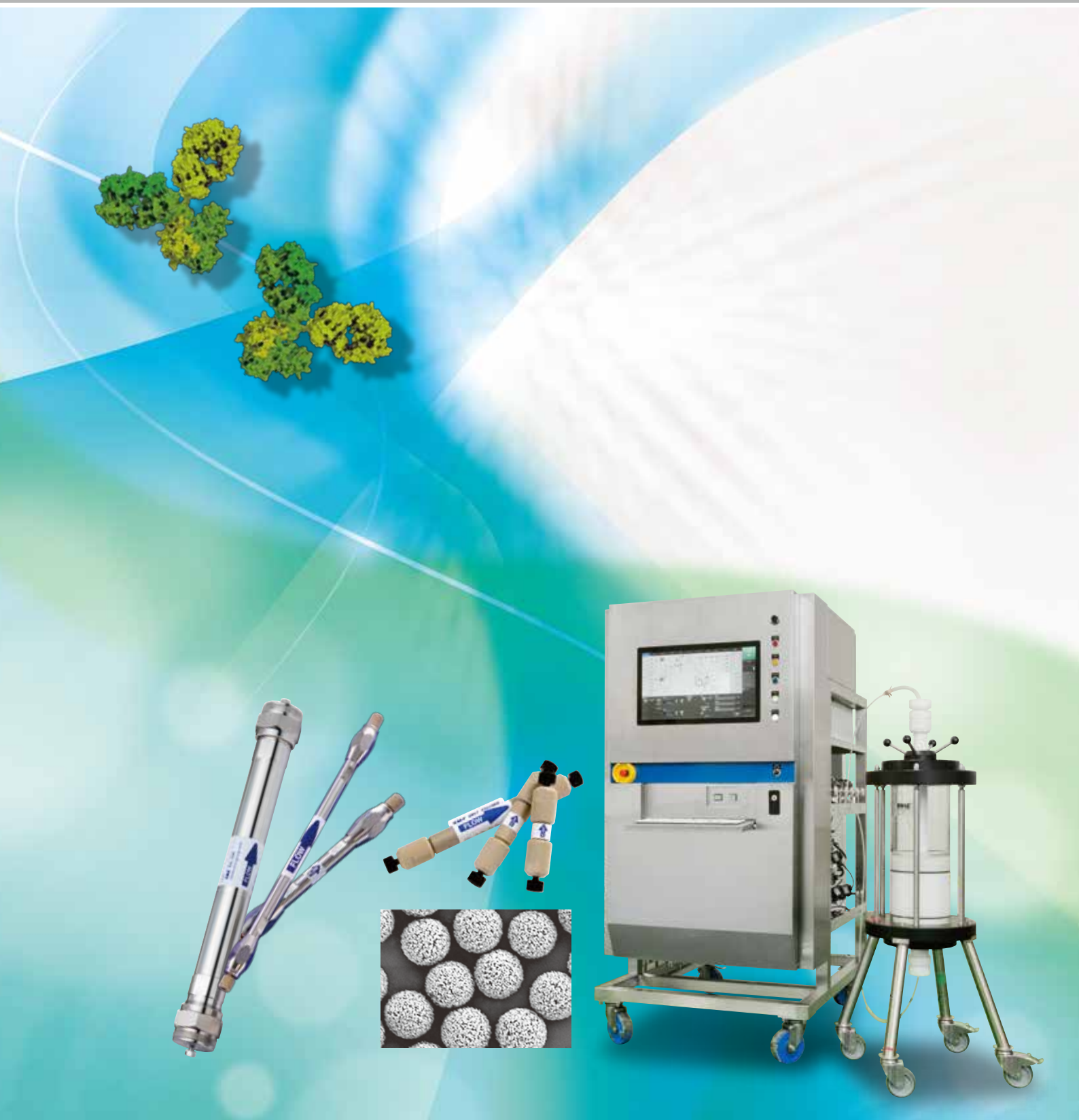


Bioseparation



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