



# HALO<sup>®</sup>

BIOCLASS

INNOVATION YOU CAN TRUST – PERFORMANCE YOU CAN RELY ON

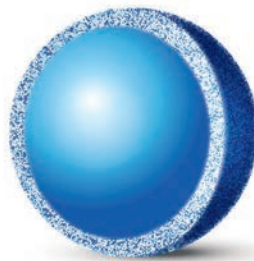
## TABLE OF CONTENTS

3	HALO <sup>®</sup> PROTEIN, PEPTIDE AND GLYCAN OVERVIEW
4	PROTEIN SOLUTIONS
5	HALO 1000 Å PROTEIN BONDED PHASE PORTFOLIO
8	HALO 400 Å PROTEIN BONDED PHASE PORTFOLIO
10	PEPTIDE SOLUTIONS
11	HALO <sup>®</sup> PEPTIDE BONDED PHASE PORTFOLIO
14	GLYCAN SOLUTIONS
16	HALO <sup>®</sup> UHPLC AND HPLC GUARD COLUMNS
17	HALO <sup>®</sup> BIOCLASS SPECIFICATIONS TABLES
18	PART NUMBER LISTING

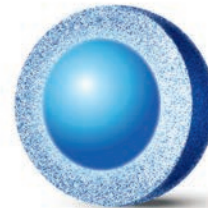
### BIOCLASS



1000 Å 2.7 micron particle



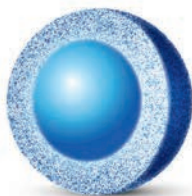
400 Å 3.4 micron particle



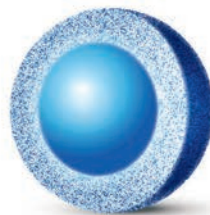
90 Å 2.7 micron particle

PROTEIN

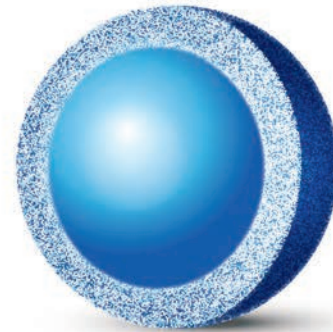
GLYCAN



160 Å 2 micron particle



160 Å 2.7 micron particle



160 Å 5 micron particle

PEPTIDE

## HALO® PROTEIN, PEPTIDE AND GLYCAN

Advanced Materials Technology led the revolution in Fused-Core® particle technology with the development of the first commercially available sub-3 µm superficially porous particle (SPP) with the original HALO® 2.7 µm particle. Our manufacturing expertise has carried forward into being the wide pore particle leader. This innovative culture has been transferred to developing novel materials for the biotherapeutic market. HALO® particles are ready to solve the characterization challenges of these complex separations.

Today, researchers are keenly interested in both fast and high resolution separations of numerous biomolecules to support the development of novel therapeutic proteins and peptides in pharmaceutical drug development, advance academic understanding in modern University laboratories, characterize protein post-translation modifications, and to fully assess subtle differences

in biosimilars and other products of bioengineering and manufacturing. HALO® BioClass columns have been developed to simplify and promote a more comprehensive understanding - faster.

- Intact proteins, monoclonal antibodies (mAbs), biosimilars, and other large biomolecules such as pegylated proteins, antibody drug conjugates (ADCs), etc.
- Peptide mapping (analysis of enzyme digests) for characterization and monitoring of synthetic protein drugs
- Analysis of therapeutic peptides and peptide biomarkers (protein surrogates)
- High resolution separations of complex mixtures of glycans released from N- and O-linked glycoproteins

The HALO® BioClass column family is comprised of the following product lines.

### PROTEIN

- Wide pore portfolio for unrestricted bonded phase access capable of characterizing very large proteins with good peak shape and recovery
- Compatible chemistries for UHPLC, HPLC, and mass spectrometry
- Variety of bonded phase options for a tailored solution

### PEPTIDE

- SPP technology for fast, high resolution peptide separations
- High peak capacities delivering rugged, reliable performance for use with either UHPLC, HPLC, or LC-MS
- Extensive portfolio of particle sizes and chemistries

### GLYCAN

- Improved retention of acidic and zwitterionic analytes
- Very low sensitivity to buffer concentration
- Able to separate isobaric oligosaccharides with different linkages

### Guidance for Pore Size Selection

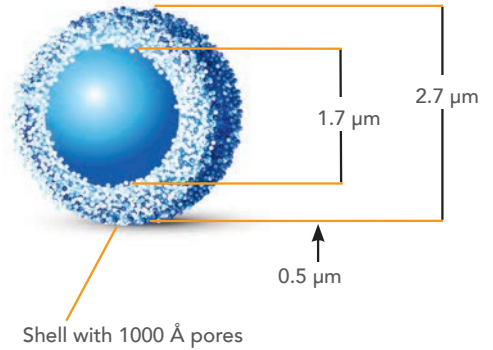
Molecule Size	Pore Size (Å)	Application	Particle Sizes (µm)
SMALL (< 20 kDa*)	90	Glycan	2.7
MEDIUM (100 Da < MW < 15 kDa)	160	Peptide	2, 2.7, 5
LARGE (2 kDa < MW < 500 kDa)	400	Protein	3.4
LARGE (> 50 kDa)	1000		2.7

\* for glycans, glycopeptides and glycoproteins

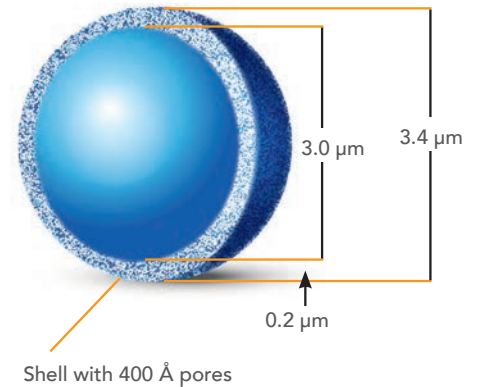
## PROTEIN SOLUTIONS

- The advantages of using wide pore silica based superficially porous particles (SPP) for high resolution analysis of large proteins has been well established and as the innovator of the 1000 Å Fused-Core® particle, AMT recognizes the benefit of unrestricted pore access which combines the power of ultrafast and high resolution separations to the biologics workflow
- Fused-Core® particles provide narrower peak widths and improved resolution for characterization of biomolecules in comparison to fully porous particles (FPPs)
- As complex biotherapeutics development continues to grow, understanding structural modifications requires separation options. Often these minor variants consist of subtle differences in protein chains, glycosylation sites and free sulfhydryl groups
- HALO® delivers a comprehensive portfolio of both 400 Å and 1000 Å silica phase selectivities to choose from

HALO® PROTEIN 2.7 µm



HALO® PROTEIN 3.4 µm



### APPLICATIONS

- mAbs
- ADCs
- Biosimilars
- H/D exchange
- Fragments

### FEATURES

- Outstanding temperature stability up to 90 °C
- Compatible with UHPLC, HPLC and MS
- Elution of very large proteins with excellent peak shape and recovery
- Very low LC-MS bleed

