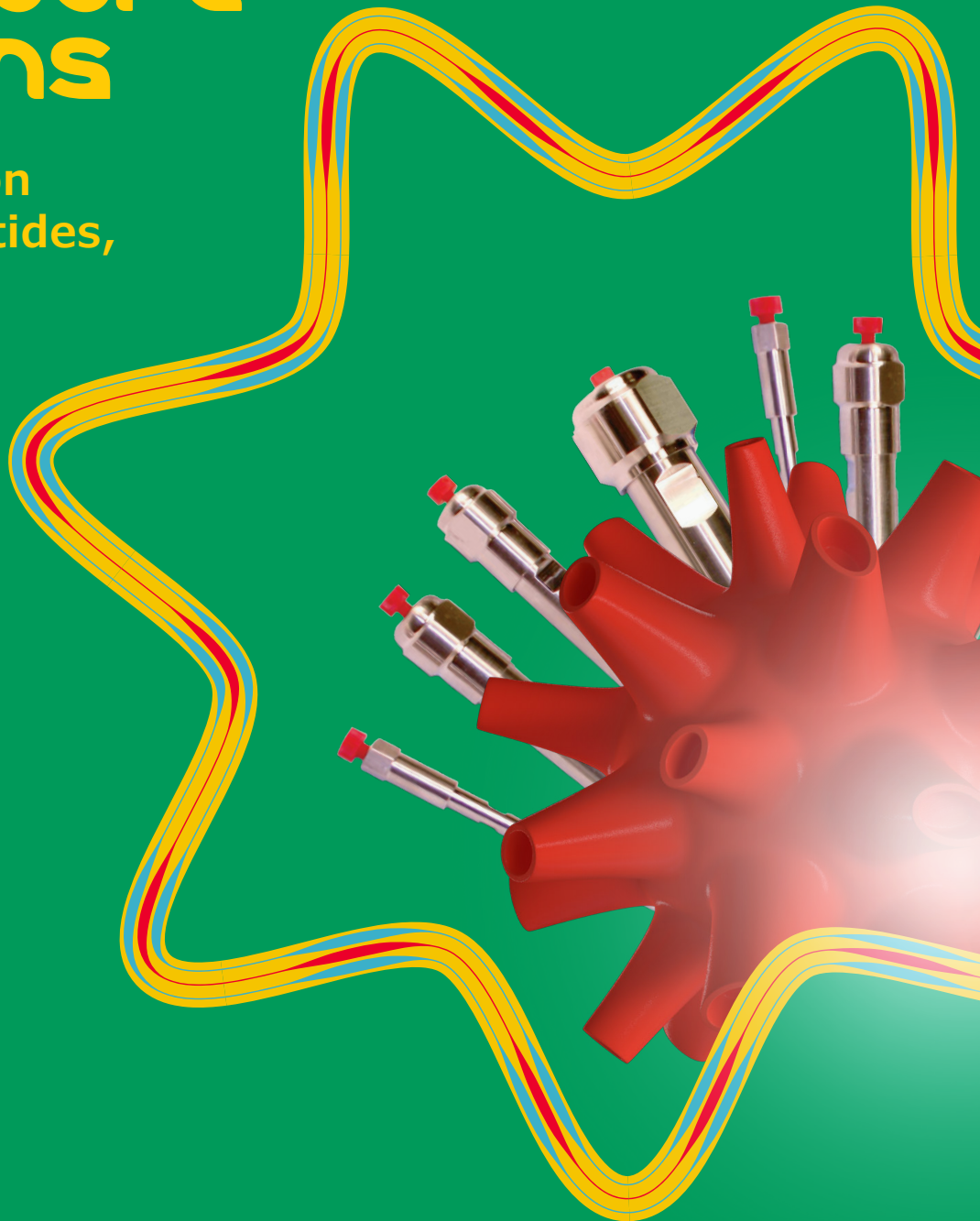


Supelco

Quality Control Products

BioshellTM Fused-Core[®] Columns

Faster Separation
of Proteins, Peptides,
and Glycans



The life science business of Merck KGaA,
Darmstadt, Germany operates as
MilliporeSigma in the U.S. and Canada.

**MILLIPORE
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The Life Science business of Merck KGaA, Darmstadt, Germany, which operated as MilliporeSigma in the U.S. and Canada, unites the innovative products, world-class services, and exceptional talents of EMD Millipore and Sigma-Aldrich to create a global leader in the life science industry.

Supelco®

In the complex world of analytical chemistry, a dependable resource is key to covering the vast array of techniques, instrumentation, devices and consumables involved.




This is why we have created the Supelco® Portfolio of Analytical Products, one of the broadest portfolios in the industry.

