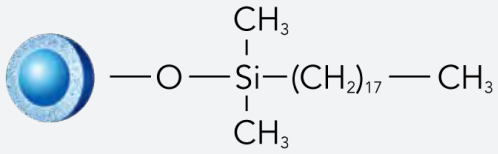
HALO®

OLIGO C18

Bringing the speed and efficiency of Fused-Core® performance to oligonucleotide separations.

A BETTER PATH TO SEPARATIONS

HALO® OLIGO C18



Built upon proven Fused-Core® particle technology for speed and efficiency, the HALO® OLIGO C18 incorporates surface modified organo-silane technology for alkaline resistance resulting in excellent stability under elevated pH operating conditions common in oligonucleotide separation methods.

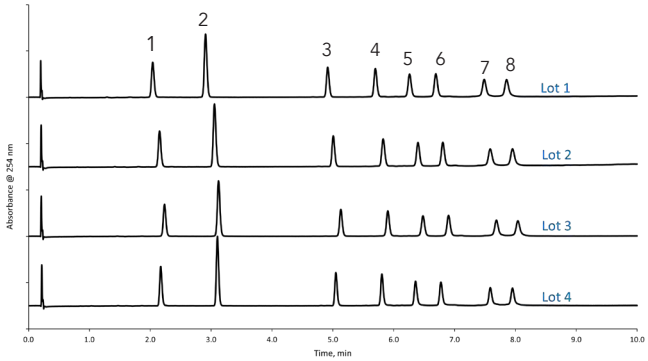
Loaded into surface passivated column hardware to address adsorption concerns, the HALO® OLIGO columns are ready for use with standard or bio inert instrumentation.

FEATURES OF HALO® OLIGO C18

- 120 Å pore size, enables separations of oligomers up to 60 bases in length
- High pH and temperature stability, designed for conditions suited for oligonucleotide separations
- UHPLC and mass spectrometry compatible stationary phase
- Surface passivated column hardware to reduce potential of stainless steel sample adsorption.

EXCELLENT LOT-TO-LOT REPRODUCIBILITY

Four different lots of HALO® OLIGO C18 were tested using a ladder of single stranded DNA ranging in base length from 10 mer to 60 mer.



PEAK IDENTITIES:

1.	10 mer	4.	25 mer	7.	50 mer
2.	15 mer	5.	30 mer	8.	60 mer
3.	20 mer	6.	40 mer		

TEST CONDITIONS:

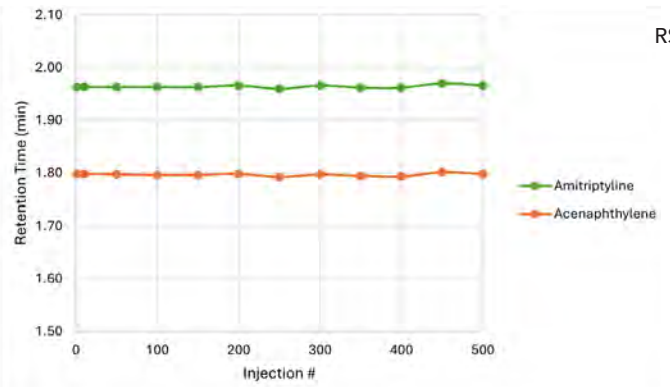
Columns: HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 50 mm
Part Number: P2A62-402
Mobile Phase A: 10mM TEAA, pH 8.5
Mobile Phase B: Acetonitrile
Gradient:

Time	%B
0.0	5
10.0	11
11.0	11
11.5	5

Flow Rate: 0.5 mL/min
Back Pressure: 125 bar
Temperature: 60 °C
Injection Volume: 1.0 µL
Sample Solvent: 10mM Tris HCl/1mM EDTA
Detection: UV/PDA, 254 nm
Flow Cell: 1 µL
Data Rate: 100 Hz
Response Time: 0.025 sec.
LC System: Shimadzu Nexera X2

PERFORMANCE YOU CAN RELY ON!

Testing the packing material stability that is used in the HALO® OLIGO C18 column, a less than 1% change in retention is achieved over 20,000 column volumes. This stability run was performed at both high pH (10) and high T (60 °C).