ANTIBODY-DRUG CONJUGATES

BIOSIMILARS H/D EXCHANGE

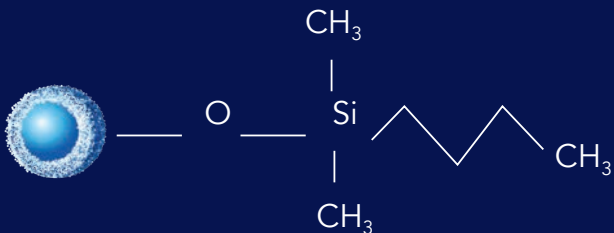
FRAGMENTS

mAb

PROTEIN

# HALO 1000 Å PROTEIN BONDED PHASE PORTFOLIO

## HALO 1000 Å C4



**Ligand:** DIMETHYLBUTYLSILANE

**USP Designation:** L26

**Available Particle Sizes:** 2.7 μm

**Pore Size:** 1000 Å

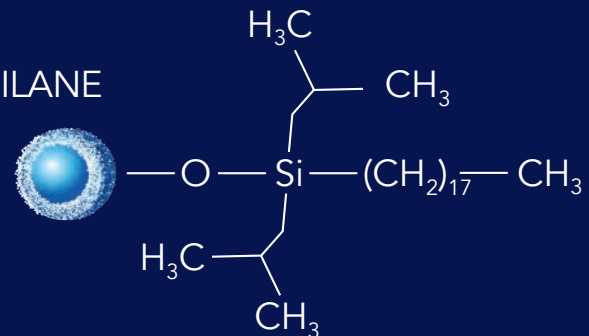
## HALO 1000 Å ES-C18

**Ligand:** DIISOBUTYLOCTADECYLSILANE

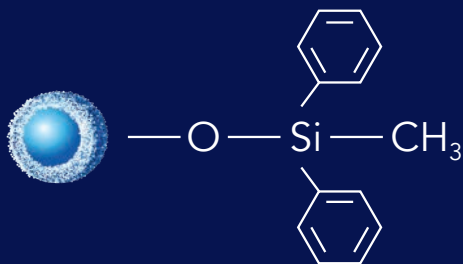
**USP Designation:** L1

**Available Particle Sizes:** 2.7 μm

**Pore Size:** 1000 Å



## HALO 1000 Å DIPHENYL



**Ligand:** DIPHENYLMETHYL

**USP Designation:** L11

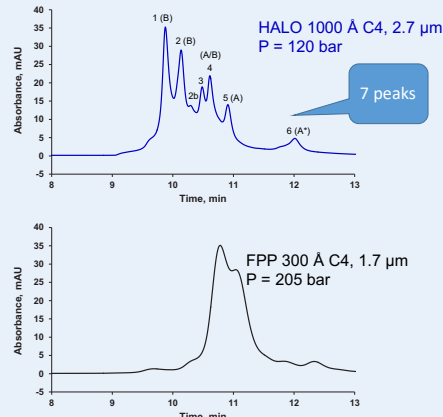
**Available Particle Sizes:** 2.7 μm

**Pore Size:** 1000 Å

## INADAQUATE PORE SIZE RESULTS IN BROADER PEAKS AND LOWER RESOLUTION

The large pores of the HALO 1000 Å C4 column allow improved access to the stationary phase and increased resolution for IgG2 isoforms compared to the smaller 300 Å pores of the FPP C4 column.

Twice the peaks  
Almost half the back pressure



### TEST CONDITIONS:

Columns: HALO 1000 Å C4, 2.7 μm, 2.1 x 150 mm  
Part Number: 92712-714  
Mobile Phase A: 88/10/2 water/ACN/n-propanol/0.1% DFA  
Mobile Phase B: 70/20/10 n-propanol/ACN/water/0.1% DFA  
Gradient: 14-24% B in 20 min  
Flow Rate: 0.2 mL/min  
Temperature: 80 °C  
Injection: 2 μL of 2 mg/mL denosumab in water/0.1% DFA  
Detection: 280 nm, PDA  
LC System: Shimadzu Nexera

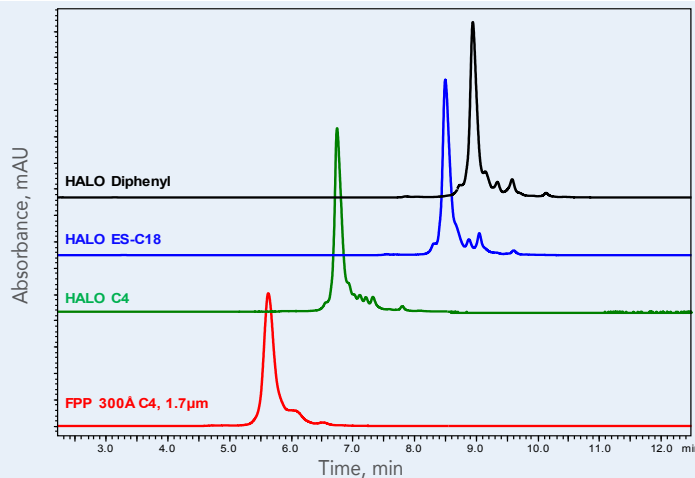
### PEAK IDENTITIES

- |             |             |
|-------------|-------------|
| 1. IgG2-B   | 4. IgG2-A/B |
| 2. IgG2-B   | 5. IgG2-A   |
| 2b. IgG2-B  | 6. IgG2-A*  |
| 3. IgG2-A/B |             |

Comparative results presented here may not be representative for all applications.

## WIDE PORE BONDED PHASE OPTIONS

AMT recognizes mAbs are unique and; therefore, developed three 1000 Å bonded phase options for tailored characterization screening.



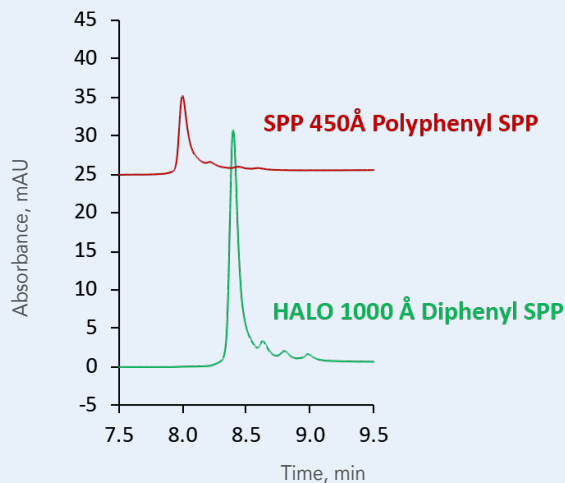
### TEST CONDITIONS:

Columns: as indicated, 2.1 x 150 mm  
Mobile phase A: water/0.1% TFA  
Mobile phase B: ACN/0.1% TFA  
Gradient: 32-40% B in 16 min  
Flow rate: 0.4 mL/min  
Temperature: 80 °C  
Injection volume: 2 μL  
Instrument: Shimadzu Nexera  
Detection: 280 nm, PDA

Comparative results presented here may not be representative for all applications.

## HALO® OUTPERFORMS THE COMPETITION

While many protein separations occur at higher temperatures, the desire to carry them out at lower temperature exists. In this example of the HALO® Diphenyl versus a competitor phenyl phase note the exemplary performance of the HALO® Diphenyl.



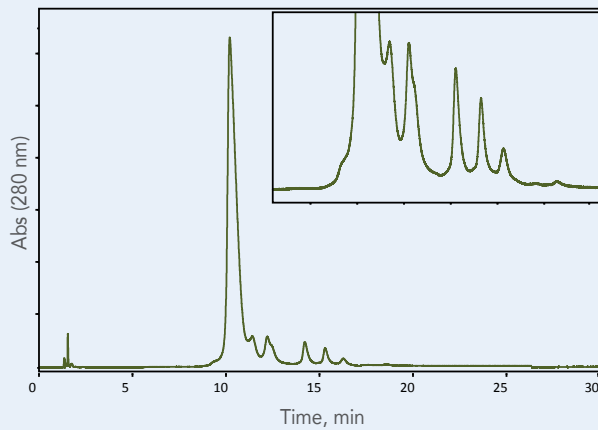
### TEST CONDITIONS:

Columns: 2.1 x 150 mm  
Mobile Phase A: water/0.1% TFA  
Mobile Phase B: ACN/0.1% TFA  
Gradient: 30-45% B in 15 min  
Flow rate: 0.4 mL/min  
Temperature: 40 °C  
Injection volume: 2 μL of 2 mg/mL trastuzumab in water/0.1% TFA  
Detection: 280 nm, PDA

Comparative results presented here may not be representative for all applications.

## OPTIMIZED TRASTUZUMAB SEPARATION USING A HALO 1000 Å DIPHENYL COLUMN

The separation using this highly efficient HALO 1000 Å Protein Diphenyl column is completed in less than 30 minutes, while being compatible with both UV detection, as well as online high resolution MS detection.

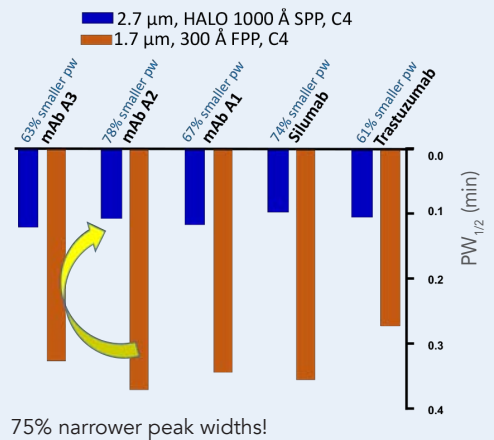


### TEST CONDITIONS:

Mobile phase A: water (0.1% DFA)  
 Mobile phase B: 50/50 ACN/n-propanol/0.1% DFA  
 Gradient: 29–33% B in 29 min  
 Flow rate: 0.25 mL/min  
 Temperature: 60 °C  
 Injection volume: 2 µL of 2mg/mL trastuzumab in water/0.1% TFA  
 Instrument: Shimadzu Nexera  
 Detection: 280 nm, PDA

## IMPROVED PEAK WIDTH ACROSS VARIOUS MABS

The improved performance of ultra wide pores is not just realized with one mAb. Note the advantage to five various large proteins.

