



HALO®

HALO® 1000 Å Method Development Kit

Fused-Core® Particle Technology

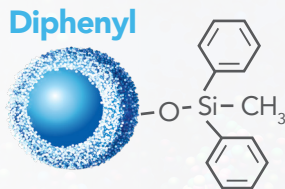
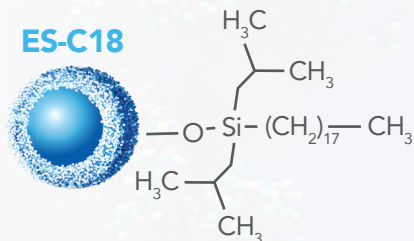
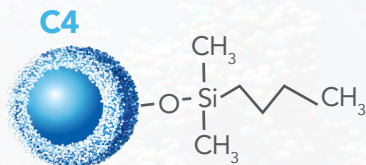


BIOCLASS

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HALO® 1000 Å UHPLC and HPLC Method Development Kit

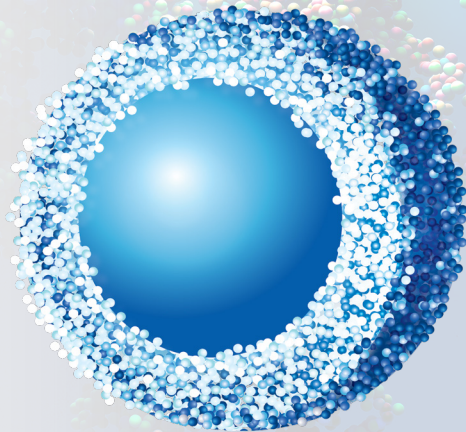
- Method development kit is available in 50 and 150 mm lengths with 2.1 and 4.6 mm IDs
- The kit is comprised of three different phases with 2.7 µm, 1000 Å Fused-Core® particles
- The kit allows you to adjust the selectivity of your separation with three unique bonding chemistries that provide options for method development and optimization
- All columns provide excellent peak shape, efficiency, reproducibility and column lifetime for challenging separations



Advantages of 1000 Å Pore Size

Reasons for the 1000 Å Pore Size

- Surface area only matters if the analytes can access it. With a 1000 Å pore size, large, bulky protein structures have unrestricted access to the bonded phase, which resides primarily in the pores
- Monoclonal antibodies (mAbs) are very large biomolecules
 - General guideline: the particle pore size should be ~10 fold larger than the analyte of interest in order to avoid (or minimize) restricted diffusion
- Inadequate pore size results in broader peaks and lower resolution



Advantages of Superficially Porous Particles (SPP) for Large Biomolecule Separations

- The superficially porous layer provides a shorter diffusion path (vs. fully porous sub-2- μm particles) for large biomolecules, which have very slow diffusion coefficients, while maintaining necessary loading capacity
- Improved resolution vs. fully porous particles (FPP) thanks to total pore access is evident in this comparison of IgG2 variants

Test Conditions:

Columns: 2.1 x 150 mm

Flow Rate: 0.2 mL/minute

Mobile Phase A: 88/10/2 H_2O /ACN/
n-Propanol + 0.1% DFA

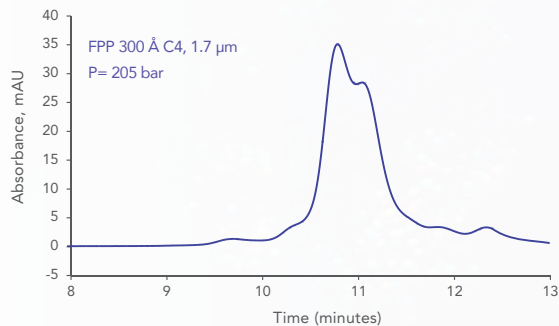
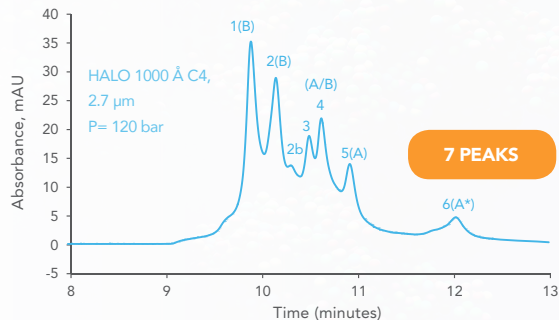
Mobile Phase B: 70/20/10 n-Propanol/ACN/
 H_2O + 0.1% DFA