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Life Science – View at a Glance



Our core strengths:

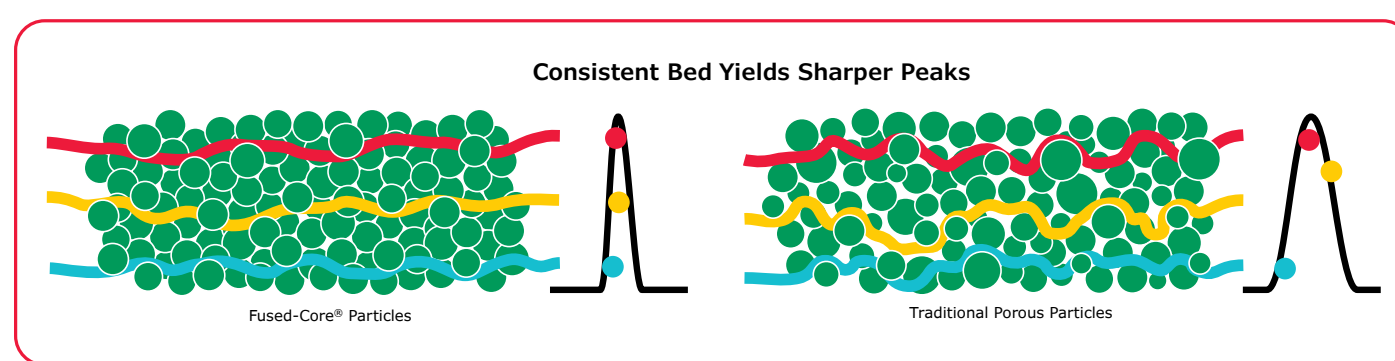
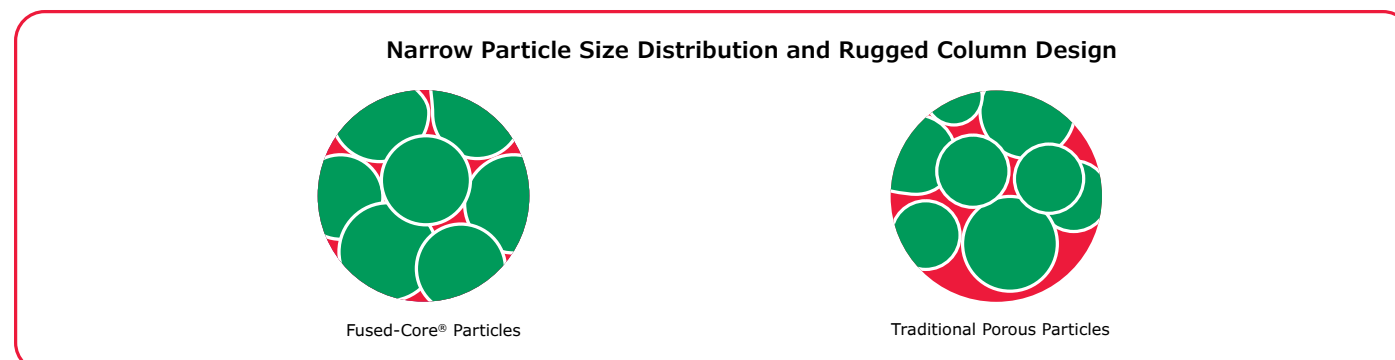
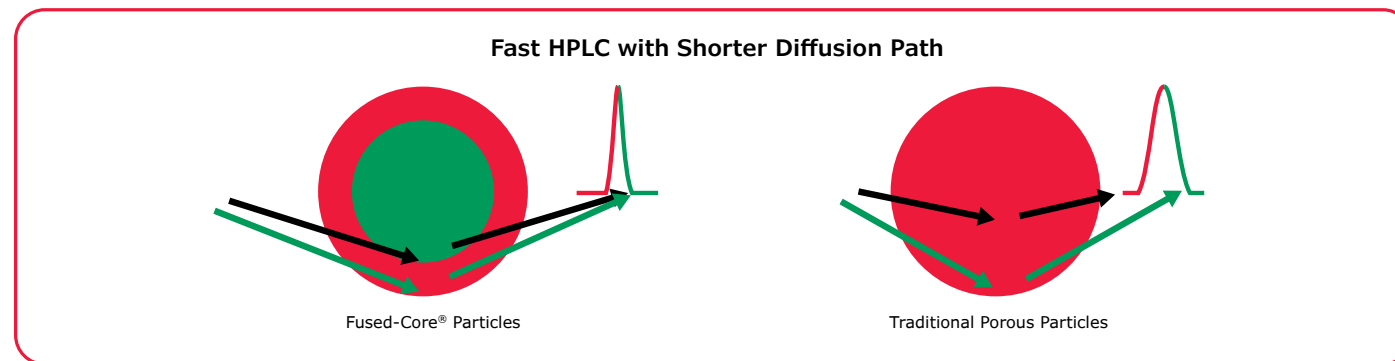
-  Comprehensive portfolio and customer-focused innovation
-  Balanced geographic reach
-  Well-differentiated and industry-leading capabilities

*€5.4B in pro forma sales, including Sigma-Aldrich, in 2015

(This net sales calculation would have resulted had the first-time consolidation of Sigma-Aldrich already taken place on January 1, 2015).

Faster Separation

Using BIOshell™ Fused-Core® Technology



Testimonial

“The partially porous stationary phase material has demonstrated equivalent resolving power to sub-2 μm materials under the ballistic gradient chromatography conditions employed, and shown to exhibit excellent resilience and performance over the analysis of thousands of protein precipitated plasma extracts, suggesting that this type of column is a valuable tool for pharmaceutical bioanalysts.”

D.N. Mallett, C. Ramírez-Molina / Journal of Pharmaceutical and Biomedical Analysis (2009) 100-107

BIOshell™ Fused-Core® Columns

Faster Separation of Proteins, Peptides, and Glycans

BIOshell™ columns are the most recent innovation in Fused-Core® particle technology: high efficiency U/HPLC columns for protein, peptide, and glycan separations. BIOshell™ columns can be operated in HPLC or UHPLC instrumentation equipped with a mass spectrometer or any other detector.