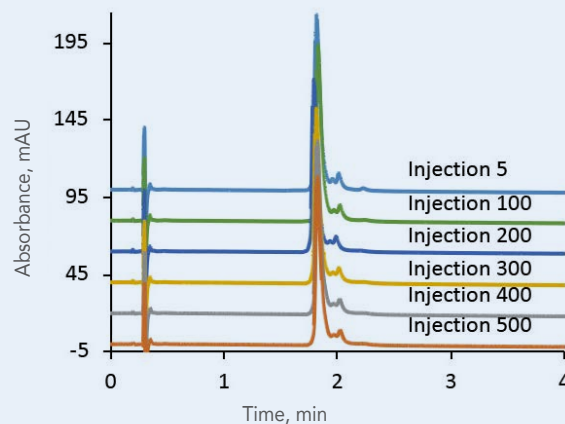


### TEST CONDITIONS:

Columns: 2.1 x 150 mm  
 Mobile Phase A: water/0.1% DFA  
 Mobile Phase B: ACN/0.1% DFA  
 Gradient: 27–37% B in 20 min  
 Flow rate: 0.4 mL/min  
 Temperature: 80 °C  
 Injection Volume: 2 µL (1 µg)  
 Instrument: Shimadzu Nexera  
 Detection: 280 nm, PDA

## RUGGEDNESS AND RELIABILITY

The HALO 1000 Å stationary phases offer rugged and reliable performance every time. In the example of HALO 1000 Å ES-C18, the retention times for trastuzumab show extreme phase stability for over 500 injections.

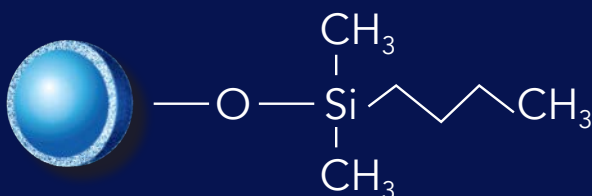


### TEST CONDITIONS:

Columns: HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 50 mm  
 Mobile Phase A: water/ 0.1% TFA  
 Mobile Phase B: ACN/ 0.1% TFA  
 Gradient: 32–60% B in 6 min  
 Flow Rate: 0.4 mL/min  
 Injection Volume: 1.0 µL  
 Temperature: 80 °C  
 Detection: 280 nm, PDA  
 Sample: trastuzumab

## HALO 400 Å PROTEIN BONDED PHASE PORTFOLIO

### HALO 400 Å C4



**Ligand:** DIMETHYLBUTYLSILANE

**USP Designation:** L26

**Available Particle Sizes:** 3.4 μm

**Pore Size:** 400 Å

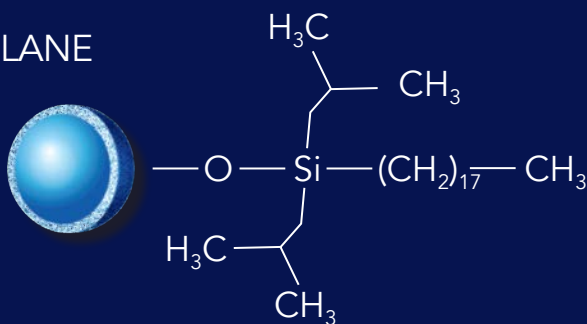
### HALO 400 Å ES-C18

**Ligand:** DIISOBUTYLOCTADECYLSILANE

**USP Designation:** L1

**Available Particle Sizes:** 3.4 μm

**Pore Size:** 400 Å



ANTIBODY-DRUG CON

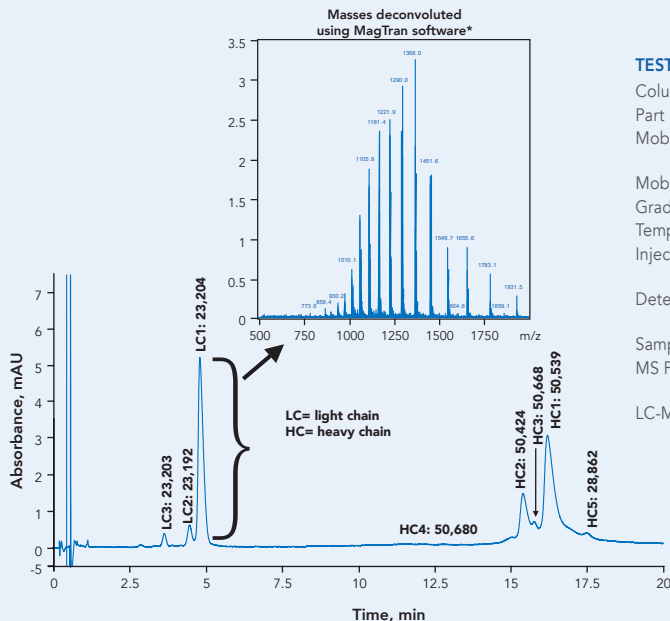
ANTIBODY FRAGMENTS

POLYPEPTIDE



## HIGH RESOLUTION OF LIGHT AND HEAVY CHAIN VARIANTS OF IgG1

Very high resolution is obtained between variants of light and heavy chains of a reduced and alkylated monoclonal antibody (IgG1) sample using a HALO 400 Å Protein C4 column.

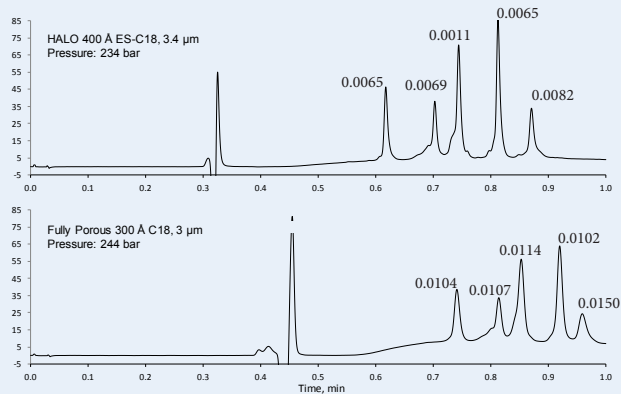


### TEST CONDITIONS:

Columns: HALO 400 Å C4, 3.4 μm, 2.1 x 100 mm  
Part Number: 93412-614  
Mobile Phase A: 0.5% formic acid with 20 mM Ammonium Formate  
Mobile Phase B: 45% ACN/45% IPA/10% A solvent  
Gradient: 29–32% B in 20 min  
Temperature: 80 °C  
Injection Volume: 2 μL of 2 μg/μL reduced and alkylated IgG1  
Detection: 280 nm, UV and MS using 2pps scan rate from 500 to 2000 m/z  
Sample Solvent: 0.25% (v/v) formic acid in water  
MS Parameters: Positive ion mode, ESI at +4.5 kV, 400 °C heatblock, 225 °C capillary  
LC-MS System: Shimadzu Nexera and LCMS-2020 (single quadrupole MS)

## PROTEIN SEPARATIONS: 3.4 μm FUSED-CORE VS. 3 μm FULLY POROUS PARTICLE

Improved peak widths are observed with the HALO 400 Å versus the competitor 300 Å fully porous column.



Comparative results presented here may not be representative for all applications.

### TEST CONDITIONS:

Columns: 4.6 x 100 mm  
Mobile Phase A: water/0.1% TFA  
Mobile Phase B: ACN/0.1% TFA  
Gradient: 23–85% B in 1 min  
3 + 6 μL heat exchangers  
Flow rate: 3 mL/min  
Temperature: 60 °C  
Injection Volume: 5 μL  
Instrument: Agilent 1200 SL  
Detection: 215 nm, PDA

### PEAK IDENTITIES

- |                   |   |
|-------------------|---|
| 1. Ribonuclease A | 13.7 kDa                                |
| 2. Cytochrome c   | 12.4 kDa                                |
| 3. Lysozyme       | 14.3 kDa                                |
| 4. α-Lactalbumin  | 14.2 kDa                                |
| 5. Catalase       | 250 kDa total; tetramer of ~60 kDa each |

JUGATES

BIOSIMILARS

PROTEIN

FRAGMENTS

mAb

## PEPTIDE SOLUTIONS

- HALO<sup>®</sup> BioClass Peptide solutions offer an extensive portfolio of particle sizes and phases to tailor a solution ideal for both ultrafast and ultrahigh resolution separations of peptides and polypeptides up to 20 kDa
- Fast, high resolution, high peak capacity peptide separations at 40-50% the back pressure compared to sub-2  $\mu\text{m}$  particle columns
- Due to the lower back pressure of Fused-Core<sup>®</sup> design, columns can be used in series to maximize peak capacity for UHPLC and HPLC analyses of complex tryptic digest samples
- Compatible for UHPLC and LC-MS applications offering high efficiency and stability
- ~20% higher peak capacity than sub-2  $\mu\text{m}$  non-core columns (2  $\mu\text{m}$ )