RSD <1% for both analytes

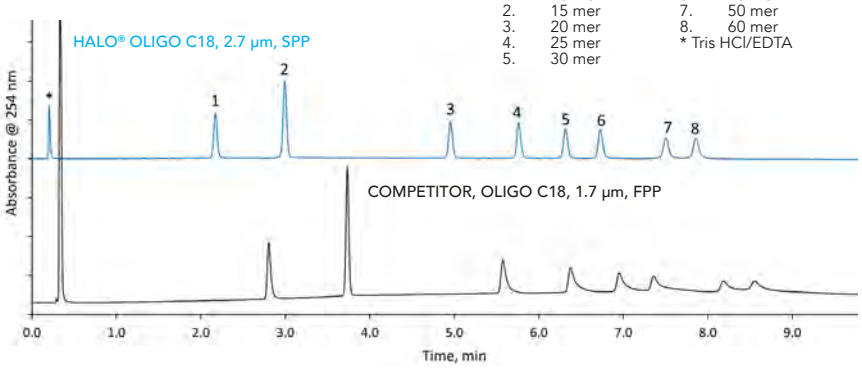
COMPETITIVE ADVANTAGE OF HALO® OLIGO

An oligonucleotide ladder of mixed sequence and length is separated on the HALO® OLIGO C18 and a competitor oligonucleotide column under high pH conditions. The oligomers of 20 base length AND higher begin to tail significantly on the competitor column. The same oligomers show no tailing on the HALO® column demonstrating the chromatographic efficiency and speed of Fused-Core®. Note: Tailing of the competitor column could represent poor column loading, however, both the HALO® and competitor columns were QC tested and passed prior to analysis.

PEAK IDENTITIES:

1. 10 mer
2. 15 mer
3. 20 mer
4. 25 mer
5. 30 mer
6. 40 mer
7. 50 mer
8. 60 mer

* Tris HCl/EDTA



TEST CONDITIONS:

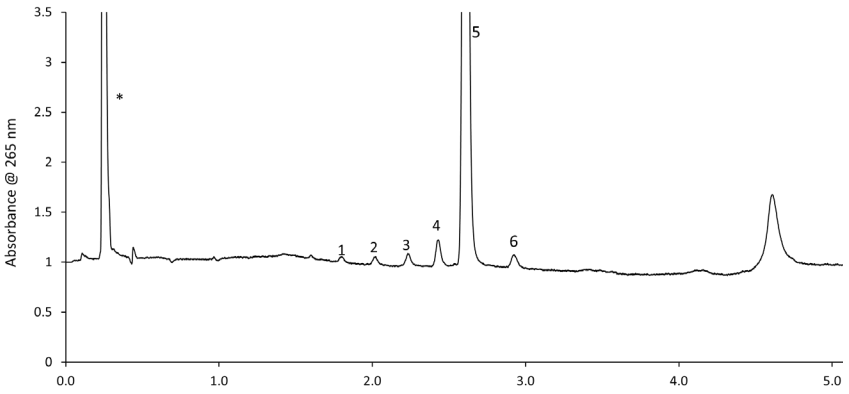
Column: HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 50 mm
Competitor: Oligo 130 Å C18, 1.7 µm, 2.1 x 50 mm
Mobile Phase A: 100mM TEAA, Adjusted to pH = 8.5
Mobile Phase B: ACN

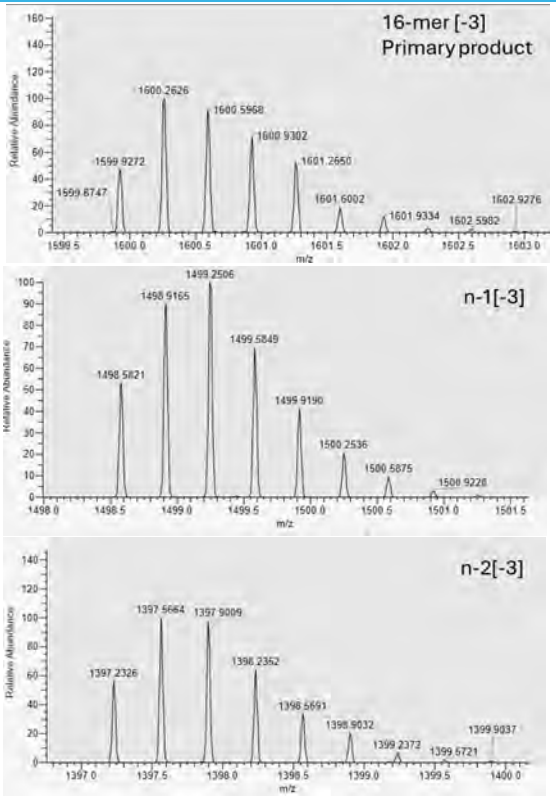
Gradient: Time	%B
0.0	5
10.0	11
11.0	11
11.5	0
16.5	0