The complete BIOshell™ column lineup includes:

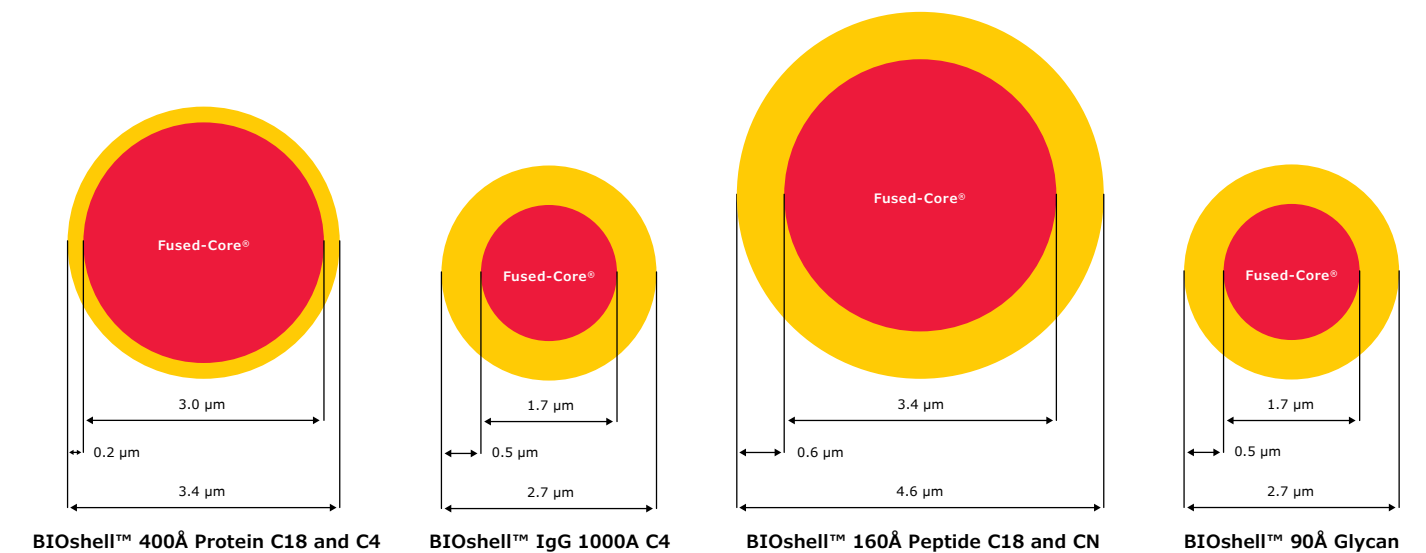
- BIOshell™ A160 Peptide
- BIOshell™ A400 Protein
- BIOshell™ A90 Glycan
- BIOshell™ A1000 IgG

Particle Characteristics

BIOshell™ Columns	Particle Size (μm)	Core Size (μm)	Shell Thickness (μm)	Pore Size (Å)	SBET (m ² /g)	Capacity vs. Porous*
2.7 μm A90 Glycan	2.7	1.7	0.5	90	135	75%
2.7 μm A160 Peptide C18	2.7	1.7	0.5	160	80	75%
2.7 μm A160 Peptide CN	2.7	1.7	0.5	160	80	75%
5 μm A160 Peptide C18	4.6†	3.5	0.6	160	80	59%
5 μm A160 Peptide CN	4.6†	3.5	0.6	160	80	59%
3.4 μm A400 Protein C18	3.4	3.0	0.2	400	15	31%
3.4 μm A400 Protein C4	3.4	3.0	0.2	400	15	31%
2.7 μm A1000 IgG C4	2.7	TBC	0.5	1000	22	TBC

* Calculated capacity based on value of core diameter and shell thickness
 † Nominal particle size is 5 micron

BIOshell™ Fused-Core® Particles



BIOshell™ Protein, Peptide and Glycan columns are packed with Fused-Core® particles that have been specifically engineered for fast, high-resolution separation of biomolecules.