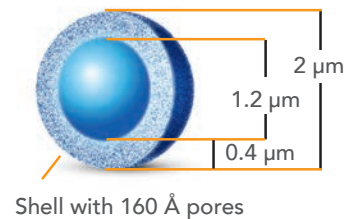
### APPLICATIONS

- Tryptic digests
- Post-Translational Modifications (PTMs)
- Variants
- Polypeptides

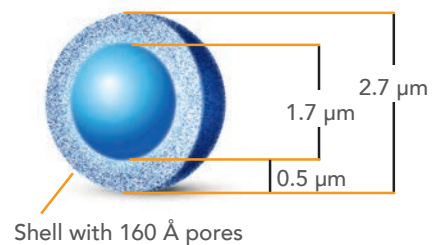
### FEATURES

- Fast separations
- High peak capacity
- Rugged, reliable performance
- Alternative selectivity with ES-C18, ES-CN, and Phenyl-Hexyl

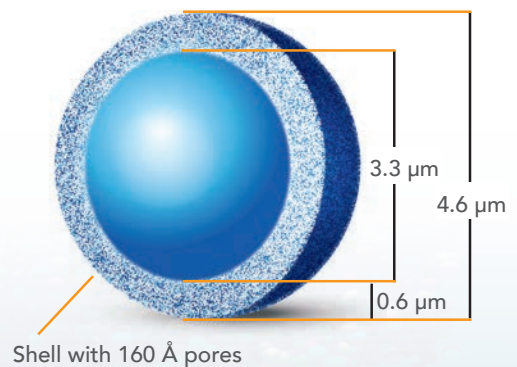
### HALO<sup>®</sup> 2 $\mu\text{m}$ Peptide



### HALO<sup>®</sup> 2.7 $\mu\text{m}$ Peptide



### HALO<sup>®</sup> 5 $\mu\text{m}$ Peptide



POLYPEPTIDES

2  $\mu\text{m}$

ES-C18

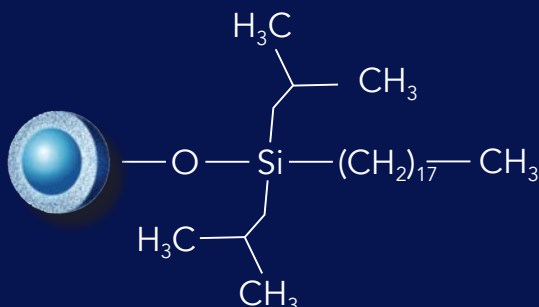
VARIANTS

PTM



## HALO® PEPTIDE BONDED PHASE PORTFOLIO

### HALO 160 Å ES-C18



**Ligand:** DIISOBUTYLOCTADECYLSILANE

**USP Designation:** L1

**Available Particle Sizes:** 2, 2.7, 5 μm

**Pore Size:** 160 Å

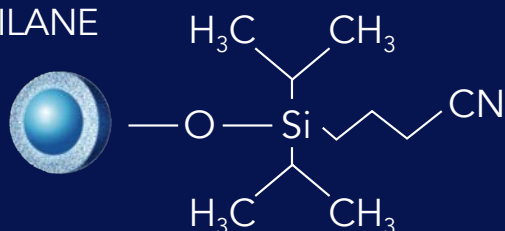
### HALO 160 Å ES-CN

**Ligand:** DIISOPROPYLCYANOPROPYLSILANE

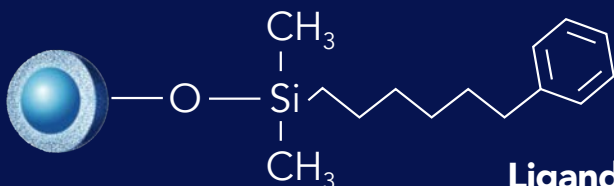
**USP Designation:** L10

**Available Particle Sizes:** 2.7, 5 μm

**Pore Size:** 160 Å



### HALO 160 Å Phenyl-Hexyl



**Ligand:** DIMETHYLPHENYL-HEXYLSILANE

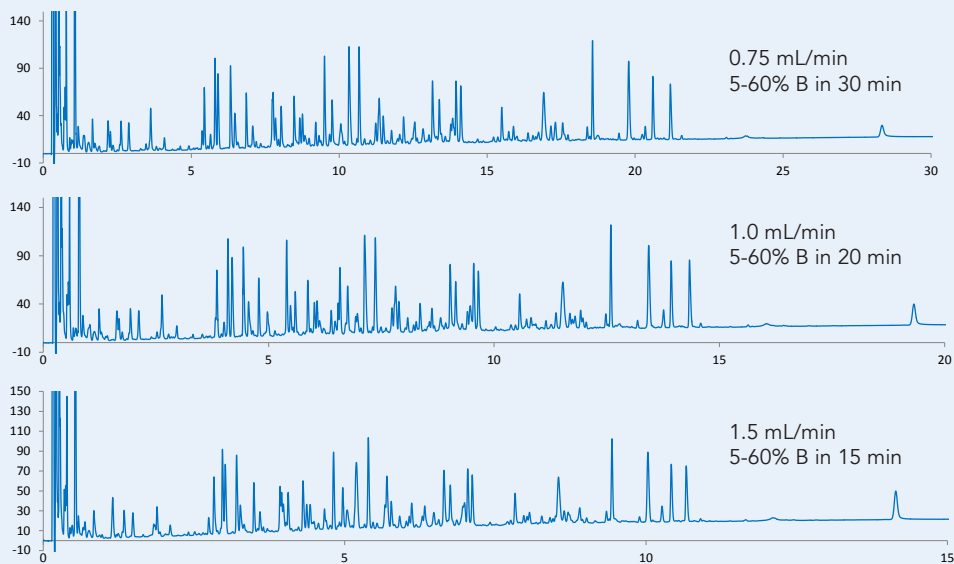
**USP Designation:** L11

**Available Particle Sizes:** 2.7 μm

**Pore Size:** 160 Å

## FAST TRYPTIC DIGEST SEPARATIONS WHILE MAINTAINING RESOLUTION

HALO® Peptide separations can be increased 2-fold while maintaining high resolution due to the Fused Core® particle design.

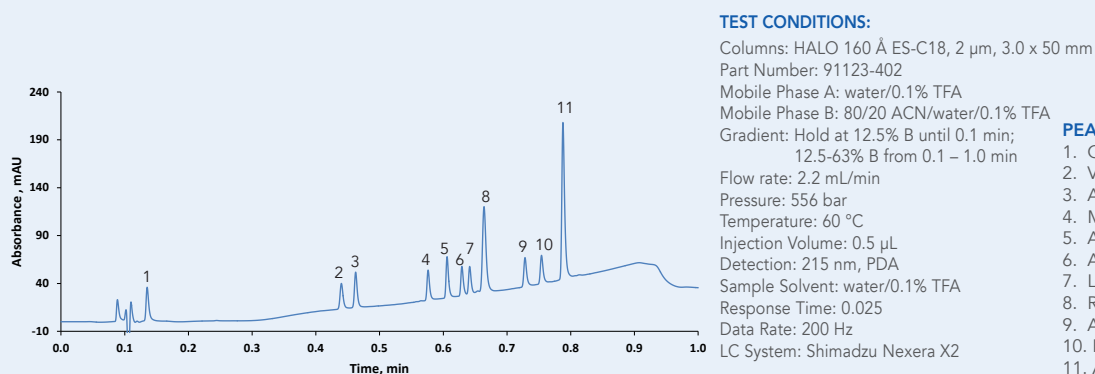


### TEST CONDITIONS:

Columns: 2.1 x 100 mm HALO 160 Å ES-C18, 2.7 μm  
 Mobile Phase A: water/0.1% TFA  
 Mobile Phase B: 80% ACN/0.1% TFA  
 Gradient: as indicated  
 Temperature: 60 °C  
 Injection volume: 15 μL  
 Detection: 215 nm, PDA  
 Sample: apotransferrin tryptic digest

## FAST, HIGH RESOLUTION SEPARATION WITH HALO®

Due to the Fused-Core® design and excellent mass transfer ability, ultra fast peptide separations are achievable with the HALO® Peptide



