

# HALO® OLIGO C18

# TAKING SEPARATIONS TO A HIGHER LEVEL

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.

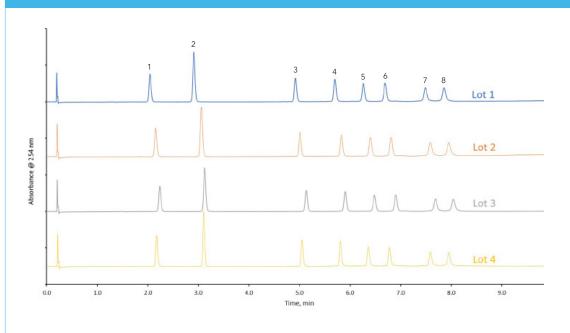
Loaded into surface passivated column hardware to address adsorption concerns, the HALO® OLIGO columns are ready for use with standard or bio inert instrumentation.

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

### **TEST CONDITIONS:**

Columns: HALO 120 Å OLIGO C18, 2.7  $\mu$ m, 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

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 HCI/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

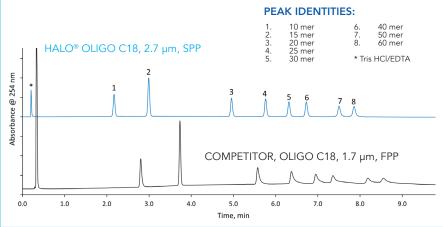
# **APPLICATIONS**



### **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: As tailing of the competitor column could represent poor column loading, both the HALO® and competitor columns were QC tested to ensure a fair comparison.

Both columns passed QC which verified each column.



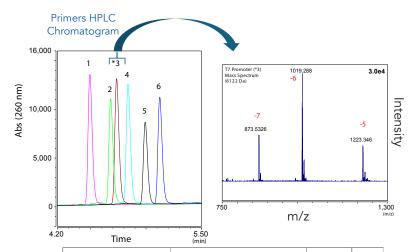
#### **TEST CONDITIONS:**

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 = 8.5Mobile Phase B: ACN %В Gradient: Time 0.0 5 10.0 11 11.0 11 0 11.5 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
HCI/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 <sub>m</sub> (°C)
1	T7 Terminator <sub>19mer</sub>	GCT AGT TAT TGC TCA GCG G	4.49	60.3
2	16s rRNA <sub>20mer</sub>	AGA GTT TGA TCC TGG CTC AG	4.68	61.3
3	T7 Promoter <sub>20mer</sub>	TAA TAC GAC TCA CTA TAG GG	4.74	55.2
4	16s rRNA <sub>21mer</sub>	ACG GCT ACC TTG TTA CGA CTT	4.84	63.5
5	dT <sub>15mer</sub>	TTT TTT TTT TTT TTT	4.99	38.6
6	dT <sub>16mer</sub>	TTT TTT TTT TTT TTT T	5.12	40.9

#### **TEST CONDITIONS:**

Column: HALO 120 Å OLIGO C18, 2.7  $\mu$ m, 2.1 x 50 mm Mobile Phase A: 5mM TEA/50mM HFIP, pH 8.35

Temperature: 50 °C Injection: 1.0 µL, 10ng on-column

Sample Solvent: 10mM Tris HCI/1mM EDTA pH=8.0

Wavelength: PDA, 260 nm Flow Cell: 1 µL Data Rate: 100 Hz Response Time: 0.025 sec. LC System: Shirmadzu 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

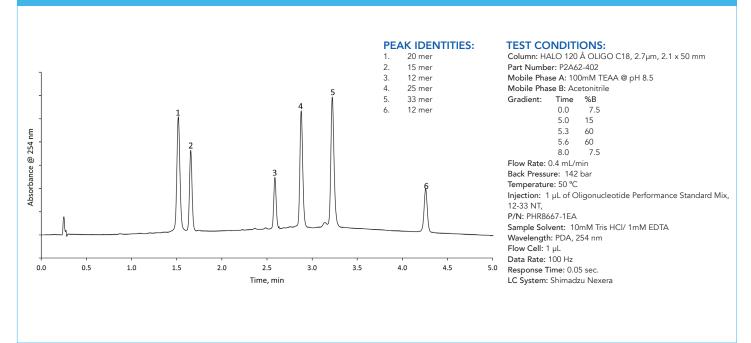


# **APPLICATIONS**



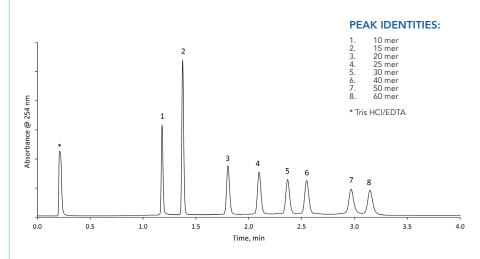
### **OLIGONUCLEOTIDE PERFORMANCE MIX**

By using the OLIGO C18 column under high pH conditions a sample of 6 different oligonucleotides can be separated in under 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.



## **OLIGONUCLEOTIDE SIZE STANDARD SEPARATION**

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.



#### **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 %В 0.0 7.4 0.5 10.7 3.5 3.6 20 4.1 20 4.2 9.0

Flow Rate: 0.5 mL/min Back Pressure: 137 bar Temperature: 60 °C Injection: 2.0 µL, (10µg)

Sample Solvent: 10mM Tris HCl/1mM EDTA pH=8.0

Wavelength: PDA, 254 nm Flow Cell: 1 ul 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 m²/g Endcapped: YES Low pH Limit: 2 High pH limit: 9 Temp limit @ low pH: 90 °C Temp limit @ high pH: 85 °C

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

# **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		







# Manufactured by:



Scantec Nordic

Analys & Mätteknik

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