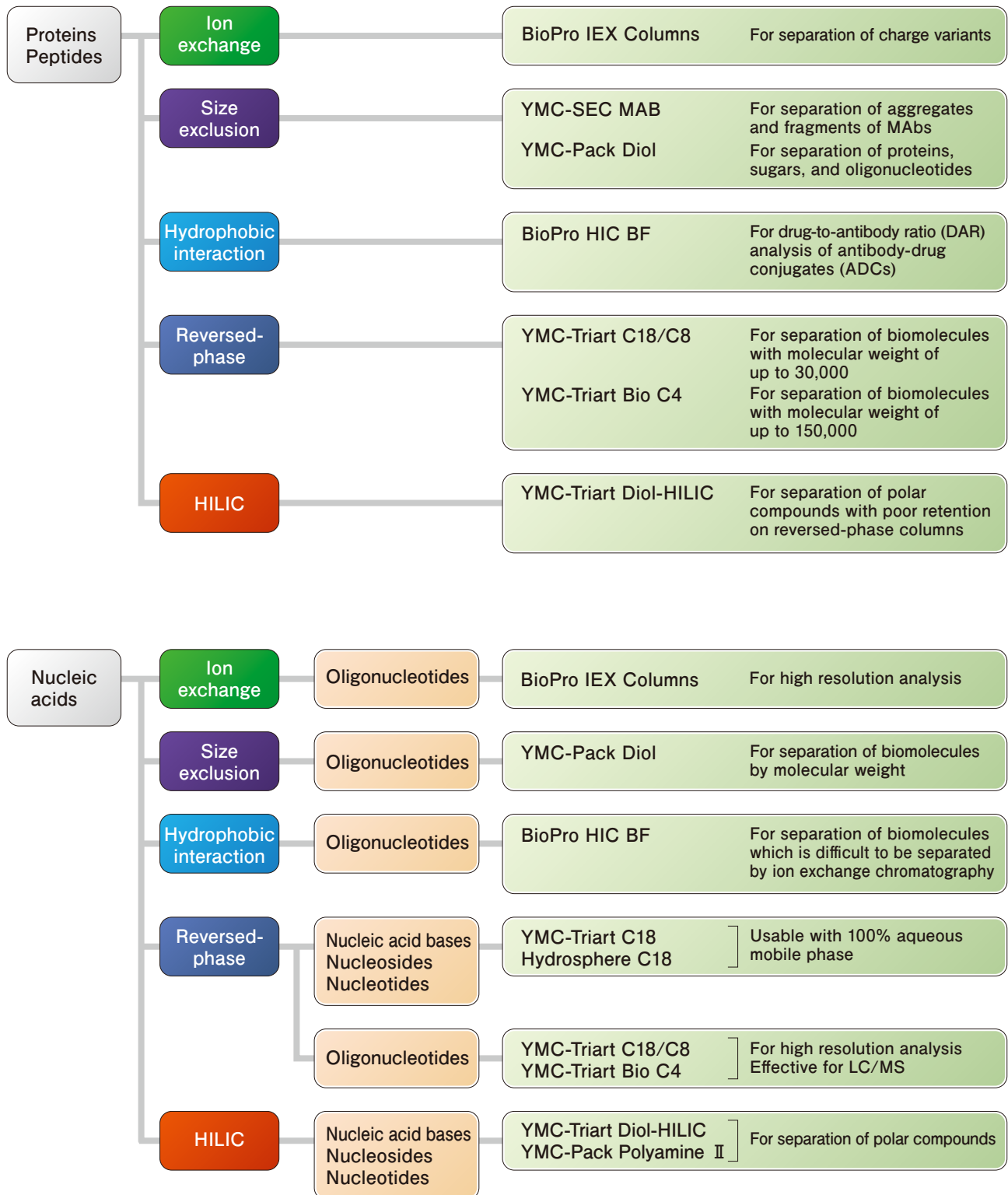
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Column Selection Guide