Flow Rate: 0.5 mL/min
Back Pressure:
HALO® - 140 bar
Competitor - 255 bar
Temperature: 60 °C
Injection: 1.0 µL, 10µg on column
Sample Solvent: 10mM Tris HCl/1mM EDTA pH=8.0
Wavelength: PDA, 254 nm
Flow Cell: 1 µL
Data Rate: 100 Hz
Response Time: 0.025 sec.
LC System: Shimadzu Nexera X2

IMPURITY ANALYSIS OF POLY dT OLIGONUCLEOTIDES

A 16-mer ssDNA poly dT oligonucleotide was analyzed on a HALO® OLIGO C18 column under UV conditions. The chromatogram showed smaller peaks around the main peak, which were further examined using LC/MS. Mass spectrometry data confirmed these smaller peaks as truncated poly dT sequences (n-1 to n-6) from the synthesis process. The later-eluting peak was identified as a 16-mer poly dT with a cyanoethyl protecting group, typically removed in the final deprotection step. These results demonstrate the HALO® OLIGO C18 column's effectiveness for analyzing oligonucleotide impurities and offering insights into synthesis and purification.





TEST CONDITIONS:

Column: HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 50 mm
Mobile Phase A: 5mM TEA/50mM HFIP @ pH 8.3
Mobile Phase B: 25/75 ACN/MeOH

Gradient: Time	%B
0.0	5
0.5	8
3.5	13
5.0	20
5.4	20
5.5	5
9.0	5

Flow Rate: 0.4 mL/min
Back Pressure: 219 bar
Temperature: 60 °C
Injection: 1.0 µL, Primer (8µg/mL)
Sample Solvent: 10mM Tris HCl/1mM EDTA
Wavelength: PDA, 265 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2

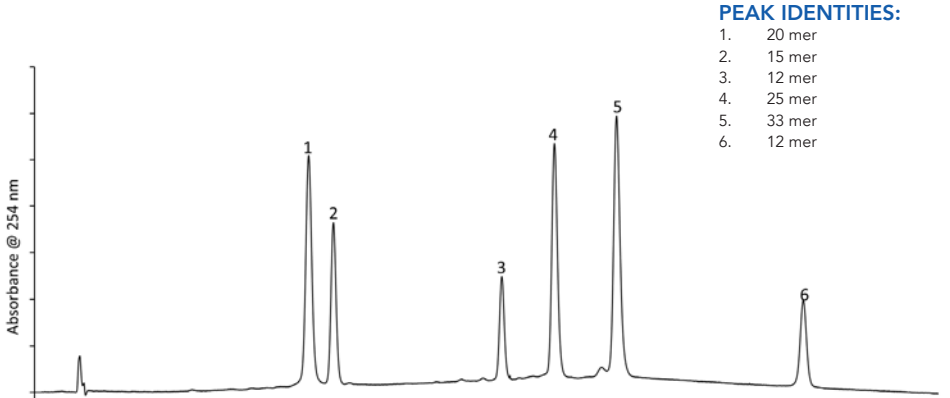
MS CONDITIONS:

Polarity: Negative mode
Sheath Gas Flow: 25
Auz Gas Flow: 10
Sweep Gas: 1
Spray Voltage: 2.5kV
Capillary Temperature: 325 °C
Aux Gas Heater Temp: 300 °C
S-Lens RF Level: 60
m/z Scan Range: 450-2000
Resolution: 120,000 MS1

APPLICATIONS

OLIGONUCLEOTIDE PERFORMANCE MIX

By using the HALO® OLIGO C18 column under high pH conditions a sample of 6 different oligonucleotides can be separated in under 4.5 minutes. Using the SigmaAldrich Oligonucleotide Performance Standard Mix, the HALO® OLIGO C18 demonstrates utility as part of system suitability testing. The standard, with a range of 12 to 33 oligomers in base length, and having two 12 base length oligos, serves as an ideal performance mix.