### TEST CONDITIONS:

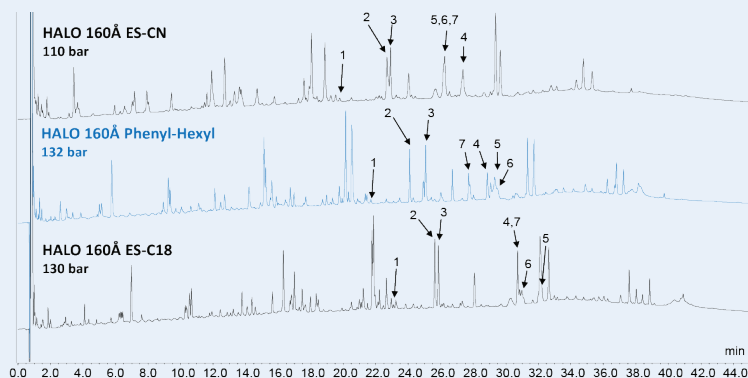
Columns: HALO 160 Å ES-C18, 2 μm, 3.0 x 50 mm  
 Part Number: 91123-402  
 Mobile Phase A: water/0.1% TFA  
 Mobile Phase B: 80/20 ACN/water/0.1% TFA  
 Gradient: Hold at 12.5% B until 0.1 min;  
 12.5-63% B from 0.1 – 1.0 min  
 Flow rate: 2.2 mL/min  
 Pressure: 556 bar  
 Temperature: 60 °C  
 Injection Volume: 0.5 μL  
 Detection: 215 nm, PDA  
 Sample Solvent: water/0.1% TFA  
 Response Time: 0.025  
 Data Rate: 200 Hz  
 LC System: Shimadzu Nexera X2

### PEAK IDENTITIES

1. Gly-Tyr
2. Val-Tyr-Val
3. Angiotensin 1/2 (107) amide
4. Met-enk
5. Angiotensin 1/2 (1-8) amide
6. Angiotensin II
7. Leu-enk
8. Ribonuclease A
9. Angiotensin (1-12) (mouse)
10. Porcine Insulin
11. Angiotensin (1-12) (human)

## ENHANCE SELECTIVITY WITH HALO 160 Å PHENYL-HEXYL FOR A TRYPTIC DIGEST

The HALO 160 Å Phenyl-Hexyl column provides improved resolution between tryptic digest fragments 2 and 3 compared to the 160 Å ES-CN column and the 160 Å ES-C18 column. Peptide identification accomplished by using MS-MS fragmentation spectra.



### PEAK IDENTITIES:

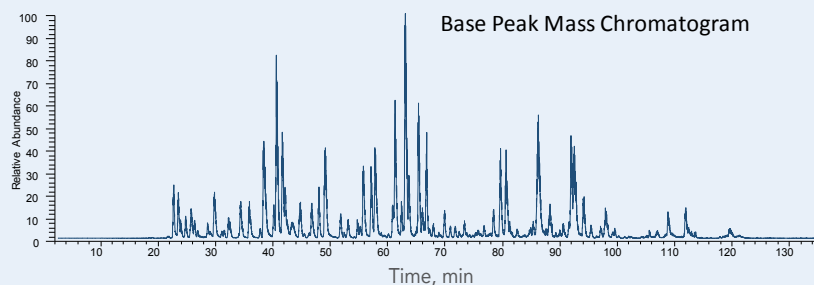
- |                                     |   |
|-------------------------------------|---|
| 1. FTISADTSKNTAYLQMNSLR (754 m/z)   | 4. LLIYSASFLYSGVPSR (592 m/z)             |
| 2. LScAASGFNIKDTYIHWVR (747 m/z)    | 5. SGTASVvcLLNNFYPR (899 m/z)             |
| 3. GFYPSDIAVEWESNGQPENNYK (849 m/z) | 6. ScDKTHtPcPAPELLGGPSVFLFPPKPK (834 m/z) |
|                                     | 7. VVSVLTVLHQDWLNGKEYK (1115 m/z)         |

### TEST CONDITIONS:

Columns: HALO 160 Å ES-CN, 2.7  $\mu$ m, 2.1 x 100 mm  
 Part Number: 92122-604 HALO 160 Å Phenyl-Hexyl, 2.7  $\mu$ m, 2.1 x 100 mm  
 Part Number: 92112-606 HALO 160 Å ES-C18, 2.7  $\mu$ m, 2.1 x 100 mm  
 Part Number: 92122-602  
 Mobile Phase A: water + 10 mM difluoroacetic acid (DFA)  
 Mobile Phase B: ACN + 10 mM difluoroacetic acid  
 Gradient: 2-50% B in 60 min  
 Flow Rate: 0.3 mL/min  
 Temperature: 60 °C  
 Injection Volume: 5  $\mu$ L of 0.2 mg/mL digest  
 Detection: 220 nm, PDA  
 Sample Solvent: 50 mM Tris-HCl/1.5 M Guanidine-HCl with 0.25% formic acid  
 LC System: Shimadzu Nexera  
 Flow Cell: 2.5  $\mu$ L semi-micro

## NANOSCALE HIGH RESOLUTION LC-MS ANALYSIS OF TRASTUZUMAB TRYPTIC DIGEST

In this example, two 0.2 x 250 mm HALO 160 Å ES-C18, 5  $\mu$ m nanoscale columns were connected in series to provide additional resolving power for LC analysis of the tryptic digest of reduced and alkylated trastuzumab.



HALO 160 Å columns are also available in capillary and nano dimensions for proteomics experiments and other sample-limited situations.

### TEST CONDITIONS:

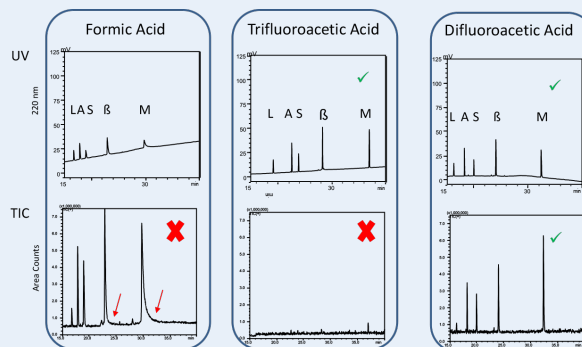
Columns: HALO 160 Å ES-C18, 5  $\mu$ m two 0.2 mm x 250 mm columns in series  
 Mobile Phase A: 0.1% difluoroacetic acid in water  
 Mobile Phase B: 0.1% difluoroacetic acid in ACN  
 Flow Rate: 3  $\mu$ L/min  
 Gradient:

Time (min)	% B
0	5
2	5
137	42.5

Temperature: 60 °C  
 Injection Volume: 5  $\mu$ L  
 Sample: 10  $\mu$ g/ $\mu$ L trastuzumab tryptic peptides (Sample loaded at 2% B at 10  $\mu$ L/min)  
 HPLC: Dionex Ultimate 3000  
 Mass Spectrometer: Thermo Orbitrap Velos Pro™ Hybrid Ion Trap-Orbitrap

## BENEFITS OF DFA

Switching to Difluoroacetic Acid (DFA), a less fluorinated ion pairing acid mobile phase modifier provides MS sensitivity improvement relative to TFA, particularly with small to mid size molecules. DFA has the practical advantage of similar chromatographic benefits of TFA, (including excellent peak shape and recovery), along with easier removal from instrument components. DFA can be easily removed in 10-15 minutes with 50:50 ACN/water.



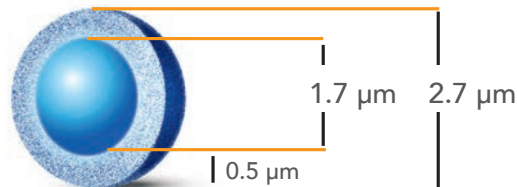
L = leucine enkephalin, A = angiotensin I, S = substance P,  $\beta$  =  $\beta$ -endorphin, M = melittin

## GLYCAN SOLUTIONS

The HALO<sup>®</sup> Glycan incorporates a highly polar ligand that contains 5 hydroxyl groups tethered to 2.7  $\mu\text{m}$  Fused-Core<sup>®</sup> silica particles via novel, proprietary linkage chemistry resulting in a high-resolution separation of complex glycan mixtures.