Column Selection Guide



Features of Separation Modes

	Ion exchange BioPro IEX Columns	Size exclusion YMC-SEC MAB YMC-Pack Diol	Hydrophobic interaction BioPro HIC BF	Reversed-phase YMC-Triart etc.
Principle of separation	Electric charge	Molecular weight	Hydrophobicity	Hydrophobicity
Molecular weight range	Up to several millions	Up to about 1,000,000	Up to several millions	Up to about 150,000
Resolution	+++	++	+++	+++
Speed	++ ~ +++	+	+++	+++
Loading	+++	++	+++	++
Sample stability	+++	+++	+++	+ ~ ++
Typical applications	Charge variants analysis	Separation of aggregates Separation of fragments	Drug-to-antibody ratio analysis of antibody-drug conjugates	Peptide mapping LC/MS Structural analysis

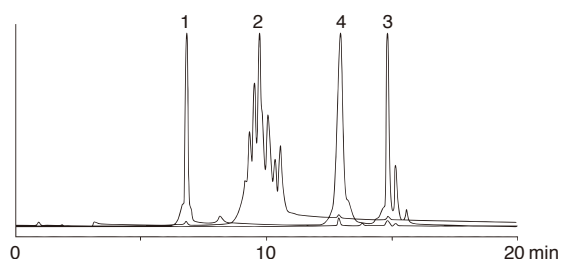
Separation of Antibodies/Proteins/Peptides

Charge variants analysis of monoclonal antibodies

Ion exchange

BioPro IEX SF 5 µm, 100 X 4.6 mm I.D.

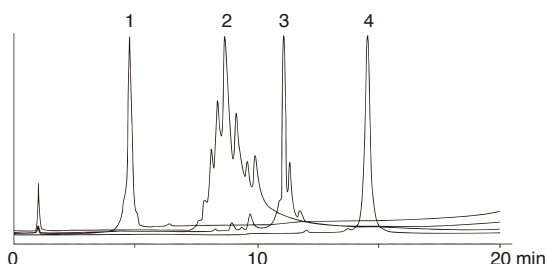
Salt gradient mode



1. Natalizumab (Humanized IgG4, pI=7.3)
2. Cetuximab (Chimeric IgG1, pI=7.9)
3. Adalimumab (Human IgG1, pI=8.4)
4. Denosumab (Human IgG2, pI=8.8)

Eluent : A) 10 mM MES-NaOH (pH 5.7)
 B) 10 mM MES-NaOH (pH 5.7) containing 1 M NaCl
 0-20%B (0-20 min)
 Flow rate: 0.6 mL/min

pH gradient mode



Eluent : A) CX-1 pH Gradient Buffer A* (pH 5.6)
 B) CX-1 pH Gradient Buffer B* (pH 10.2)
 0-100%B (0-20 min)
 Flow rate: 0.6 mL/min

*Purchased from Thermo Fisher Scientific Inc.

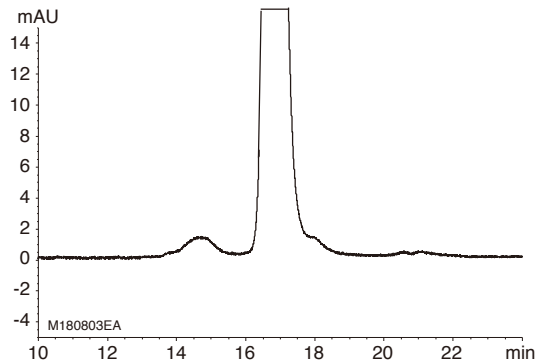
J. Pharm. Biomed. Anal., 2015, 111, 169-176.

Monoclonal antibodies were separated by ion exchange chromatography in salt gradient mode and in pH gradient mode. Sharp peaks were obtained in both modes with BioPro IEX columns since they have extremely low nonspecific adsorption. This shows that BioPro IEX columns are effective for separation of charge variants and isoforms.

Aggregates analysis of antibody-drug conjugate (ADC)

Size exclusion

YMC-SEC MAB 3 μm , 300 X 4.6 mm I.D.