Bonded Phase and Operational Characteristics of BIOshell™ Fused-Core® Columns

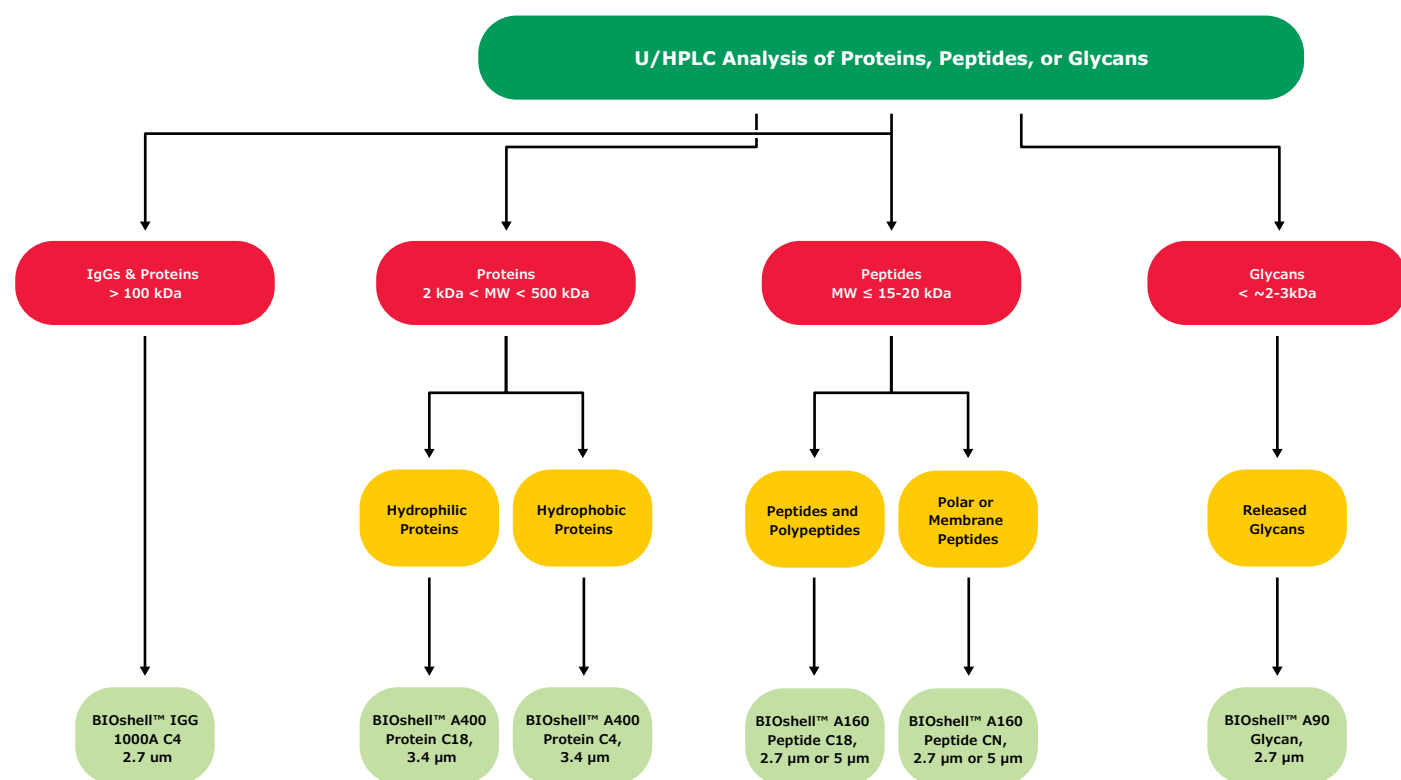
BIOshell™ Columns	Bonded Phase Ligand	End Cap	Max. Temp. (°C)*	pH Range	Pmax (bar)	Frit Porosity (µm)
2.7 µm A90 Glycan	proprietary penta-hydroxysilane	No	65	2–9	1000	2
2.7 µm A160 Peptide C18	di-isobutyl-octadecylsilane	No	100	1–8	600	2
2.7 µm A160 Peptide CN	di-isopropyl-cyanopropylsilane	Yes	90	1–8	600	2
5 µm A160 Peptide C18	di-isobutyl-octadecylsilane	No	100	1–8	600	2
5 µm A160 Peptide CN	di-isopropyl-cyanopropylsilane	Yes	90	1–8	600	2
3.4 µm A400 Protein C18	di-isobutyl-octadecylsilane	Yes	90	1–9	600	2
3.4 µm A400 Protein C4	di-methyl-butylsilane	Yes	90	1–9	600	2

* Temperature at which each bonded phase type was tested for long-term physical and chemical stability.

Features and Benefits

BIOshell™ Protein, Peptide and Glycan columns are packed with Fused-Core® particles that have been specifically engineered for fast, high-resolution separation of biomolecules. These columns offer a tremendous advantage over conventional column formats by providing increased resolution, faster separations, and lower back pressure. In addition, BIOshell™ columns offer a level of ruggedness that is not often associated with columns exhibiting such a high level of performance. Some of the benefits realized when using a BIOshell™ Fused-Core® column are as follows:

- 40% higher efficiency than fully porous particles of the same size
- Heightened bonded phase stability
- High temperature stability enabling increased throughput and improved peak shape
- Operation at high flow rates
- Decreased column pluggage
- Compatibility with UHPLC as well as conventional HPLC systems



Applications

Selected BIOshell™ Applications

The examples shown here demonstrate that BIOshell™ Fused-Core® columns provide very high efficiency separations for peptide, protein, and glycan samples that are routinely analyzed in academic, biopharmaceutical, clinical and diagnostic laboratories. These new column types provide the scientist with the option of either using the extra efficiency to reduce analysis time or obtain better quality data by separating more components per unit time.

Figures 1-3 show the utility of the BIOshell™ columns. The BIOshell™ A400 Protein C4 column is used to provide fast, high resolution separations of monoclonal antibody (mAb) fragments, subunits, as well as the intact protein. The latest addition to the portfolio, the Bioshell IGG A1000 Column (Figure 3) is an excellent alternative with higher efficiency. The 1000 A Pore size also allow better access to mAb aggregates with possibility to higher resolution of larger proteins.

Figures 4-5 highlight the separation potential of the BIOshell™ Peptide columns. In Figure 4, a BIOshell™ A160 Peptide C18 was used to perform a peptide digest on the phosphoprotein casein with an exceptional level of resolution. Figure 5 shows the selectivity differences realized between the BIOshell™ A160 Peptide C18 and BIOshell™ A160 Peptide CN columns. The alternate selectivity of the cyano phase enables improved separations for peak pairs that are not well resolved on the C18 bonded phase.

