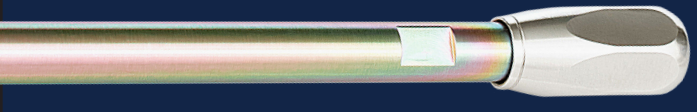
# HALO®

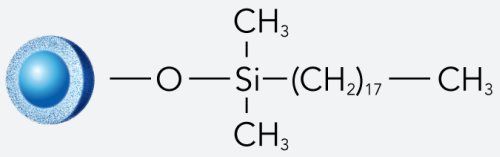
# OLIGO C18

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.

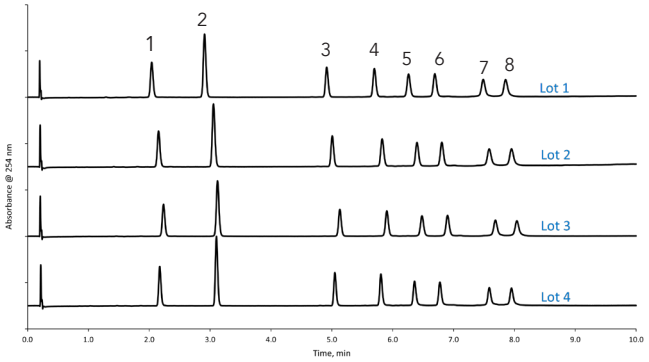
Loaded into surface passivated column hardware to address adsorption concerns, the HALO® OLIGO columns are ready for use with standard or bio inert instrumentation.

## 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:

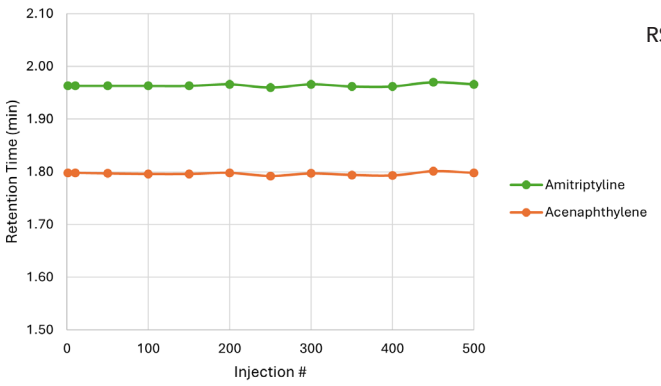
**Columns:** HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 50 mm
**Part Number:** P2A62-402
**Mobile Phase A:** 10mM TEAA, pH 8.5
**Mobile Phase B:** Acetonitrile
**Gradient:**

| 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).

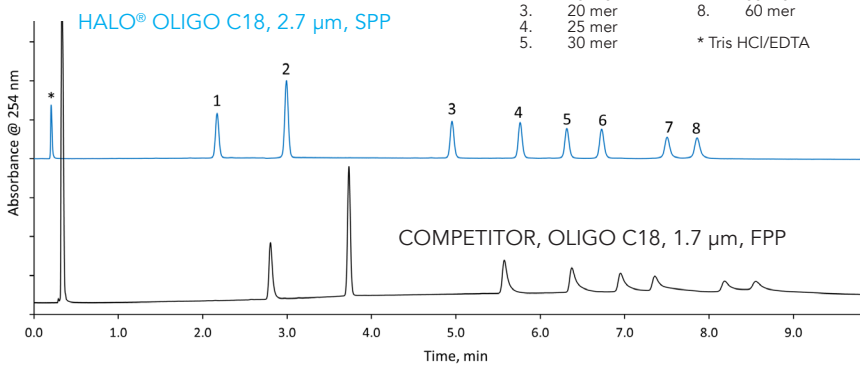



RSD <1% for both analytes

# APPLICATIONS

## 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:**

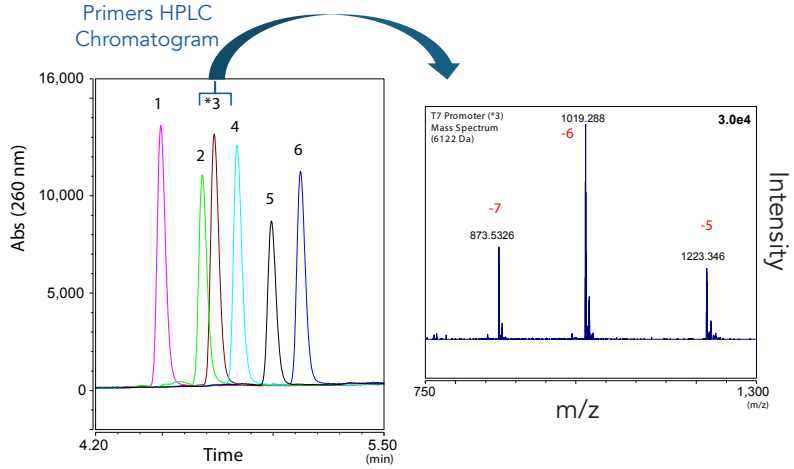
**Column:** HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 50 mm
**Competitor:** Oligo 130 Å C18, 1.7 µm, 2.1 x 50 mm
**Mobile Phase A:** 100mM TEAA, Adjusted to pH = 8.5
**Mobile Phase B:** ACN

| 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

## OLIGONUCLEOTIDE SEPARATION WITH LC/MS DETECTION CONDITIONS

Resolution of intermediate length synthetic oligonucleotides and impurities, can be conducted using shallow gradients of methanol in MS compatible IP-RP conditions. This example shows synthetic oligonucleotides of 15-21 bases resolved using 5 mM TEA as the IP reagent, with 50 mM HFIP buffer additive (left side, Abs 260 nm). In series, online ESI-MS analysis was obtained (right side), showing the charge states for the 6122 amu MW T7 promoter synthetic oligonucleotide primer. Retention of this ssDNA is determined by length and sequence (composition), which permits closely related impurities and failure sequences (n-1) to be well resolved using the HALO OLIGO C18 column.