- Improved retention of acidic and zwitterionic analytes
- Ideal for hydrophilic interaction liquid chromatography (HILIC) separations of oligosaccharides, and particularly, of released and labeled glycans from glycoproteins and proteoglycans
- Each lot of HALO<sup>®</sup> Glycan material is tested for quality assurance by separation of a procainamide-reducing-end-labeled glycan ladder of oligosaccharides having 2–25 glucose units (GU)
  - Peaks for oligosaccharides composed of 5 and 10 GU must meet tight specifications for retention and peak width before lot is approved for glycan analysis

### HALO<sup>®</sup> Glycan



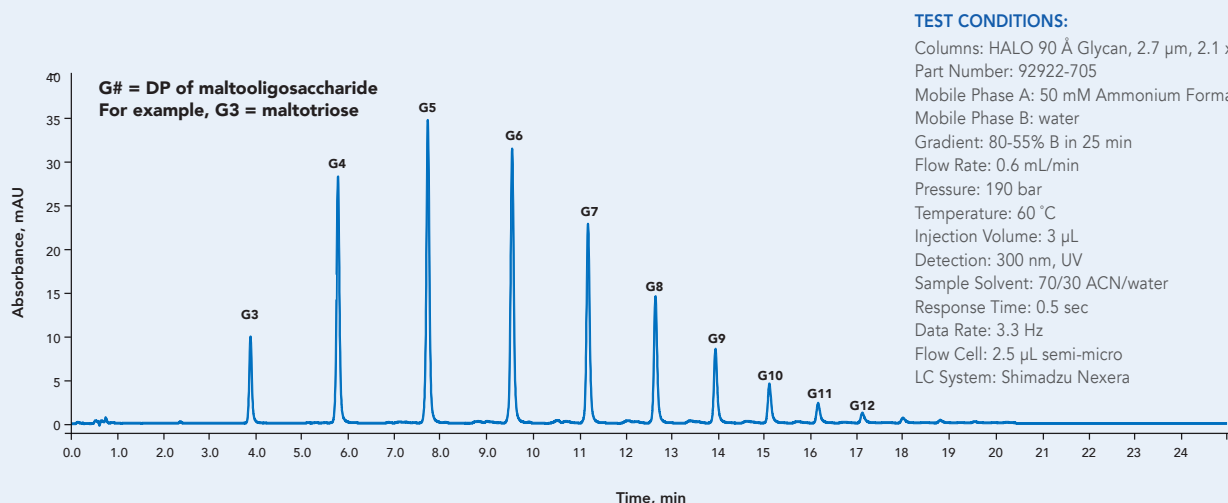
Shell with 90 Å pores

### HALO 90 Å GLYCAN

**Ligand:** PROPRIETARY POLY-HYDROXY  
**USP Designation:** L95  
**Available Particle Sizes:** 2.7  $\mu\text{m}$   
**Pore Size:** 90 Å

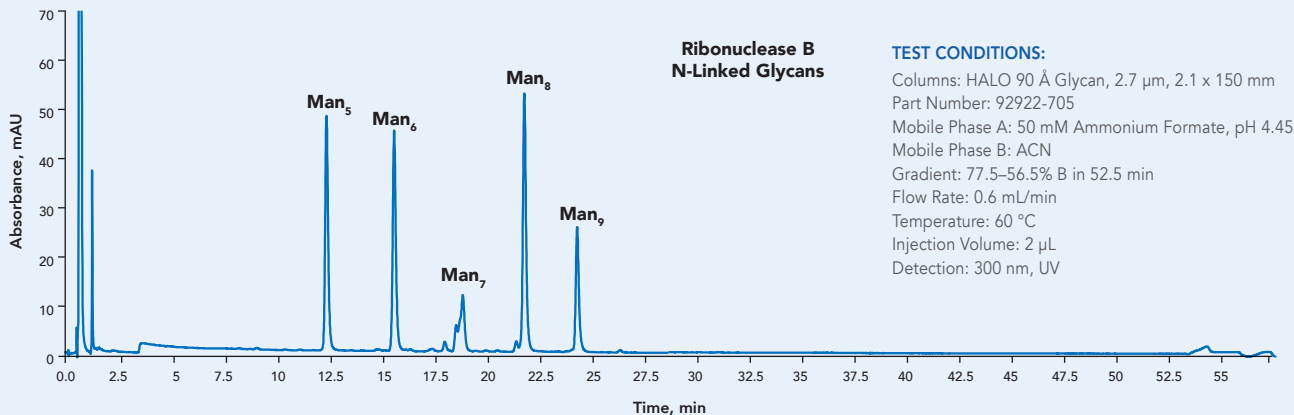
### QA ANALYSIS OF HALO<sup>®</sup> GLYCAN

Example QA Chromatogram for HALO<sup>®</sup> Glycan column. Each HALO<sup>®</sup> Glycan packing lot is tested using this glycan ladder mixture to assess and ensure lot-to-lot reproducibility.



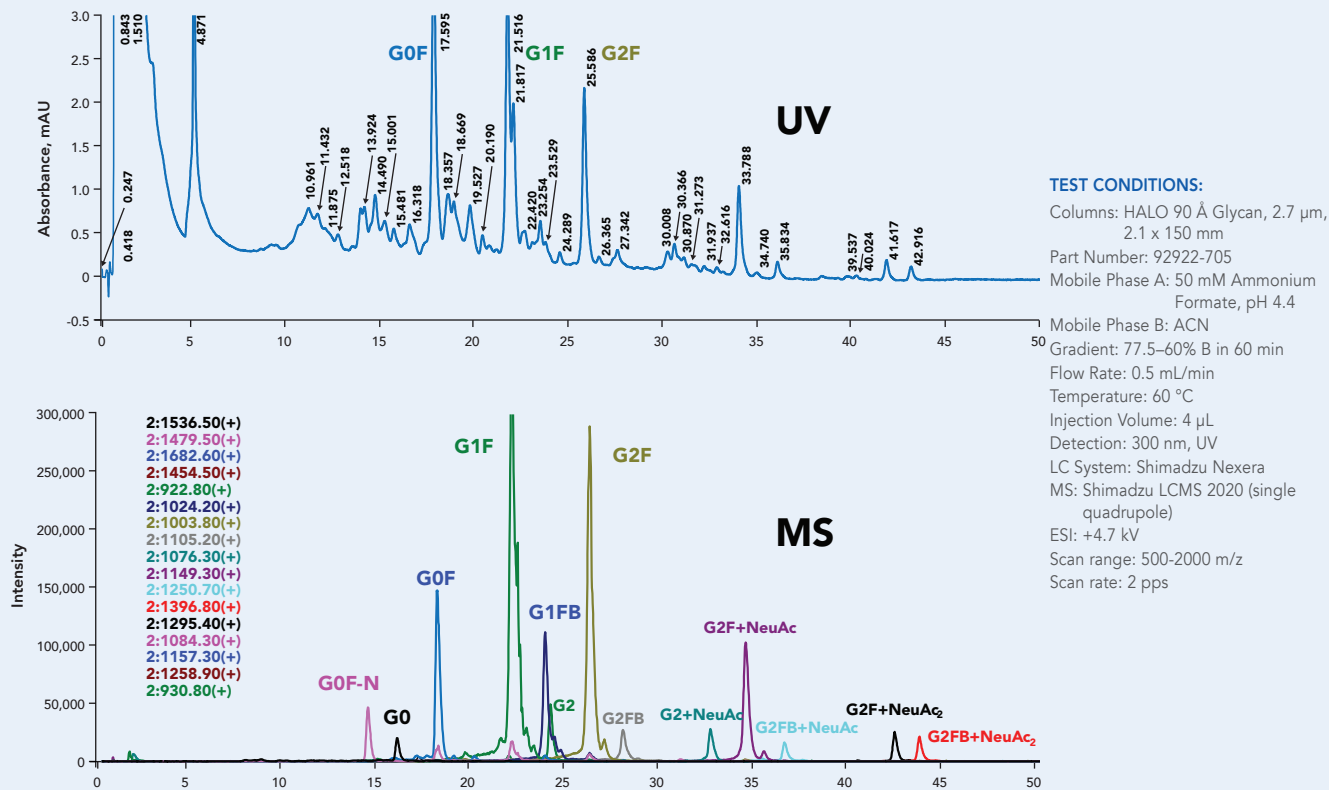
## SEPARATION OF N-LINKED GLYCANS FROM RIBONUCLEASE B

Gradient HILIC-MS separation of N-linked glycans, which had been released using PNGase from ribonuclease B, using the HALO<sup>®</sup> Glycan column.



## SEPARATION OF N-LINKED GLYCANS FROM HUMAN IgG

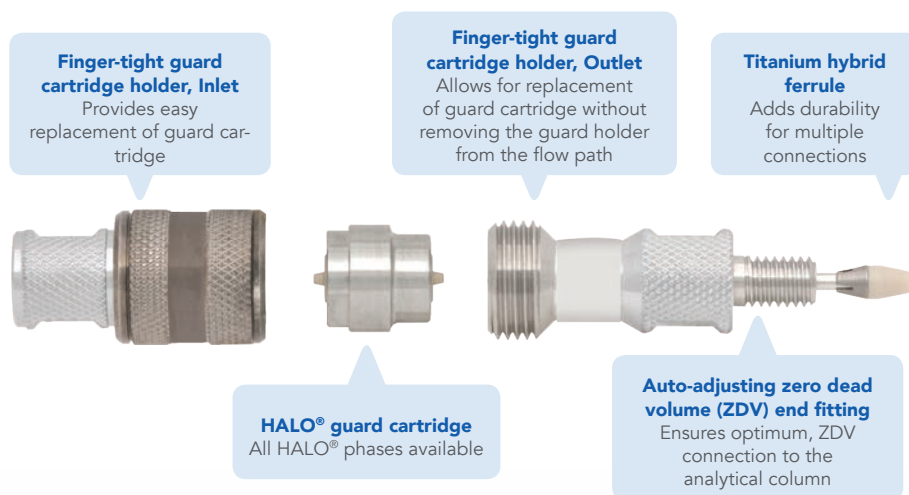
Released- and procainamide-labeled glycans from human IgG were separated using a 2.1 x 150 mm HALO<sup>®</sup> Glycan column and detected using UV and selected-ion-monitoring MS detection.