The separation capabilities of the BIOshell™ Glycan column are displayed in Figures 6-7. The glycan profiles of the procainamide-labeled biotherapeutic, cetuximab, as well as the monoclonal antibody, human IgG, are highlighted.

Proteins

Antibody Fragments on Wide Pore Reversed Phase Columns Operated at Maximum Recommended Temperature

Column	as indicated, 10 cm × 2.1 mm
Mobile phase	[A] 0.1% TFA in 80:20 (v/v) water:acetonitrile; [B] 0.1% TFA in 50:50 (v/v) water:acetonitrile
Gradient	30–70% B in 12 min
Flow	0.3 mL/min
Column temp.	as indicated
Detection	UV, 215 nm
Injection	1 µL, after sample diluted in mobile phase A

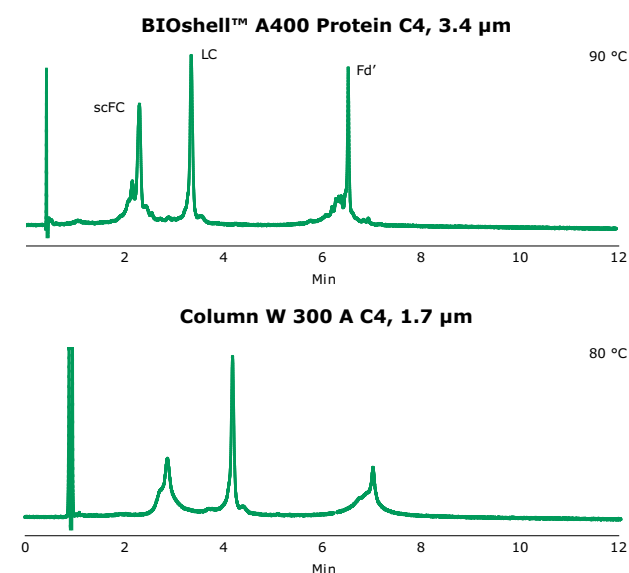


Figure 1.

SigmaMAb Monoclonal Antibody Subunits on BIOshell™ A400 Protein C4

Column	BIOshell™ A400 Protein C4, 5 cm × 2.1 mm I.D., 3.4 µm particles (66824-U)
Mobile phase	[A] 80:20, 0.1% TFA in water:0.1% TFA in acetonitrile; [B] 40:60, 0.1% TFA in water:0.1% TFA in acetonitrile
Gradient	30–42% B in 5 min
Flow	0.3 mL/min
Pressure	720 psi (initial)
Column temp.	75°C
Detection	UV, 215 nm
Injection	1 µL

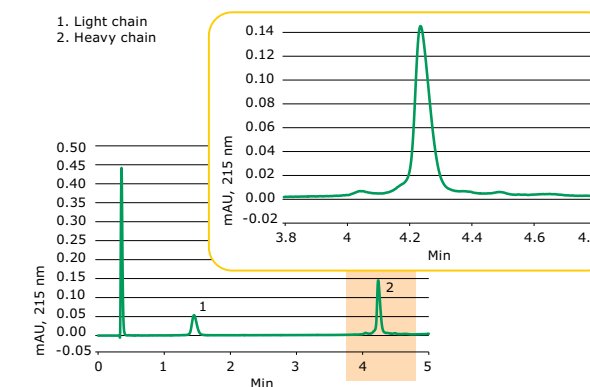
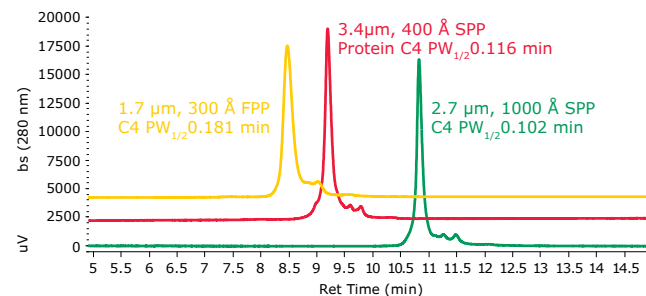


Figure 2.

BIOshell™ IgG HPLC Columns for mAbs

High Efficiency Separation of Trastuzumab

Column	BIOshell™ IgG 1000A C4 2.1 x 150 mm, 2.7 µm (63288-U)
Mobile phase A	water/0.1% DFA
Mobile phase B	acetonitrile/0.1% DFA
Gradient	27-37% B in 20 min
Flow rate	0.4 mL/min
Injection Volume	2 µL (1 µg)
Temp.	80°C



Separation of Denosumab (Prolia)

Column	BIOshell™ IgG 1000A C4 2.1 x 150 mm, 2.7 µm (63288-U)
Mobile phase A	water/5% n-PrOH/0.1% DFA
Mobile phase B	70% n-PrOH/20% AcN/10% water/0.1% DFA
Gradient	14-24% B in 20 min
Flow rate	0.4 mL/min
Injection Volume	4 µL (2 µg)
Temp.	80°C