PEAK IDENTITIES:

1. 20 mer
2. 15 mer
3. 12 mer
4. 25 mer
5. 33 mer
6. 12 mer

TEST CONDITIONS:

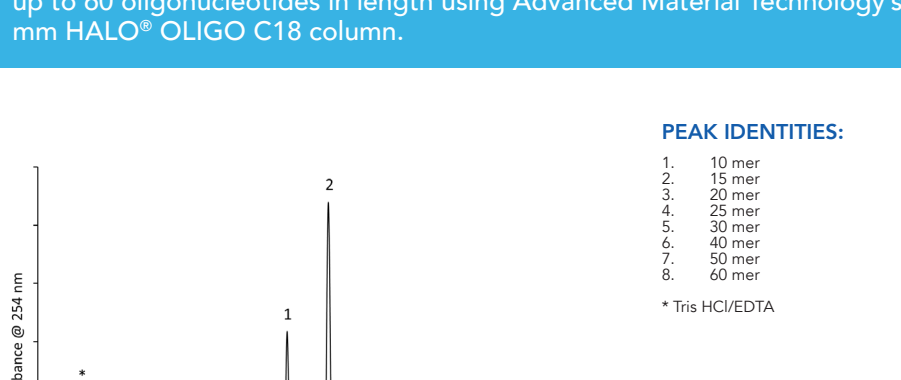
Column: HALO 120 Å OLIGO C18, 2.7µm, 2.1 x 50 mm
Part Number: P2A62-402
Mobile Phase A: 100mM TEAA @ pH 8.5
Mobile Phase B: Acetonitrile
Gradient:

Time	%B
0.0	7.5
5.0	15
5.3	60
5.6	60
8.0	7.5

Flow Rate: 0.4 mL/min
Back Pressure: 142 bar
Temperature: 50 ºC
Injection: 1 µL of Oligonucleotide Performance Standard Mix, 12-33 NT
P/N: PHR8667-1EA
Sample Solvent: 10mM Tris HCl/ 1mM EDTA
Wavelength: PDA, 254 nm
Flow Cell: 1 µL
Data Rate: 100 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2

RAPID SEPARATION OF OLIGONUCLEOTIDE LADDER

This example demonstrates the resolution of an Oligonucleotide Standard mixture (10- to 60-mer ssDNA) using triethylammonium acetate (TEAA) with absorbance detection and gradient elution with acetonitrile. This fast separation (less than 3.5 minutes) illustrates the excellent peak shape, and high resolution of oligonucleotides of up to 60 oligonucleotides in length using Advanced Material Technology's Fused-Core® technology in the 2.1 x 50 mm HALO® OLIGO C18 column.



PEAK IDENTITIES:

1. 10 mer
2. 15 mer
3. 20 mer
4. 25 mer
5. 30 mer
6. 40 mer
7. 50 mer
8. 60 mer

* Tris HCl/EDTA

TEST CONDITIONS:

Column: HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 50 mm
Part Number: P2A62-402
Mobile Phase A: 100mM TEAA, pH 8.5
Mobile Phase B: Acetonitrile
Gradient:

Time	%B
0.0	5
0.5	7.4
3.5	10.7
3.6	20
4.1	20
4.2	5
9.0	5

Flow Rate: 0.5 mL/min
Back Pressure: 137 bar
Temperature: 60 ºC
Injection: 2.0 µL, (10µg)
Sample Solvent: 10mM Tris HCl/1mM EDTA pH=8.0
Wavelength: PDA, 254 nm
Flow Cell: 1 µL
Data Rate: 100 Hz
Response Time: 0.025 sec.
LC System: Shimadzu Nexera X2

PRODUCT CHARACTERISTICS

Ligand: dimethyloctadecylsilane, surface modified
Particle Size: 2.7 µm
Pore Size: 120 Å

USP Designation: L1
Carbon Load: 5.6%
Surface Area: 75 m^2/g
Endcapped: YES

Low pH Limit: 2
High pH limit*: 9
Temp limit @ low pH: 90 °C
Temp limit @ high pH*: 85 °C

PART NUMBERS

2.7 µm ANALYTICAL COLUMNS	
Dimensions: ID x Length (in mm)	Part Number
2.1 x 50	P2A62-402
2.1 x 100	P2A62-602
2.1 x 150	P2A62-702
4.6 x 50	P2A64-402
4.6 x 100	P2A64-602
4.6 x 150	P2A64-702

SURFACE PASSIVATED **HARDWARE**

*Column lifetime will vary depending on the operating temperature and the type and concentration of buffers used. Operation at extreme specifications of temperature and pH may reduce column lifetime. Consult the column Care and Use document for more information.

INNOVATION YOU CAN TRUST – PERFORMANCE YOU CAN RELY ON

HALO®

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advancedmaterialstechnology

halocolumns.com

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AMT24_OLIGO_REV1

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