## HALO<sup>®</sup> UHPLC AND HPLC GUARD COLUMNS

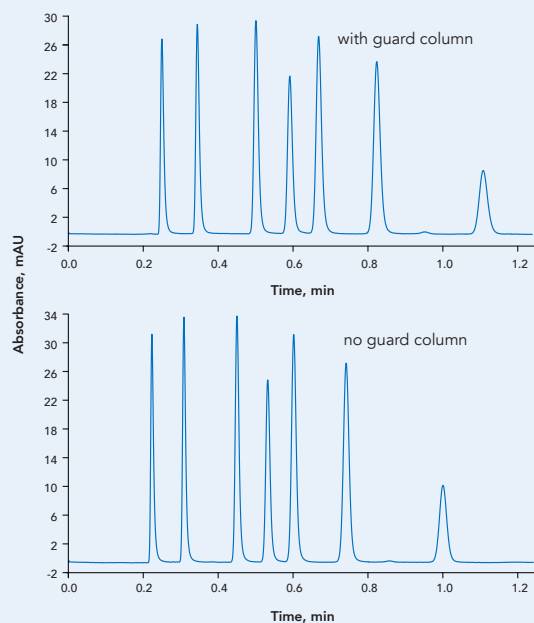
- Collect strongly retained compounds from the sample and minimizes column fouling
- Ultra-low dispersion, easy to use, operate at pressures up to 1000 bar
- Finger-tight, direct-connect units that auto-adjust to any column with a 10–32 inlet port
- Easily replace guard cartridge without removing guard holder from the flow path
- Available for all HALO<sup>®</sup> analytical geometries (2.1, 3.0 and 4.6 mm ID) and phases

See below for an exploded view of the HALO<sup>®</sup> guard cartridge and guard holder. Please see pages 18-19 for ordering information.



### HALO<sup>®</sup> GUARD COLUMNS: PROTECTION + PERFORMANCE

HALO<sup>®</sup> guard columns provide optimum protection for your HALO<sup>®</sup> HPLC and UHPLC column without sacrificing column efficiency.



#### TEST CONDITIONS:

Columns: HALO 90 Å C18, 2.7 μm, 4.6 x 50 mm  
 Mobile Phase: 60/40 ACN/water  
 Flow Rate: 1.8 mL/min  
 Temperature: 30 °C  
 Injection Volume: 1 μL  
 Detection: 254 nm, UV  
 Pressure: 158 bar with guard column  
 146 bar without guard column  
 Instrument: Optimized Agilent 1100  
 bypassed semi-micro flow cell  
 0.05" ID tubing  
 14 Hz data rate

The Optimize Technologies EXP<sup>®</sup> Direct Connect Holder: U.S. Patent No. 8,201,854 & 8,696,902 and Foreign Patents Pending.



# HALO® BIOCLASS SPECIFICATIONS TABLES

## PROTEIN SPECIFICATIONS

Bonded Phase	Pore Size (Å)	Particle Sizes (s) (µm)	USP Designation	Carbon Load (%)	Surface Area (m <sup>2</sup> /g)	Low pH/T Limit	High pH/T Limit	Endcapped
C4	1000	2.7	L26	0.6	22	2/90 °C	9/40 °C	Yes
	400	3.4		0.4	15			
ES-C18	1000	2.7	L1	1.4	22	1/90 °C	8/40 °C	Yes
	400	3.4		1.0	15			
Diphenyl	1000	2.7	L11	1.0	22	2/90 °C	9/40 °C	Yes

## PEPTIDE SPECIFICATIONS

Bonded Phase	Pore Size (Å)	Particle Sizes (s) (µm)	USP Designation	Carbon Load (%)	Surface Area (m <sup>2</sup> /g)	Low pH/T Limit	High pH/T Limit	Endcapped
ES-C18	160	2	L1	4.0	65	1/90 °C	8/40 °C	No
		2.7		4.6	90			
		5		4.0	60			
ES-CN	160	2.7	L10	2.2	90	1/90 °C	8/40 °C	Yes
		5		1.5	60			
Phenyl-Hexyl	160	2.7	L11	4.7	90	2/90 °C	9/40 °C	Yes

## GLYCAN SPECIFICATIONS

Bonded Phase	Pore Size (Å)	Particle Sizes (s) (µm)	USP Designation	Carbon Load (%)	Surface Area (m <sup>2</sup> /g)	Low pH/T Limit	High pH/T Limit	Endcapped
Proprietary Poly-Hydroxy	90	2.7	L95	3.2	135	2/65 °C	9/40 °C	No

## HALO 1000 Å AND 400 Å PROTEIN COLUMNS

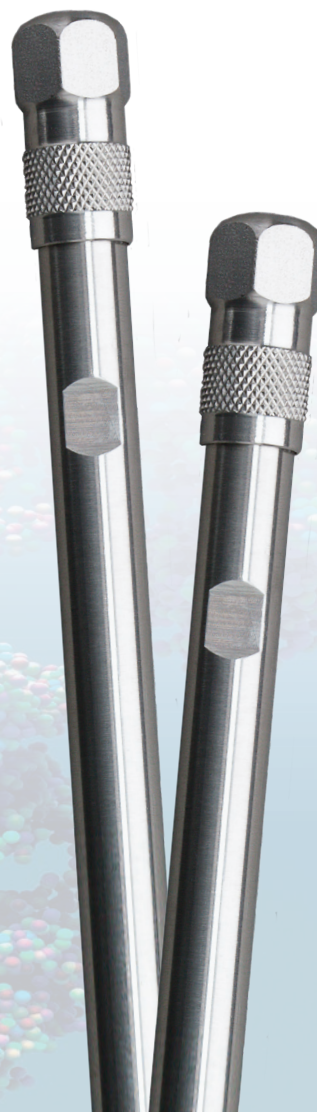
Part numbers for nano, capillary, analytical and semi-preparative HALO 1000 Å and 400 Å in 2.7 and 3.4 µm phases are provided below. Guard columns are available in 2.1, 3.0 and 4.6 mm IDs for UHPLC and HPLC applications to provide additional column protection when desired.

Dimensions ID x Length (in mm)	400 Å, 3.4 µm		1000 Å, 2.7 µm		
	C4	ES-C18	C4	ES-C18	Diphenyl
0.075 x 50	94319-414	94319-402	97219-414	97219-402	97219-426
0.075 x 100	94319-614	94319-602	97219-614	97219-602	97219-626
0.075 x 150	94319-714	94319-702	97219-714	97219-702	97219-726
0.1 x 50	94318-414	94318-402	97218-414	97218-402	97218-426
0.1 x 100	94318-614	94318-602	97218-614	97218-602	97218-626
0.1 x 150	94318-714	94318-702	97218-714	97218-702	97218-726
0.2 x 50	94317-414	94317-402	97217-414	97217-402	97217-426
0.2 x 100	94317-614	94317-602	97217-614	97217-602	97217-626
0.2 x 150	94317-714	94317-702	97217-714	97217-702	97217-726
0.3 x 50	94316-414	94316-402	97216-414	97216-402	97216-426
0.3 x 100	94316-614	94316-602	97216-614	97216-602	97216-626
0.3 x 150	94316-714	94316-702	97216-714	97216-702	97216-726
0.5 x 50	94315-414	94315-402	97215-414	97215-402	97215-426
0.5 x 100	94315-614	94315-602	97215-614	97215-602	97215-626
0.5 x 150	94315-714	94315-702	97215-714	97215-702	97215-726
1.0 x 30	93411-314	93411-302	92711-314	92711-302	92711-326
1.0 x 50	93411-414	93411-402	92711-414	92711-402	92711-426
1.0 x 75	93411-514	93411-502	92711-514	92711-502	92711-526
1.0 x 100	93411-614	93411-602	92711-614	92711-602	92711-626
1.0 x 150	93411-714	93411-702	92711-714	92711-702	92711-726
2.1 x 20	93412-214	93412-202	92712-214	92712-202	92712-226
2.1 x 30	93412-314	93412-302	92712-314	92712-302	92712-326
2.1 x 50	93412-414	93412-402	92712-414	92712-402	92712-426
2.1 x 75	93412-514	93412-502	92712-514	92712-502	92712-526
2.1 x 100	93412-614	93412-602	92712-614	92712-602	92712-626
2.1 x 150	93412-714	93412-702	92712-714	92712-702	92712-726
2.1 x 250	93412-914	93412-902	92712-914	92712-902	92712-926
3.0 x 20	93413-214	93413-202	92713-214	92713-202	92713-226
3.0 x 30	93413-314	93413-302	92713-314	92713-302	92713-326
3.0 x 50	93413-414	93413-402	92713-414	92713-402	92713-426
3.0 x 75	93413-514	93413-502	92713-514	92713-502	92713-526
3.0 x 100	93413-614	93413-602	92713-614	92713-602	92713-626
3.0 x 150	93413-714	93413-702	92713-714	92713-702	92713-726
3.0 x 250	93413-914	93413-902	92713-914	92713-902	92713-926
4.6 x 20	93414-214	93414-202	92714-214	92714-202	92714-226
4.6 x 30	93414-314	93414-302	92714-314	92714-302	92714-326
4.6 x 50	93414-414	93414-402	92714-414	92714-402	92714-426
4.6 x 75	93414-514	93414-502	92714-514	92714-502	92714-526
4.6 x 100	93414-614	93414-602	92714-614	92714-602	92714-626
4.6 x 150	93414-714	93414-702	92714-714	92714-702	92714-726
4.6 x 250	93414-914	93414-902	92714-914	92714-902	92714-926
10.0 x 50	93410-414	93410-402	92710-414	92710-402	92710-426
10.0 x 75	93410-514	93410-502	92710-514	92710-502	92710-526
10.0 x 100	93410-614	93410-602	92710-614	92710-602	92710-626
10.0 x 150	93410-714	93410-702	92710-714	92710-702	92710-726
<b>Guard Columns, 3-Pack</b>					
Dimensions ID x Length (in mm)	C4	ES-C18	C4	ES-C18	Diphenyl
2.1 x 5	93412-114	93412-102	92712-114	92712-102	92712-126
3.0 x 5	93413-114	93413-102	92713-114	92713-102	92713-126
4.6 x 5	93414-114	93414-102	92714-114	92714-102	92714-126
<b>Guard Column Holder</b> 94900-001					