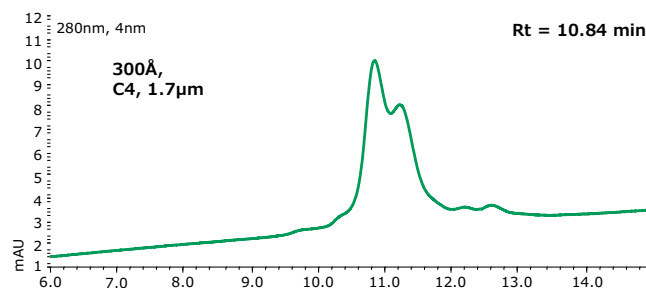
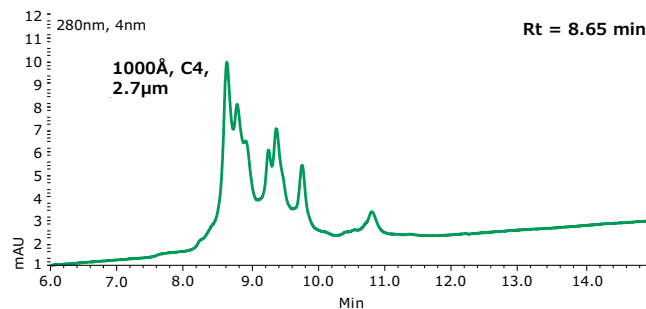


Figure 3.

Peptides

Peptide Digest Mixture on BIOshell™ A160 Peptide C18 Column

Column	BIOshell™ A160 Peptide C18, 15 cm x 2.1 mm I.D., 2.7 µm (66905-U)
Mobile phase	[A] 0.1% formic acid; [B] 0.4% formic acid:acetonitrile (25:75)
Gradient	6–70% B/40 min
Flow rate	0.3 mL/min
Column temp.	35°C
Det.	ESI(+)-TOF
Injection	2 µL
Sample	10 pmol/µL

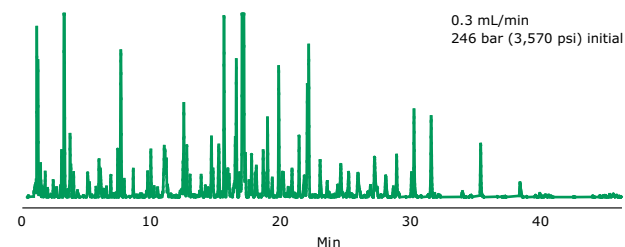


Figure 4.

Peptides on BIOshell™ A160 Peptide C18 and CN

Columns	BIOshell™ A160 Peptide C18, 15 cm x 4.6 mm I.D., 5 µm particles (67015-U) and BIOshell™ A160 Peptide CN, 15 cm x 4.6 mm I.D., 5 µm particles (67081-U)
Mobile phase	(A):water:0.1% TFA (B):acetonitrile:0.1% TFA
Gradient	5–50% B in 30 minutes
Flow rate	1 mL/min
Pressure	126 bar, 250 bar
Temp.	40°C
Injection vol	10 µL
Detector	UV, 215 nm
Sample	0.01 mg/mL in water/0.1% TFA

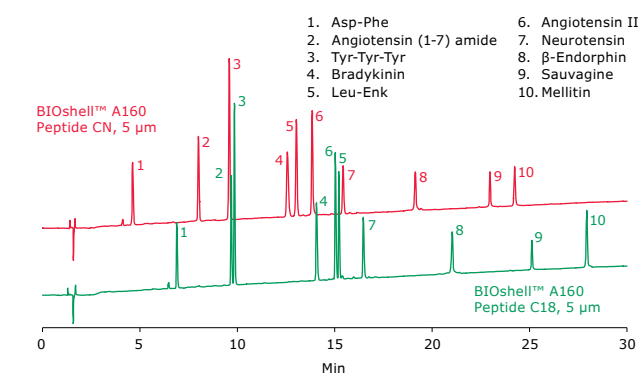


Figure 5.

Glycans

Procainamide-Labeled Cetuximab Glycans on BIOshell™ Glycan using HILIC-FLR

Column	BIOshell™ Glycan, 15 cm x 2.1 mm I.D., 2.7 µm particles (55094-U)
Mobile phase	[A] 50 mM ammonium formate, pH 4.4 (50 mM ammonium hydroxide, adjusted to pH 4.4 with formic acid); [B] acetonitrile
Gradient	75–59% B in 75 min
Flow rate	0.3 mL/min
Temp.	58°C
Injection vol	10 µL
Detector	FLR, ex 308 nm, em 359 nm
Sample	Cetuximab

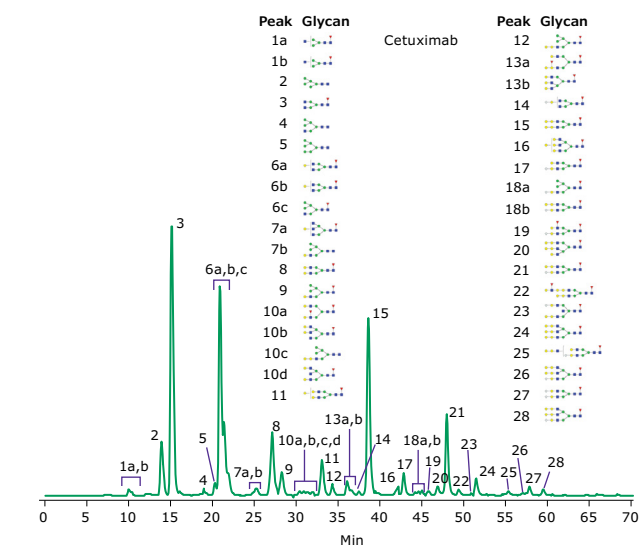


Figure 6.

