Agilent AdvanceBio Oligonucleotide Columns
and Oligonucleotide Standards

IMPROVE RELIABILITY. REDUCE COSTS. INCREASE FLEXIBILITY.

The Measure of Confidence

Agilent Technologies

HIGH-RESOLUTION OLIGONUCLEOTIDE SEPARATIONS WITH LONG COLUMN LIFETIME ON HPLC AND UHPLC SYSTEMS

Synthetic oligonucleotides are promising therapeutic agents for the treatment of many diseases, including viral infections and cancer. Several classes of nucleic acids, such as antisense oligonucleotides, small interfering RNAs (siRNAs), and aptamers, are being investigated for 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.

A key challenge in the development and manufacture of oligonucleotide therapeutics is the need for analytical methods to separate and identify impurities.

- **Improve reliability of results** – high-resolution separations delivered by efficient Poroshell particle morphology.
- **Reduce costs** – long column lifetimes from robust, high-pH resistant chemically modified silica
- **Increase flexibility** – compatibility with HPLC and UHPLC systems via 2.7 µm diameter particles

OLIGONUCLEOTIDE SEPARATIONS

There are three UHPLC/HPLC techniques routinely used for oligonucleotide separations:

- **Ion-pair reversed-phase separation of the trityl-on oligos:** This procedure is relatively simple to perform and separates the full-length target oligo, which still has the dMT group attached, from the deprotected failure sequences. The analytical information obtained is limited and this is generally considered to be a purification method.
- **Ion-exchange separations of the trityl-off, deprotected oligos:** This method uses the negative charge on the backbone of the oligo to facilitate the separation. Resolution is good for the shorter oligos but decreases with increasing chain length. Aqueous eluents are used but oligos are highly charged, and high concentrations of salt are needed to achieve elution from the column, making the technique unsuitable for use with LC/MS.
- **Ion-pair reversed-phase separation of the trityl-off, deprotected oligos:** This technique uses organic solvents and mobile phase additives such as triethylammonium acetate (TEAA) or triethylamine and hexafluoroisopropanol (TEA-HFIP) to ion pair with the negatively charged phosphodiester backbone of the oligonucleotide. High performance columns deliver excellent resolution. What's more, methods with volatile mobile phase constituents such as TEA-HFIP are suitable for use with LC/MS, providing useful information to help characterize oligonucleotide structures and sequences.

Agilent AdvanceBio Oligonucleotide columns are designed for ion-pair reversed-phase separation of the trityl-off, deprotected oligos using either TEAA or TEA-HFIP.

Agilent offers solutions for the other oligonucleotide techniques. See back page for details.

COMBINING RESOLUTION WITH LIFETIME

THE NEED FOR RESOLUTION AND LIFETIME

Successful ion-pair reversed-phase separation of the trityl-off, deprotected oligos requires columns that have high resolving power and are robust enough to withstand the relatively aggressive analysis conditions.

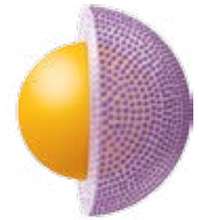
Without sufficient resolution, the accuracy and precision of measurements can be compromised, leading to a lack in confidence in the analytical results.

Columns that are not robust will have a short lifetime, resulting in frequent replacement with the related disruption to workflows and increased costs.

Agilent AdvanceBio Oligonucleotide columns feature high-efficiency, 2.7 µm superficially porous Poroshell particles. The particles are chemically modified using proprietary technology that makes them very resistant to high pH mobile phases. They are bonded with an endcapped C18 phase that delivers excellent selectivity for oligonucleotides.

To ensure performance for your separations every batch of AdvanceBio Oligonucleotide media is tested with an Agilent Oligonucleotide Resolution standard. See page 6 for further details.

Poroshell 2.7 µm particles with high pH resistance and C18 endcapping provide high-resolution separations of oligonucleotides with long column lifetime on HPLC and UHPLC systems improve reliability of results and reduce costs.



Bonded Phase	Pore Size	Temp. Limits	pH Range	Endcapped
C18	100Å	65 °C	3.0 - 11.0	Double

Agilent innovation: The first high-pH stable superficially porous particle based LC column for oligonucleotide analysis

The use of 2.7 µm diameter Poroshell particles in AdvanceBio Oligonucleotide columns plus a pressure rating of 600 bar gives compatibility with HPLC and UHPLC systems.