## HALO 90 Å GLYCAN COLUMNS

HALO® Glycan columns are available in 2.1 and 4.6 mm diameters in the following lengths as a 2.7 µm particle size. Guard columns are available for UHPLC and HPLC applications if additional protection is desired.

Dimensions ID x Length (in mm)	HALO Glycan
2.1 x 50	92922-405
2.1 x 100	92922-605
2.1 x 150	92922-705
4.6 x 50	92924-405
4.6 x 100	92924-605
4.6 x 150	92924-705
<b>Guard Columns, 3-Pack</b>	
Dimensions ID x Length (in mm)	HALO Glycan
2.1 x 5	92922-105
4.6 x 5	92924-105
<b>Guard Column Holder</b> 94900-001	



## HALO 160 Å PEPTIDE COLUMNS

The part numbers are provided below for the nano, capillary, analytical and semi-preparative HALO 160 Å 2, 2.7 and 5 µm phases. Guard columns are available for 2.1, 3.0 and 4.6 mm internal diameters for UHPLC and HPLC applications, if additional protection is desired.

Dimensions ID x Length (in mm)	160 Å, 2 µm		160 Å, 2.7 µm			160 Å, 5 µm	
	ES-C18	ES-C18	ES-CN	Phenyl-Hexyl	ES-C18	ES-CN	
0.075 x 50	-	91229-402	91229-404	91229-406	91529-402	91529-404	
0.075 x 100	-	91229-602	91229-604	91229-606	91529-602	91529-604	
0.075 x 150	-	91229-702	91229-704	91229-706	91529-702	91529-704	
0.1 x 50	-	91228-402	91228-404	91228-406	91528-402	91528-404	
0.1 x 100	-	91228-602	91228-604	91228-606	91528-602	91528-604	
0.1 x 150	-	91228-702	91228-704	91228-706	91528-702	91528-704	
0.2 x 50	-	91227-402	91227-404	91227-406	91527-402	91527-404	
0.2 x 100	-	91227-602	91227-604	91227-606	91527-602	91527-604	
0.2 x 150	-	91227-702	91227-704	91227-706	91527-702	91527-704	
0.3 x 50	-	91226-402	91226-404	91226-406	91526-402	91526-404	
0.3 x 100	-	91226-602	91226-604	91226-606	91526-602	91526-604	
0.3 x 150	-	91226-702	91226-704	91226-706	91526-702	91526-704	
0.5 x 50	-	91225-402	91225-404	91225-406	91525-402	91525-404	
0.5 x 100	-	91225-602	91225-604	91225-606	91525-602	91525-604	
0.5 x 150	-	91225-702	91225-704	91225-706	91525-702	91525-704	
1.0 x 30	-	92121-302	92121-304	92121-306	95121-302	95121-304	
1.0 x 50	-	92121-402	92121-404	92121-406	95121-402	95121-404	
1.0 x 75	-	92121-502	92121-504	92121-506	95121-502	95121-504	
1.0 x 100	-	92121-602	92121-604	92121-606	95121-602	95121-604	
1.0 x 150	-	92121-702	92121-704	92121-706	95121-702	95121-704	
2.1 x 20	91122-202	92122-202	92122-204	92122-206	95122-202	95122-204	
2.1 x 30	91122-302	92122-302	92122-304	92122-306	95122-302	95122-304	
2.1 x 50	91122-402	92122-402	92122-404	92122-406	95122-402	95122-404	
2.1 x 75	91122-502	92122-502	92122-504	92122-506	95122-502	95122-504	
2.1 x 100	91122-602	92122-602	92122-604	92122-606	95122-602	95122-604	
2.1 x 150	91122-702	92122-702	92122-704	92122-706	95122-702	95122-704	
2.1 x 250	91122-902	92122-902	92122-904	92122-906	95122-902	95122-904	
3.0 x 20	91123-202	92123-202	92123-204	92123-206	95123-202	95123-204	
3.0 x 30	91123-302	92123-302	92123-304	92123-306	95123-302	95123-304	
3.0 x 50	91123-402	92123-402	92123-404	92123-406	95123-402	95123-404	
3.0 x 75	91123-502	92123-502	92123-504	92123-506	95123-502	95123-504	
3.0 x 100	91123-602	92123-602	92123-604	92123-606	95123-602	95123-604	
3.0 x 150	91123-702	92123-702	92123-704	92123-706	95123-702	95123-704	
3.0 x 250	91123-902	92123-902	92123-904	92123-906	95123-902	95123-904	
4.6 x 20	-	92124-202	92124-204	92124-206	95124-202	95124-204	
4.6 x 30	-	92124-302	92124-304	92124-306	95124-302	95124-304	
4.6 x 50	-	92124-402	92124-404	92124-406	95124-402	95124-404	
4.6 x 75	-	92124-502	92124-504	92124-506	95124-502	95124-504	
4.6 x 100	-	92124-602	92124-604	92124-606	95124-602	95124-604	
4.6 x 150	-	92124-702	92124-704	92124-706	95124-702	95124-704	
4.6 x 250	-	92124-902	92124-904	92124-906	95124-902	95124-904	
10.0 x 50	-	92120-402	92120-404	92120-406	95120-402	95120-404	
10.0 x 75	-	92120-502	92120-504	92120-506	95120-502	95120-504	
10.0 x 100	-	92120-602	92120-604	92120-606	95120-602	95120-604	
10.0 x 150	-	92120-702	92120-704	92120-706	95120-702	95120-704	
10.0 x 250	-	-	-	-	95120-902	95120-904	
<b>Guard Columns, 3-pack</b>							
Dimensions ID x Length (in mm)	ES-C18	ES-C18	ES-CN	Phenyl-Hexyl	ES-C18	ES-CN	
2.1 x 5	91122-102	92122-102	92122-104	92122-106	95122-102	95122-104	
3.0 x 5	91123-102	92123-102	92123-104	92123-106	95123-102	95123-104	
4.6 x 5	-	92124-102	92124-104	92124-106	95124-102	95124-104	
<b>Guard Column Holder</b> 94900-001							

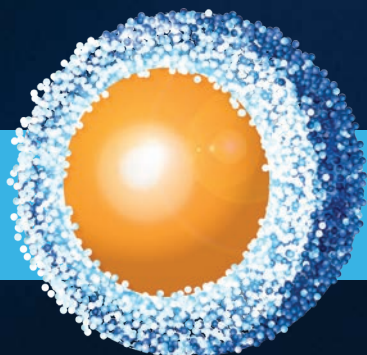
# HALO®



**infochroma ag**



Official dealer for Switzerland



**advancedmaterialstechnology**

[info@advanced-materials-tech.com](mailto:info@advanced-materials-tech.com)  
[www.advanced-materials-tech.com](http://www.advanced-materials-tech.com)

**fused-core.com**