Procainamide-Labeled Human IgG Glycans on BIOshell™ Glycan using HILIC-FLR

Column	BIOshell™ Glycan, 15 cm x 2.1 mm I.D., 2.7 µm particles (50994-U)
Mobile phase	[A] 50 mM ammonium formate, pH 4.4 (50 mM ammonium hydroxide, adjusted to pH 4.4 with formic acid); [B] acetonitrile
Gradient	75–59% B in 75 min
Flow rate	0.3 mL/min
Temp.	58°C
Injection vol	10 µL
Detector	FLR, ex 308 nm, em 359 nm
Sample	human IgG

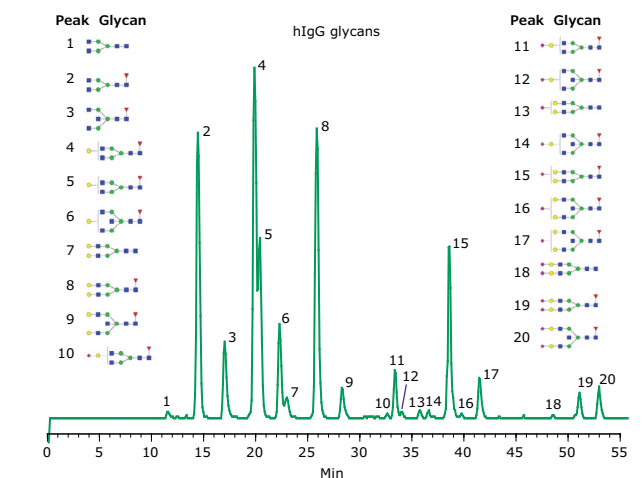


Figure 7.

BIOshell™ U/HPLC Columns

Pore Size	Particle Size	I.D.	L (cm)	C4	C18	CN	Glycan
BIOshell™ Fused-Core® Glycan Columns							
90 Å	2.7 µm	2.1 mm	5	—	—	—	50991-U
90 Å	2.7 µm	2.1 mm	10	—	—	—	50993-U
90 Å	2.7 µm	2.1 mm	15	—	—	—	50994-U
90 Å	2.7 µm	4.6 mm	5	—	—	—	50997-U
90 Å	2.7 µm	4.6 mm	10	—	—	—	50998-U
90 Å	2.7 µm	4.6 mm	15	—	—	—	50999-U
BIOshell™ Fused Core® Peptide and Protein Analytical Columns							
400 Å	3.4 µm	2.1 mm	5	66824-U	67459-U	—	—
400 Å	3.4 µm	2.1 mm	10	66825-U	67463-U	—	—
400 Å	3.4 µm	2.1 mm	15	66826-U	67469-U	—	—
400 Å	3.4 µm	4.6 mm	5	66827-U	67473-U	—	—
400 Å	3.4 µm	4.6 mm	10	66828-U	67475-U	—	—
400 Å	3.4 µm	4.6 mm	15	66829-U	67477-U	—	—
160 Å	2.7 µm	2.1 mm	3	—	66901-U	66965-U	—
160 Å	2.7 µm	2.1 mm	5	—	66902-U	66966-U	—
160 Å	2.7 µm	2.1 mm	7.5	—	66903-U	66967-U	—
160 Å	2.7 µm	2.1 mm	10	—	66904-U	66968-U	—
160 Å	2.7 µm	2.1 mm	15	—	66905-U	66969-U	—
160 Å	2.7 µm	3.0 mm	3	—	66906-U	66970-U	—
160 Å	2.7 µm	3.0 mm	5	—	66907-U	66971-U	—
160 Å	2.7 µm	3.0 mm	10	—	66908-U	66972-U	—
160 Å	2.7 µm	3.0 mm	15	—	66909-U	66973-U	—
160 Å	2.7 µm	4.6 mm	5	—	66913-U	66974-U	—
160 Å	2.7 µm	4.6 mm	10	—	66915-U	66975-U	—
160 Å	2.7 µm	4.6 mm	15	—	66917-U	66976-U	—
160 Å	5 µm	2.1 mm	3	—	67001-U	67061-U	—
160 Å	5 µm	2.1 mm	5	—	67002-U	67062-U	—
160 Å	5 µm	2.1 mm	7.5	—	67003-U	67063-U	—
160 Å	5 µm	2.1 mm	10	—	67004-U	67064-U	—
160 Å	5 µm	2.1 mm	15	—	67006-U	67065-U	—
160 Å	5 µm	3.0 mm	3	—	67007-U	67066-U	—
160 Å	5 µm	3.0 mm	5	—	67008-U	67067-U	—
160 Å	5 µm	3.0 mm	10	—	67011-U	67068-U	—
160 Å	5 µm	3.0 mm	15	—	67012-U	67069-U	—
160 Å	5 µm	4.6 mm	5	—	67013-U	67071-U	—
160 Å	5 µm	4.6 mm	10	—	67014-U	67080-U	—
160 Å	5 µm	4.6 mm	15	—	67015-U	67081-U	—
BIOshell™ Fused-Core® Peptide and Protein Capillary Columns							
400 Å	3.4 µm	75 µm	5	67031-U	67489-U	—	—
400 Å	3.4 µm	75 µm	15	67032-U	67490-U	—	—
400 Å	3.4 µm	100 µm	5	67033-U	67491-U	—	—
400 Å	3.4 µm	100 µm	15	67034-U	67493-U	—	—
400 Å	3.4 µm	200 µm	5	67036-U	67494-U	—	—

