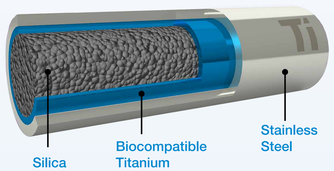
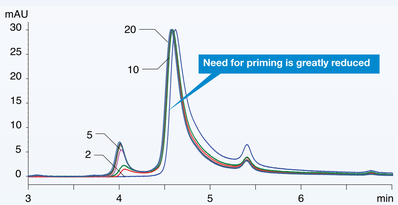
Welcome to bioZen

bioZen™ the bio series

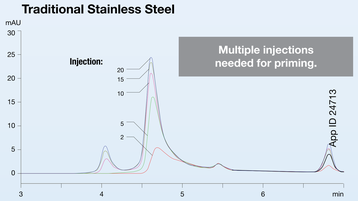
BioTi™ Hardware For Minimized Column Priming



bioZen Titanium BioTi Hardware



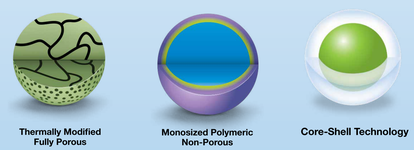
Traditional Stainless Steel



Conditions for both columns:

Column: bioZen 1.8 µm SEC-3
Dimension: 150 x 4.6 mm
Mobile Phase: 50 mM Dipotassium phosphate + 100 mM Sodium Sulfate, pH 5.0
Flow Rate: 0.3 mL/min
Temperature: Ambient
Detection: UV @ 280 nm
Injection Volumn: 2 mL
Sample: 1. γ-Globulin, 5 mg/mL
2. Ovalbumin, 1 mg/mL

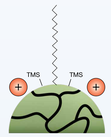
3 Particle Platforms



Thermally Modified Fully Porous | **Monosized Polymeric Non-Porous** | **Core-Shell Technology**

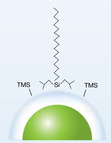
Peptide Mapping

Peptide



bioZen Peptide PS-C18
1.6 µm and 3 µm

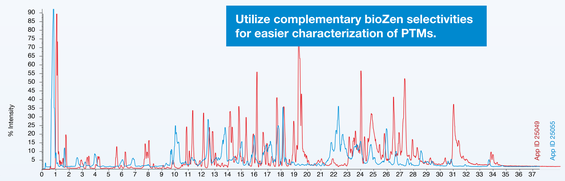
Excellent retention by combined positively charged surface ligand and C18 ligand.



bioZen Peptide XB-C18
1.7 µm and 2.6 µm

Overall retention of both acidic and basic peptides through C18 stationary phase with di-isobutyl side chains.

Trastuzumab Biosimilar Peptide Map



Conditions for all columns:

Columns: bioZen 1.6 µm Peptide PS-C18
bioZen 2.6 µm Peptide XB-C18
Dimension: 150 x 2.1 mm
Part No.: 00F-4770-AN
00F-4768-AN
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

Gradient: Time (min)	% B
0	1
0.5	1
50	50
55	50
56	95

Flow Rate: 0.3 mL/min
Temperature: 40 °C
Detection: QTOF (SCIEX® X500B)

Size Exclusion

Size Exclusion (SEC)

bioZen SEC-2
1.8 µm

Extremely inert, high density fully porous particle with high efficiency and low molecular weight (LMW) separation range of 1 k – 450 kDa.

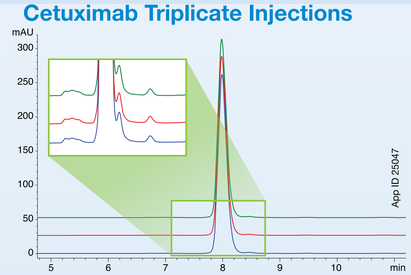
bioZen SEC-3
1.8 µm

Extremely inert, high density fully porous particle with high efficiency and high molecular weight (HMW) separation range of 10 k – 700 kDa.

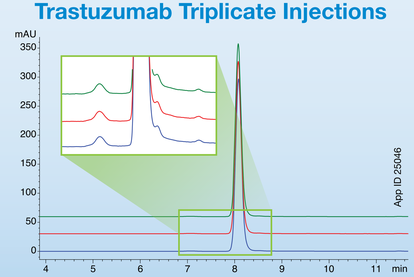
Conditions same for all samples:

Column: bioZen 1.8 µm SEC-3
Dimension: 300 x 4.6 mm
Part No.: 00H-4772-E0
Mobile Phase: 50 mM Dipotassium phosphate + 100 mM Sodium Sulfate, pH 6.8
Flow Rate: 0.2 mL/min
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: As Noted

Cetuximab Triplicate Injections

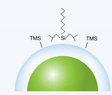


Trastuzumab Triplicate Injections



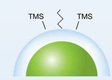
Intact/Fragment Analysis

Intact



bioZen Intact XB-C8
3.6 µm

Large pore core-shell particle for fast intact biologic entry. C8 provides highly useful moderate hydrophobic selectivity.



bioZen Intact C4
3.6 µm

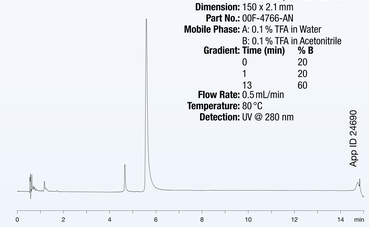
Large pore core-shell particle for fast intact biologic entry. C4 stationary phase provides highly sought after low hydrophobic retention, especially important for highly retentive biologics.