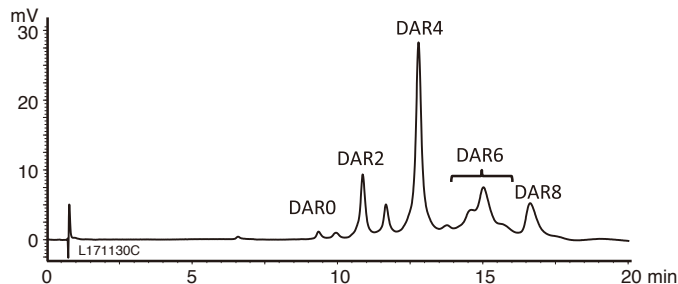
Eluent	: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl /2-propanol (85/15)
Flow rate	: 0.165 mL/min
Temperature	: 25°C
Detection	: UV at 280 nm
Injection	: 4 μL
Sample	: Cysteine-conjugated ADC mimic (2.5 mg/mL)

Size exclusion chromatography is useful for separation of substances with different molecular weights such as MABs, their dimers and aggregates or ADCs, their dimers and aggregates.

Drug-to-antibody ratio (DAR) analysis of ADC

Hydrophobic interaction

BioPro HIC BF 4 μm , 100 X 4.6 mm I.D.



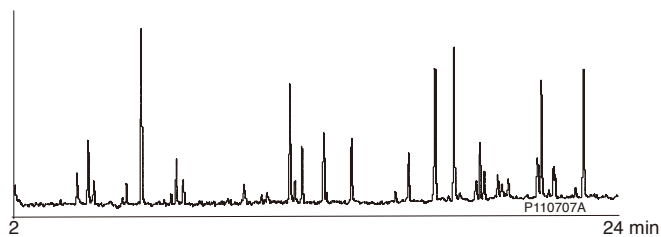
Eluent	: A) 50 mM NaH_2PO_4 - Na_2HPO_4 (pH 7.0) containing 1.5 M $(\text{NH}_4)_2\text{SO}_4$ /2-propanol (95/5) B) 50 mM NaH_2PO_4 - Na_2HPO_4 (pH 7.0)/2-propanol (80/20)
Flow rate	: 1.0 mL/min
Temperature	: 25°C
Detection	: UV at 280 nm
Injection	: 5 μL
Sample	: Cysteine-conjugated ADC mimic (5 mg/mL)

Hydrophobic interaction chromatography column BioPro HIC BF provides superior separation for ADCs and is effective for DAR determination.

Peptide mapping

Reversed-phase

YMC-Triart C18 1.9 μm , 200 X 2.0 mm I.D. (100 X 2.0 mm I.D. two coupled)



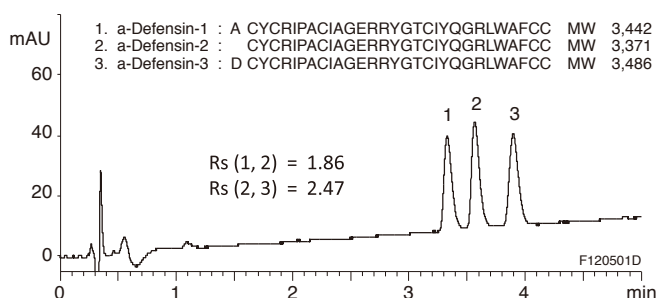
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.08) 5-40%B (0-30 min)
Flow rate	: 0.4 mL/min
Temperature	: 70°C
Detection	: UV at 220 nm
Injection	: 20 μL
Sample	: Tryptic digest of Bovine Hemoglobin

The outstanding efficiency obtained by a coupling of two 100 mm length of Triart 1.9 μm columns allows the precise separation in peptide mapping.

Analysis of antimicrobial peptides

Reversed-phase

YMC-Triart C18 1.9 μm , 50 X 2.0 mm I.D.



Eluent	: A) water/formic acid (100/0.1) B) acetonitrile/2-propanol/formic acid (50/50/0.08) 10-25%B (0-10 min)
Flow rate	: 0.4 mL/min
Temperature	: 70°C
Detection	: UV at 220 nm