Gradient: 14 - 24% B in 20 minutes

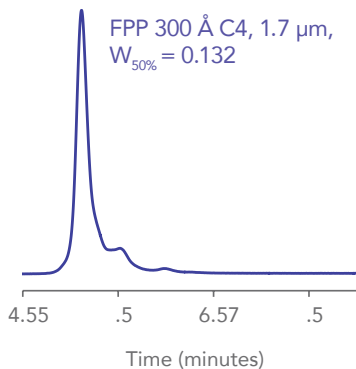
Injection Volume: 2 μL of 2 mg/mL denosumab
in H_2O + 0.1% DFA

Temp: 80 $^{\circ}\text{C}$

Detection: PDA at 280 nm



Smaller IgG1 Peak Widths with 1000 Å SPP Compared to 300 Å FPP



Test Conditions:

Columns: 2.1 x 150 mm

Flow Rate: 0.4 mL/minute

Mobile Phase A: H_2O /0.1% TFA

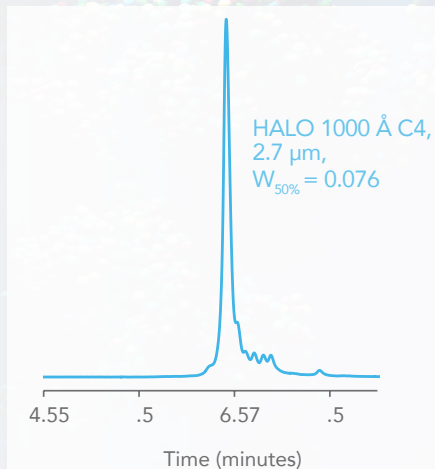
Mobile Phase B: Acetonitrile/0.1% TFA

Gradient: 32 - 38% B in 12 minutes

Injection Volume: 2 μL (1 μg)

Temp: 80 $^{\circ}\text{C}$

Detection: 280 nm



Improved resolution of an IgG1 (trastuzumab) is demonstrated by the 2.7 μm particle with 1000 Å pores.
This is not unique to this antibody.



HALO® 1000 Å C4 (Dimethylbutylsilane)

Analyte Interactions

- Hydrophobic

Advantages

- Outstanding high temperature stability up to 90 °C at low pH
- Unrestricted access to bonded phase
- Exceptional mass transfer kinetics
- Compatible with UHPLC and HPLC
- Low LC-MS bleed

Target Analytes

- Monoclonal antibodies
- Antibody-drug conjugates
- Antibody fragments
- Large proteins with MWs ≥ 50 kDa

Example Applications

- High resolution separations of monoclonal antibodies and their variants
- Antibody-drug conjugates



HALO® 1000 Å ES-C18 (Di-isobutyloctadecylsilane)

Analyte Interactions

- Hydrophobic

Advantages

- Sterically-protected di-isobutyloctadecylsilane ligands are resistant to hydrolysis at low pH and high temperatures (up to 90 °C)
- Unrestricted access to bonded phase
- Exhaustively endcapped
- Compatible with UHPLC and HPLC
- Low LC-MS bleed

Target Analytes

- Monoclonal antibodies
- Antibody-drug conjugates
- Antibody fragments
- Large proteins with MWs ≥ 50 kDa

Example Applications

- High resolution separations of monoclonal antibodies and their variants
- Antibody-drug conjugates