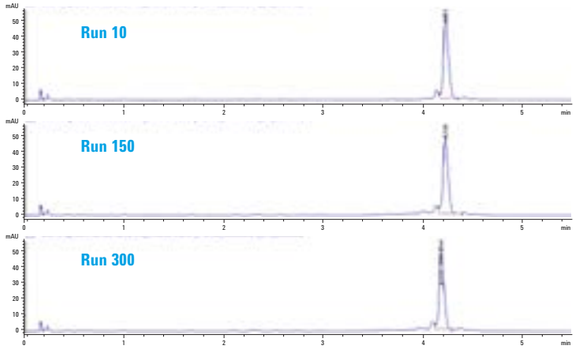
Agilent 1260 Infinity LC System

Agilent 1260 Infinity Bio-inert Quaternary LC System

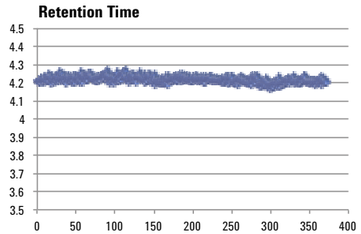
Agilent 1290 Infinity II LC System

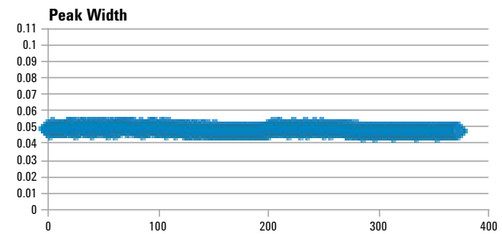
OLIGONUCLEOTIDE SEPARATIONS USING TRIETHYLAMMONIUM ACETATE (TEAA)

Unmodified silica particles are prone to dissolution in basic mobile phases, leading to reduced column lifetimes. AdvanceBio Oligonucleotide columns have excellent stability with a high pH TEAA-containing mobile phase.



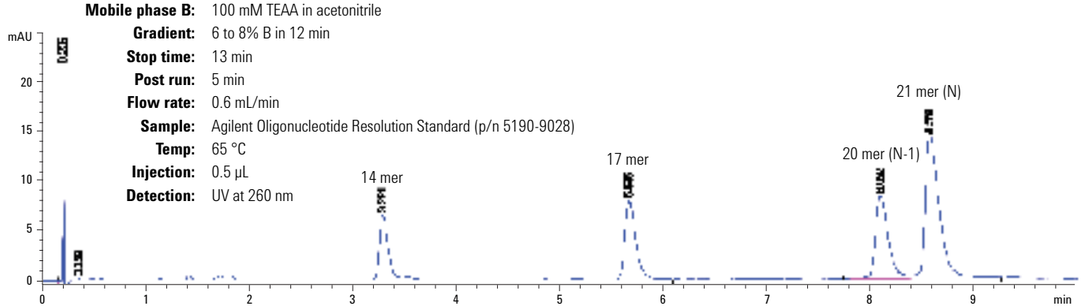
Column: AdvanceBio Oligonucleotide, 2.1 x 50 mm (p/n 659750-702)
Mobile phase A: 100 mM TEAA in water
Mobile phase B: 100 mM TEAA in acetonitrile
Flow rate: 0.69 mL/min
Gradient: 7% B to 11% B in 5 min
11% B to 80% B in 5.01 min
Hold at 80% B for 5.50 min
80% B to 7% B in 5.56 min
Total run time 8.5 min
Sample: 25 mer DNA
Injection: 1 µL of 0.5mg/mL
Temp: 65 °C
Detection: UV at 260 nm