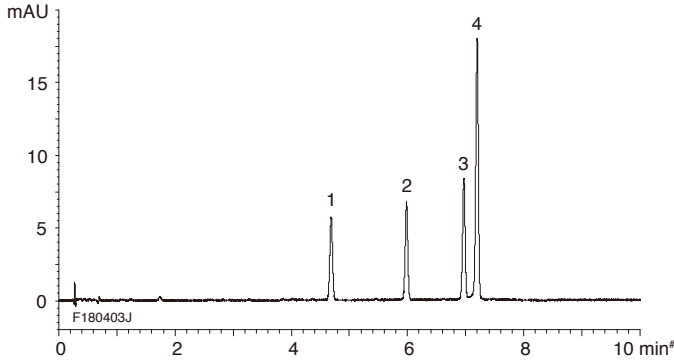
High temperature condition improves separation of peptides and proteins on reversed-phase chromatography. Since Triart columns are highly durable at high temperatures, they are effective for analysis of peptides and proteins under such harsh conditions.

Separation of Nucleic Acids

Analysis of oligonucleotides

Reversed-phase

YMC-Triart C18 1.9 μ m, 50 X 2.1 mm I.D.



1. 5'-CAC UGA AUA CCA AU-3' (14 mer)
2. 5'-UCA CAC UGA AUA CCA AU-3' (17 mer)
3. 5'-UCA UCA CAC UGA AUA CCA AU-3' (20 mer)
4. 5'-GUC AUC ACA CUG AAU ACC AAU-3' (21 mer)

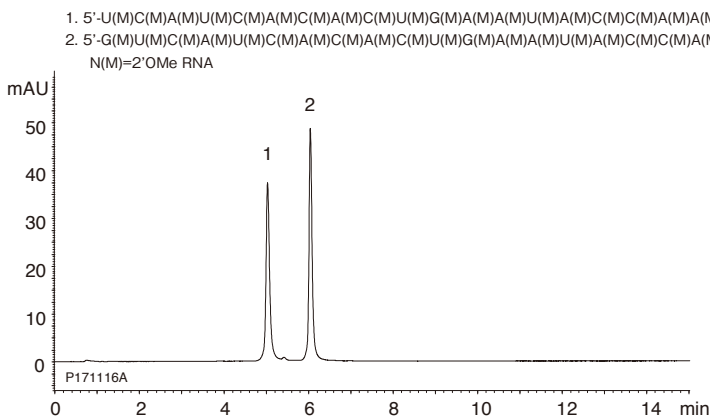
Eluent : A) 200 mM HFIP*8 mM triethylamine
 B) methanol
 10-20%B (0-10 min)
 Flow rate : 0.42 mL/min
 Temperature : 65°C
 Detection : UV at 260 nm
 Injection : 1.0 μ L (2-4 nmol/mL)

*1,1,1,3,3,3-hexafluoro-2-propanol

Triart C18 is suitable for separation of hydrophilic compounds such as oligonucleotides.

Ion exchange

BioPro IEX QF 5 μ m, 100 X 4.6 mm I.D.



1. 5'-U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)A(M)A(M)U(M)-3' (20 mer)
 2. 5'-G(M)U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)A(M)A(M)U(M)-3' (21 mer)
- N(M)=2'OMe RNA

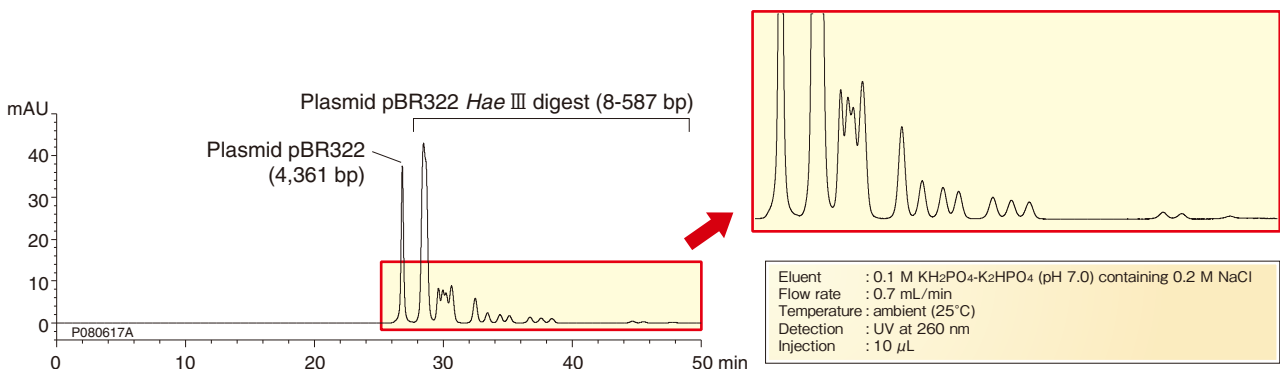
Eluent : A) 10 mM NaOH
 B) 10 mM NaOH containing 1.0 M NaClO₄
 25-55%B (0-15 min), 100%B (15-20 min)
 Flow rate : 1.0 mL/min
 Temperature : 25°C
 Detection : UV at 260 nm
 Injection : 4 μ L (5 nmol/mL)