HALO® 1000 Å Diphenyl (Diphenylmethylsilane)

Analyte Interactions

- Hydrophobic
- π - π

Advantages

- Outstanding stability from 40-90 °C
- Can elute very large proteins with good peak shape and recovery
- Compatible with UHPLC and HPLC
- Low LC-MS bleed
- Exceptional low temperature stability without loss of peak area performance

Target Analytes

- Monoclonal antibodies
- Antibody-drug conjugates
- Antibody fragments
- Large proteins with MWs > 50 kDa

Example Applications

- High resolution separations of monoclonal antibodies and their variants
- Antibody-drug conjugates



Parameters That Impact Retention and Selectivity for Gradient RPLC Separations of Proteins

- Column stationary phase
 - C4, Diphenyl, ES-C18
- Organic modifier(s) choice
 - CH₃CN, 2-propanol, n-propanol, blends
- Mobile phase additive
 - HCOOH, DFA, TFA, HOAc, etc.
- Mobile phase additive concentration
- Gradient steepness (%B/minute)
- Column temperature



Screening the Stationary Phase

Comparisons of short chain alkyl (C4), long chain alkyl (C18) and aromatic (Diphenyl) stationary phases on 1000 Å pore size columns demonstrate excellent resolution, and also show that varying the bonded phase allows one to tune selectivity for the separation of highly similar protein variants.

Test Conditions:

Columns: 1000 Å C4, ES-C18, Diphenyl, 2.7 µm,
2.1 x 150 mm

Flow Rate: 0.2 mL/minute

Temp: 80 °C

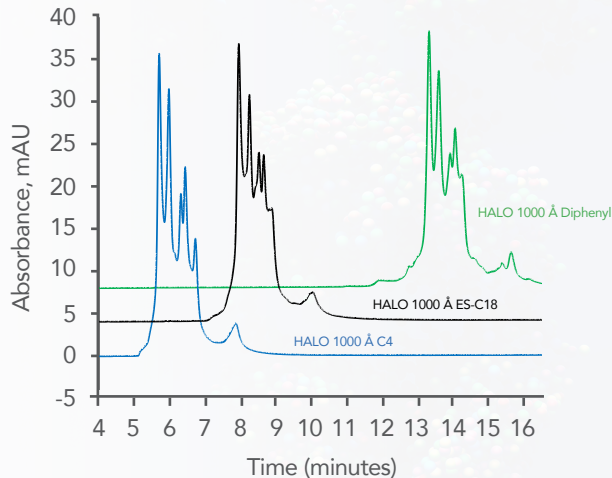
Detection: PDA at 280 nm

Mobile Phase A: 2:10:88 n-Propanol/ACN/
H₂O+ 0.1% difluoroacetic (DFA)

Mobile Phase B: 70/20/10 n-Propanol/ACN/
H₂O+ 0.1% difluoroacetic (DFA)

Gradient: 16 - 26% B in 20 minutes

Injection Volume: 2 µL of 2 mg/mL denosumab in
H₂O + 0.1% TFA



Comparison of HALO® ES-C18 vs. Competitor's FPP C18

Enhanced resolution with HALO® 1000 Å for NISTmAb

The NISTmAb material is a recombinant humanized IgG1κ expressed in murine suspension culture. It is a »150 kDa homodimer of two identical light chains and two identical heavy chains linked through both inter- and intra-chain disulfide bonds.

