-9029</a>
RNA resolution standard, oligos at 14, 17, 20, and 21 mer, 1 mL	<a href="#">5190-9028</a>



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

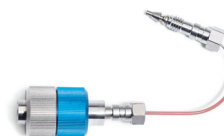
Within the InfinityLab family, Agilent offers HPLC capillaries in a variety of materials to meet your needs. Capillaries made of MP35N, PEEK-lined stainless steel and titanium are inert and corrosion resistant and are particularly suitable for bio-applications. In combination with InfinityLab Quick Connect 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](http://www.agilent.com/crosslab)



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