100 mm length column of BioPro IEX QF, which has high separation ability, is suitable for separation of oligonucleotides by one nucleotide difference.

Plasmid pBR322 *Hae* III restriction fragments

Size exclusion

YMC-Pack Diol-300 + Diol-200 5 μ m, 500 X 8.0 mm I.D. X 2



Eluent : 0.1 M KH₂PO₄-K₂HPO₄ (pH 7.0) containing 0.2 M NaCl
 Flow rate : 0.7 mL/min
 Temperature : ambient (25°C)
 Detection : UV at 260 nm
 Injection : 10 μ L

In size exclusion mode, separation is expected to be improved by using coupled columns with different pore sizes.

Columns/Packing Materials

Ion Exchange Chromatography Columns for Separation of Proteins/Nucleic Acids BioPro IEX Columns

Features

- Hydrophilic polymer beads with low nonspecific adsorption
- Ideal for analysis and laboratory-scale purification on porous type with high binding capacity and high recovery of biomolecules
- Ultra-fast analysis and high-resolution analysis on non-porous type
- Suitable for characterization of biopharmaceuticals and quality control

SEM images of polymer beads



Non-porous polymer beads



Porous polymer beads



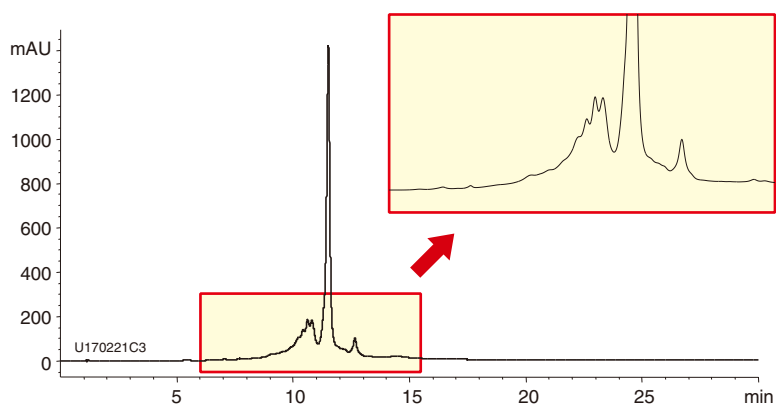
Specifications

	BioPro IEX QF	BioPro IEX SF	BioPro IEX QA	BioPro IEX SP
Matrix	Hydrophilic non-porous polymer		Hydrophilic porous polymer	
Particle size (μm)	3, 5		5	
Charged group	-CH ₂ N ⁺ (CH ₃) ₃	-CH ₂ CH ₂ CH ₂ SO ₃ ⁻	-CH ₂ N ⁺ (CH ₃) ₃	-CH ₂ CH ₂ CH ₂ SO ₃ ⁻
Counter ion	Cl ⁻	Na ⁺	Cl ⁻	Na ⁺
Ion exchange capacity* (meq/mL-resin)	0.09	0.24	0.09	0.09
Dynamic binding capacity* (mg/mL-resin)	>12 (BSA)	>10 (human-IgG)	>110 (BSA)	>70 (human-IgG)
Usable temperature	4-60°C			
Usable pH range	2-12			
Column material	PEEK			

*Reference value

High-resolution analysis of monoclonal antibody (MAb) (1)

BioPro IEX SF 3 μm, 100 X 4.6 mm I.D.