Infliximab $F(ab)_2$

Columns: bioZen 3.6 µm Intact XB-C8
Dimension: 150 x 2.1 mm
Part No.: 00F-4766-AN
Mobile Phase: A: 0.1 % TFA in Water
B: 0.1 % TFA in Acetonitrile

Gradient: Time (min)	% B
0	20
1	20
13	60

Flow Rate: 0.5 mL/min
Temperature: 80 °C
Detection: UV @ 280 nm

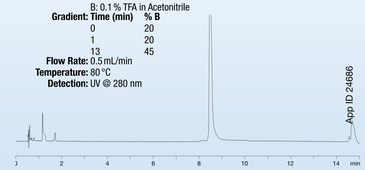


Cetuximab

Columns: bioZen 3.6 µm Intact XB-C8
Dimension: 150 x 2.1 mm
Part No.: 00F-4766-AN
Mobile Phase: A: 0.1 % TFA in Water
B: 0.1 % TFA in Acetonitrile

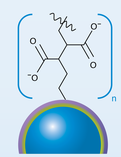
Gradient: Time (min)	% B
0	20
1	20
13	45

Flow Rate: 0.5 mL/min
Temperature: 80 °C
Detection: UV @ 280 nm



Charge Variant Analysis

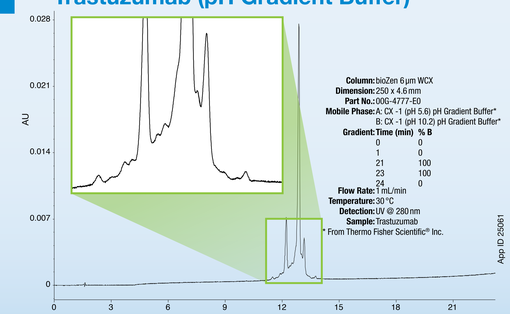
Ion-Exchange



bioZen WCX
6 µm

Monosized particles grafted with linear polycarboxylate chains to envelop and separate proteins from acidic/basic variants

Trastuzumab (pH Gradient Buffer)



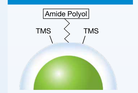
Column: bioZen 6 µm WCX
Dimension: 250 x 4.6 mm
Part No.: 00G-4777-E0
Mobile Phase: A: CX -1 (pH 5.6) pH Gradient Buffer*
B: CX -1 (pH 10.2) pH Gradient Buffer*

Gradient: Time (min)	% B
0	0
1	0
21	100
23	100
24	0

Flow Rate: 1 mL/min
Temperature: 30 °C
Detection: UV @ 280 nm
Sample: Trastuzumab
* From Thermo Fisher Scientific® Inc.

N-Glycan Analysis

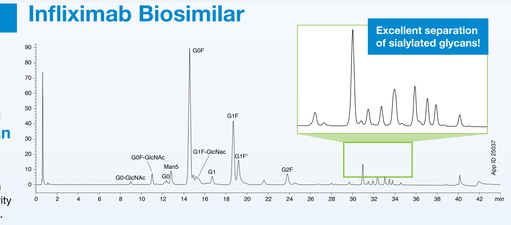
Glycan



bioZen Glycan
2.6 µm

Provides optimal combination of high efficiency and selectivity for released glycans.

Infliximab Biosimilar



Column: bioZen 2.6 µm Glycan
Dimensions: 150 x 2.1 mm
Part No.: 00F-4773-AN
Mobile Phase: A: 100 mM Ammonium Formate, pH 4.5
B: Acetonitrile

Gradient: Time (min)	% B
0	78
10	74.5
24	72
38.5	55.9
38.6	40
40.6	40
40.7	78
48	78

Flow Rate: 0.5 mL/min
Temperature: 50 °C
Detection: FLD ex/em 285/345 nm
Sample: As noted

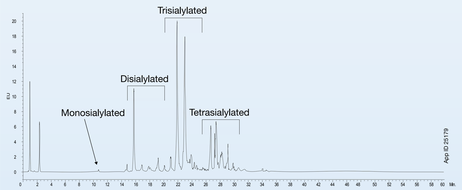
N-Glycan Clean-Up and Analysis

Pre-treatment
Glycan sample were deglycosylated and labeled using a standard labeling procedure. Samples then were diluted with 358 µL of Acetonitrile.

Solid Phase Extraction Protocol

96-Well Plate: bioZen N-Glycan Clean-Up
Part No.: 8M-S009-NGA
Condition: 200 µL Water
Equilibrate: 200 µL Water/Acetonitrile (15:85)
Load: Labeled pre-treated sample (~400 µL)
Wash: 2x 600 µL Formic acid/Water/Acetonitrile (1:9:90)
Elute: 3x 30 µL 200mM Ammonium acetate in Acetonitrile/Water (5:95)
Dilute: 100 µL Dimethyl formamide then 210 µL Acetonitrile

Alpha-1 Acid Glycoprotein (AGP)



LC Conditions
LC Column: bioZen 2.6 µm Glycan
Dimensions: 150 x 2.1 mm
Part No.: 00F-4773-AN
Mobile Phase: A: 250 mM Ammonium fomate, pH 4.5
B: Acetonitrile

Gradient: Time (min)	%B	Flow Rate (mL/min)
0	70	0.4
38	50	0.4
41.5	0	0.2
44.5	0	0.2
48.1	70	0.2
52.6	70	0.4
60	70	0.4

Column Temperature: 60 °C
LC System: ACQUITY® UPLC® H-Class Bio

Glycan	Sum of Peak Area %	Multiplication Factor	Charge Number Share
Asialo	0.28	0	0
Monosialo-	13.41	1	13.41
Disialo-	4.44	2	8.88
Trisialo-	52.49	3	157.47
Tetrasialo-	29.4	4	117.6
Total peak area	100%		Z-Value = 297.36

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