Test Conditions:

Columns: 2.1 x150 mm

Flow Rate: 0.4 mL/minute

Mobile Phase A: H₂O/0.1% TFA

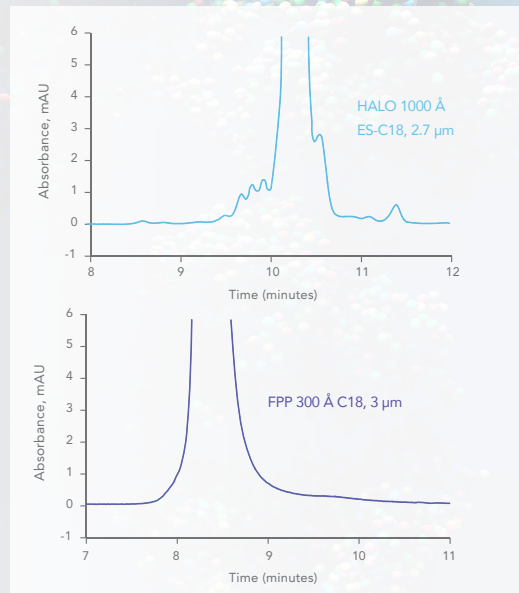
Mobile Phase B: ACN/0.1% TFA

Gradient: 36 - 44% B in 16 minutes

Injection Volume: 2 µL of 2 mg/mL NISTmAb in H₂O/0.1% TFA

Temp: 60 °C

Detection: PDA at 280 nm



Comparative results presented here may not be representative for all applications



Comparison of HALO® Diphenyl vs. Competitor's SPP Polyphenyl Column

Improved resolution, retention and peak area (recovery) are observed using the HALO 1000 Å Diphenyl column

Test Conditions:

Columns: 2.1 x 150 mm, 2.7 μ m

Flow Rate: 0.4 mL/minute

Mobile Phase A: H₂O/0.1% TFA

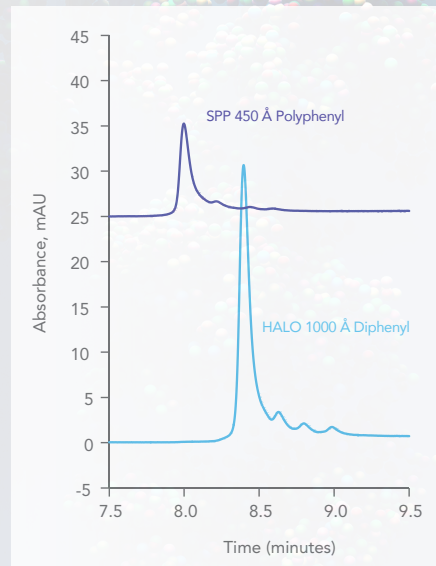
Mobile Phase B: ACN/0.1% TFA

Gradient: 30 - 45% B in 15 minutes

Injection Volume: 2 μ L of 2 mg/mL
trastuzumab in H₂O/0.1% TFA

Temp: 40 °C

Detection: PDA at 280 nm



Comparative results presented here may not be representative for all applications



HALO® 1000 Å Method

Development Kits Specifications Table

	C4	ES-C18	Diphenyl
Functional Group	Dimethylbutyl	Di-isobutyloctadecyl	Methyldiphenyl
USP Designation	L26	L1	L11
Particle Size (µm)	2.7	2.7	2.7
Pore Size (Ångstroms)	1000	1000	1000
Carbon Load (%)	0.6	1.4	1.0
Surface Area (m ² /g)	22	22	22
Endcapped	Yes	Yes	Yes
pH Range	2-9	1-8	2-9
Maximum Temperature Low pH	90	90	90
Maximum Temperature High pH	40	40	40
Maximum Pressure (Bar)	1000 600*	1000 600*	1000 600*

*for 4.6 mm IDs

HALO® 1000 Å 2.7 µm Method Development Kits Part Numbers

Column Dimensions (mm)	Phases Included	Part Number	List Price	Promotional Price Until December 31 st , 2018
2.1 x 50	C4 ES-C18 Diphenyl	92712 4KT	\$2,190	\$1,642.50
2.1 x 150	C4 ES-C18 Diphenyl	92712 7KT	\$2,760	\$2,070.00
4.6 x 50	C4 ES-C18 Diphenyl	92714 4KT	\$2,190	\$1,642.50
4.6 x 150	C4 ES-C18 Diphenyl	92714 7KT	\$2,760	\$2,070.00



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