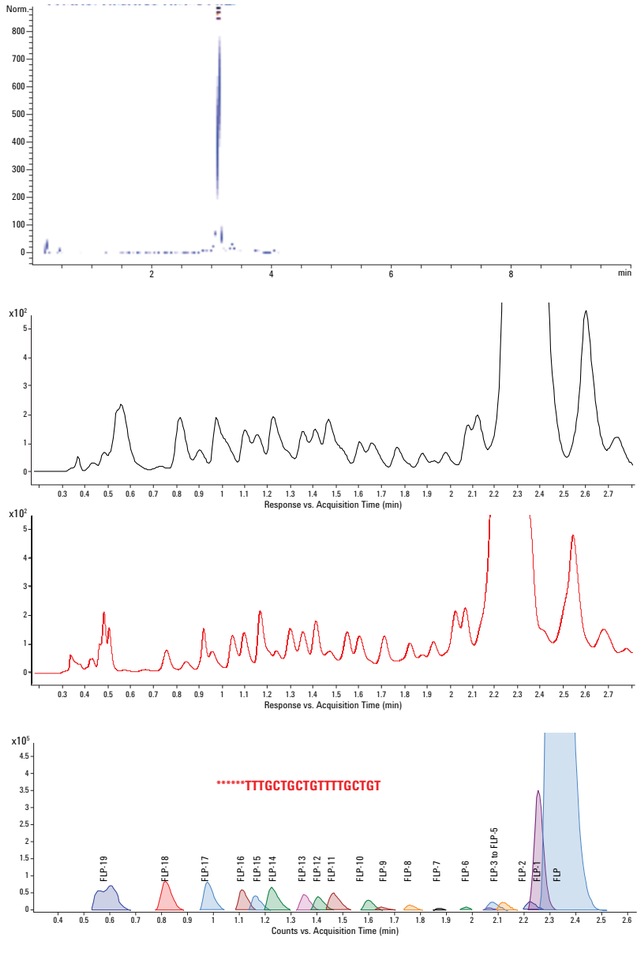
The ability to resolve oligonucleotides that differ by a single nucleotide is important for accurate characterization. The AdvanceBio Oligonucleotide column resolves N and N-1 for the Agilent Oligonucleotide Resolution Standard (14, 17, 20, and 21 mer).

Column: AdvanceBio Oligonucleotide, 2.1 x 50 mm (p/n 659750-702)
Mobile phase A: 100 mM TEAA in water
Mobile phase B: 100 mM TEAA in acetonitrile
Gradient: 6 to 8% B in 12 min
Stop time: 13 min
Post run: 5 min
Flow rate: 0.6 mL/min
Sample: Agilent Oligonucleotide Resolution Standard (p/n 5190-9028)
Temp: 65 °C
Injection: 0.5 µL
Detection: UV at 260 nm



OLIGONUCLEOTIDE SEPARATIONS USING TRIETHYLAMINE AND HEXAFLUOROISOPROPANOL (TEA-HFIP)

Mobile phase containing HFIP is compatible with MS. Combining the excellent chromatographic resolution provided by the AdvanceBio Oligonucleotide column with accurate mass MS provides characterization of the oligonucleotide structures and sequences.



Column: AdvanceBio Oligonucleotide, 2.1 x 50 mm (p/n 659750-702)
Mobile phase A: HFIP:TEA (400 mM:15mM) in water
Mobile phase B: MeOH:mobile phase A (50:50)
Flow rate: 0.4 mL/min
Gradient: 30-40% B in 0.5 min; 40-70% B in 5 min
Sample: 25 mer DNA
Temp: 65 °C
Detection: UV at 260 nm
Detection: MS
Min range: 400 m/z
Max range: 1,700 m/z
Scan rate: 3.00 spectra/s
Ion polarity: -ve
VCap: 3,500
Nozzle voltage: 1,000 V
Fragmentor: 200

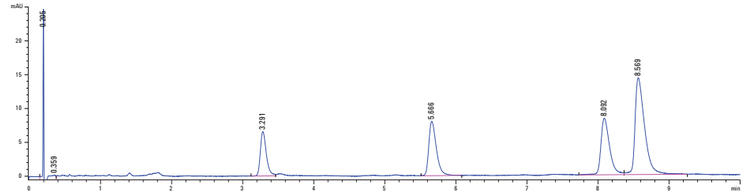
Peak	Response	%
FLP	5089897	44.33%
FLP-1	1656225	14.42%
FLP-2	304129	2.65%
FLP-3	303848	2.65%
FLP-4	218243	1.90%
FLP-5	113062	0.98%
FLP-6	104555	0.91%
FLP-7	110327	0.96%
FLP-8	134341	1.17%
FLP-9	134080	1.17%
FLP-10	186947	1.63%
FLP-11	358833	3.12%
FLP-12	251690	2.19%
FLP-13	272844	2.38%
FLP-14	416306	3.63%
FLP-15	238205	2.07%
FLP-16	304333	2.65%
FLP-17	403038	3.51%
FLP-18	459344	4.00%
FLP-19	422518	3.68%
Sum	**11482765**	**100%**

AdvanceBio Oligonucleotide columns also demonstrate excellent stability with mobile phase that contains TEA and HFIP.