| Peak Identities | | ssDNA Sequence | Ret. (min) | $T_m$ (°C) |
|---|---|---|---|---|
| 1 | T7 Terminator$_{19mer}$ | GCT AGT TAT TGC TCA GCG G | 4.49 | 60.3 |
| 2 | 16s rRNA$_{20mer}$ | AGA GTT TGA TCC TGG CTC AG | 4.68 | 61.3 |
| 3 | T7 Promoter$_{20mer}$ | TAA TAC GAC TCA CTA TAG GG | 4.74 | 55.2 |
| 4 | 16s rRNA$_{21mer}$ | ACG GCT ACC TTG TTA CGA CTT | 4.84 | 63.5 |
| 5 | dT$_{15mer}$ | TTT TTT TTT TTT TTT | 4.99 | 38.6 |
| 6 | dT$_{16mer}$ | TTT TTT TTT TTT TTT T | 5.12 | 40.9 |

**TEST CONDITIONS:**

**Column:** HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 50 mm
**Mobile Phase A:** 5mM TEA/50mM HFIP, pH 8.35
**Mobile Phase B:** Methanol

| Gradient: Time | %B |
|---|---|
| 0.0 | 5 |
| 7.0 | 18 |

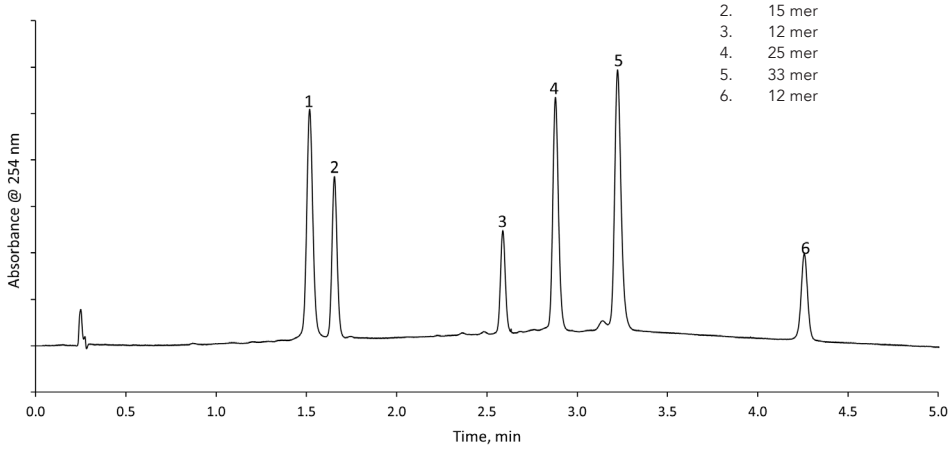
**Flow Rate:** 0.4 mL/min
**Back Pressure:** 106 bar
**Temperature:** 50 °C
**Injection:** 1.0 µL, 10ng on-column
**Sample Solvent:** 10mM Tris HCl/1mM EDTA pH=8.0
**Wavelength:** PDA, 260 nm
**Flow Cell:** 1 µL
**Data Rate:** 100 Hz
**Response Time:** 0.025 sec.
**LC System:** Shimadzu Nexera X2
**MS System:** Thermo Velos Pro Orbitrap

**MS CONDITIONS:**
**Detection:** (-) HESI
**Spray Voltage:** 2.5 kV
**Capillary temp:** 350°C
**Source Heater temp:** 300°C

# 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:**

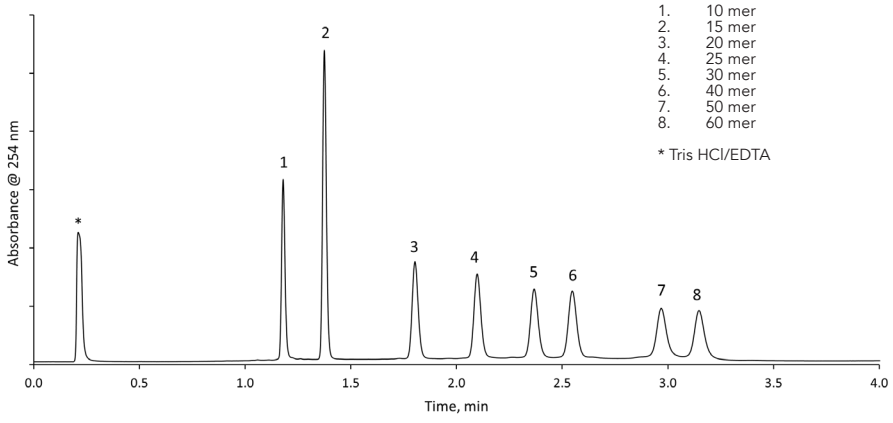
**Column:** HALO 120 Å OLIGO C18, 2.7µm, 2.1 x 50 mm
**Part Number:** P2A62-402
**Mobile Phase A:** 100mM TEAA @ pH 8.5
**Mobile Phase B:** Acetonitrile
**Gradient:**

| 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

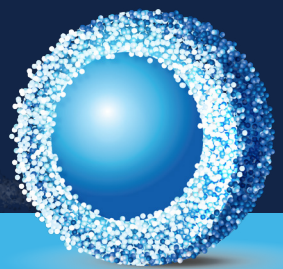
Low pH Limit: 2
High pH limit*: 9
Temp limit @ low pH: 90 °C
Temp limit @ high pH*: 85 °C

# 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**