Pore Size	Particle Size	I.D.	L (cm)	C4	C18	CN	Glycan
400 Å	3.4 µm	200 µm	15	67037-U	67495-U	—	—
400 Å	3.4 µm	300 µm	5	67038-U	67496-U	—	—
400 Å	3.4 µm	300 µm	15	67039-U	67497-U	—	—
400 Å	3.4 µm	500 µm	5	67040-U	67499-U	—	—
400 Å	3.4 µm	500 µm	15	67041-U	67502-U	—	—
400 Å	3.4 µm	1.0 mm	5	67042-U	67503-U	—	—
400 Å	3.4 µm	1.0 mm	15	67045-U	67504-U	—	—
160 Å	2.7 µm	75 µm	5	—	67085-U	67150-U	—
160 Å	2.7 µm	75 µm	15	—	67086-U	67152-U	—
160 Å	2.7 µm	100 µm	5	—	67087-U	67153-U	—
160 Å	2.7 µm	100 µm	15	—	67088-U	67155-U	—
160 Å	2.7 µm	200 µm	5	—	67089-U	67157-U	—
160 Å	2.7 µm	200 µm	15	—	67091-U	67158-U	—
160 Å	2.7 µm	300 µm	5	—	67092-U	67159-U	—
160 Å	2.7 µm	300 µm	15	—	67093-U	67160-U	—
160 Å	2.7 µm	500 µm	5	—	67095-U	67161-U	—
160 Å	2.7 µm	500 µm	10	—	67096-U	—	—
160 Å	2.7 µm	500 µm	15	—	67097-U	67163-U	—
160 Å	2.7 µm	1.0 mm	5	—	67098-U	67164-U	—
160 Å	2.7 µm	1.0 mm	15	—	67099-U	67165-U	—
160 Å	5 µm	75 µm	5	—	67201-U	67305-U	—
160 Å	5 µm	75 µm	15	—	67202-U	67307-U	—
160 Å	5 µm	100 µm	5	—	67203-U	67311-U	—
160 Å	5 µm	100 µm	15	—	67204-U	67312-U	—
160 Å	5 µm	200 µm	5	—	67205-U	67314-U	—
160 Å	5 µm	200 µm	15	—	67206-U	67315-U	—
160 Å	5 µm	300 µm	5	—	67207-U	67321-U	—
160 Å	5 µm	300 µm	15	—	67208-U	67324-U	—
160 Å	5 µm	500 µm	5	—	67209-U	67325-U	—
160 Å	5 µm	500 µm	15	—	67212-U	67326-U	—
160 Å	5 µm	1.0 mm	5	—	67215-U	67327-U	—
160 Å	5 µm	1.0 mm	15	—	67219-U	67329-U	—

BIOshell™ Fused-Core® Peptide and Protein Guard Columns, pk. of 3

400 Å	3.4 µm	2.1	0.5	66830-U	67505-U	—	—
400 Å	3.4 µm	4.6	0.5	66831-U	67508-U	—	—
160 Å	2.7 µm	2.1	0.5	—	66918-U	66977-U	—
160 Å	2.7 µm	3.0	0.5	—	66919-U	66978-U	—
160 Å	2.7 µm	4.6	0.5	—	66921-U	66979-U	—
160 Å	5 µm	2.1	0.5	—	67016-U	67082-U	—
160 Å	5 µm	3.0	0.5	—	67017-U	67083-U	—
160 Å	5 µm	4.6	0.5	—	67018-U	67084-U	—

Related Product

Description	Cat. No.
BIOshell™ Guard Cartridge Holder	66841-U

NEW!
BIOshell™ IgG 1000A C4

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Other U/HPLC Columns for Proteins and Peptides Available

Supplier, Manufacturer	dp (µm)	Matrix	Size Exclusion	Ion Exchange	Reversed Phase	HILIC	HIC	Affinity
GE Life Sciences	8, 13	dextran-agarose	Superdex™					
	>10 (dry)	dextran	Desalting					
	3, 10	PS/DVB		MiniBeads™, MonoBeads™				
	15	PS/DVB		SOURCE™	SOURCE™		SOURCE™	
Sepax Technologies	1.8	silica	Unix®					
	3	silica	Zenix®, Zenix-C					
	5	silica	SRT®, SRT-C					
	1.7, 3, 5, 10	PS/DVB		Proteomix®				
	1.7, 3, 5, 10	PS/DVB		Antibodix®				
Supelco®/ Sigma-Aldrich	2.7, 3.4, 5	silica				BIOshell™		
	3, 5, 10	silica				Discovery™ BIO		
	5	methacrylate		Discovery® BIO PolyMA				
Tosoh Bioscience: TSKgel®	3, 4, 5, 10	silica	UP-SW3000, UltraSW, SuperSW, SWxl, SW					
	2.5, 5, 10	methacrylate		NPR, STAT, 5PW				
	2.5, 10	methacrylate			NPR, 4PW, 5PW			
	3	silica			Peptide C4			
	3, 5	silica				Amide-80		
	2.5, 10	methacrylate					NPR, 5PW	
	10	methacrylate						5PW

To see the complete offering* of U/HPLC columns, including Chromolith® WP, GE Life Sciences, Sepax and Tosoh TSKgel® Columns, please visit SigmaAldrich.com/bio-hplc

*Sales restrictions may apply in some countries.

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