ASSURED PERFORMANCE

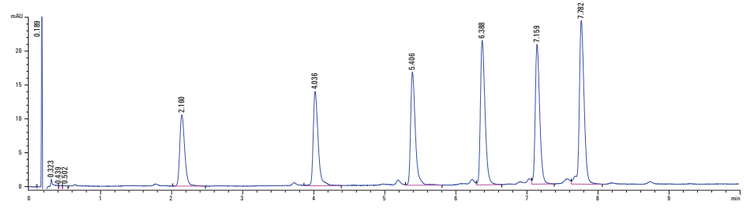
To ensure performance for your separations every batch of AdvanceBio Oligonucleotide media is tested with the Agilent Oligonucleotide Resolution standard.

The Oligonucleotide Resolution standard containing 14, 17, 20, and 21 mer synthetic oligonucleotides is designed to demonstrate N / N-1 resolution.



Column: AdvanceBio Oligonucleotide, 2.1 x 50 mm (p/n 659750-702)
Mobile phase A: 100 mM TEAA in water
Mobile phase B: 100 mM TEAA in acetonitrile
Gradient: 10 to 14%B in 10 min
Stop time: 11 min
Post run: 5 min
Flow rate: 0.6 mL/min
Col. temp: 65 °C
Sample: Agilent Oligonucleotide Ladder Standard (p/n 5190-9029)
Injection: 10 µL
Detection: UV at 260 nm

Agilent also offers an Oligonucleotide Ladder standard contains 15, 20, 25, 30, 35, and 40 mer synthetic oligodeoxythymidines, an excellent tool for demonstrating column selectivity and reproducibility.



Column: AdvanceBio Oligonucleotide, 2.1 x 50 mm (p/n 659750-702)
Mobile phase A: 100 mM TEAA in water
Mobile phase B: 100 mM TEAA in acetonitrile
Gradient: 6 to 8% B in 12 min
Stop time: 13 min
Post run: 5 min
Flow rate: 0.6 mL/min
Sample: Agilent Oligonucleotide Resolution Standard (p/n 5190-9028)
Temp: 65 °C
Injection: 0.5 µL
Detection: UV at 260 nm

See back page for ordering details of the Agilent Oligonucleotide Ladder standards.

Ordering information

Agilent AdvanceBio Oligonucleotide Columns and Oligonucleotide Standards

Description	Part Number
AdvanceBio Oligonucleotide, 2.1 x 50 mm, 2.7 µm	659750-702
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 mm Fast Guard	821725-921
AdvanceBio Oligonucleotide, 4.6 x 50 mm, 2.7 µm	659950-702
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 mm Fast Guard	820750-921
Oligonucleotide Resolution Standard	5190-9028
Oligonucleotide Ladder Standard	5190-9029

Learn more
http://www.agilent.com/chem/AdvanceBio_oligo

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Agilent Solutions for Oligonucleotides

Oligonucleotide Purification

Agilent PLRP-S and PL-SAX

For the purification of oligonucleotides, chemically and thermally stable polymeric HPLC media is required to achieve high purity with acceptable column lifetimes. There is no deterioration in column performance when PLRP-S columns are used at 80 °C with ion pairing agents, including TEA, for separating trityl-on from trityl-off oligos. PL-SAX 1000Å columns separate deprotected oligos under denaturing high pH conditions. The quaternary amine functionality on the polymeric particles enables ion-exchange separations at high pH, improving chromatography for self-complementary sequences.

TOP-DNA and TOP-RNA purification cartridges

Agilent TOP-DNA and TOP-RNA deliver outstanding yields of high purity synthetic DNA and RNA oligonucleotides by removing interfering salts, incomplete synthesis products and other impurities in a few simple steps. Perform detritylation of both DNA and RNA oligos in the cartridge. The 96-well format ensures that purification does not limit the throughput.

Nucleic Acid Solutions

Flexible Therapeutic Oligo Manufacturing & Development Services

The Agilent Nucleic Acid Solutions Division offers industry-leading experience to efficiently advance your lead oligo candidates from the clinic to market with a common goal of patient health and safety. Agilent has experience with all classes of oligo APIs, and supports customers from toxicology through commercialization. Agilent's GMP facility, located in Boulder, Colorado houses a broad range of synthesis and purification equipment for production of grams for toxicology and pre-clinical use, to 10's of kilograms for late-stage clinical trials as well as commercialization.

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