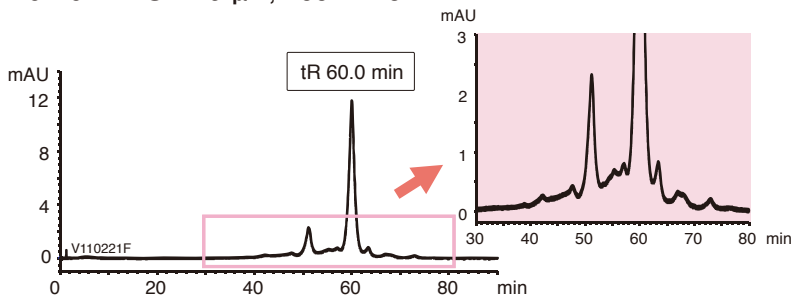


Eluent : A) 20 mM NaH₂PO₄-Na₂HPO₄ (pH 6.8)
 B) 20 mM NaH₂PO₄-Na₂HPO₄ (pH 6.8) containing 0.2 M NaCl
 0-50%B (0-30 min)
 Flow rate : 0.5 mL/min (180 cm/hr)
 Temperature : 25°C
 Detection : UV at 215 nm
 Injection : 10 μL
 Sample : Humanized monoclonal IgG1 (2.5 mg/mL)

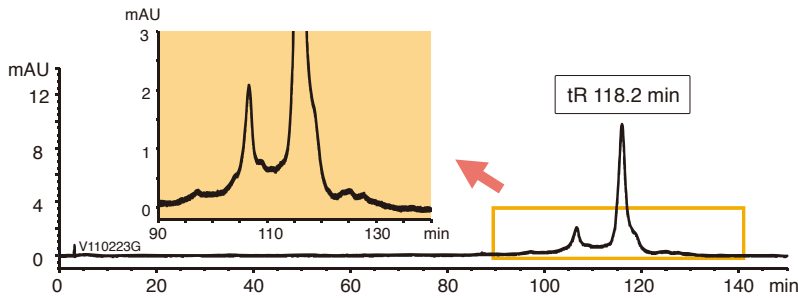
BioPro IEX SF column with 3 μm, 100 mm length achieved high resolution analysis of MAb.

High-resolution analysis of monoclonal antibody (MAB) (2)

BioPro IEX SF 5 μm, 100 X 4.6 mm.I.D.



Competitor's WCX column 10 μm, 250 X 4.0 mm.I.D.

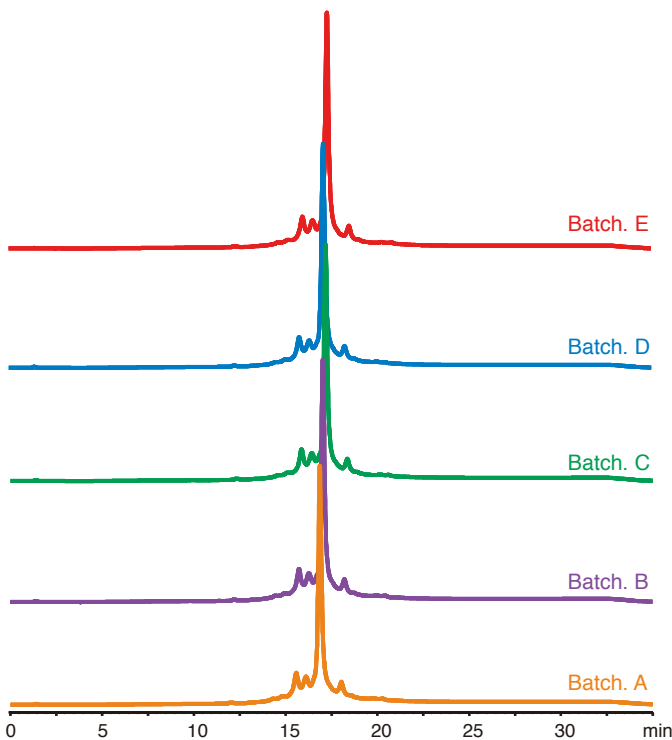


Eluent	: A) 20 mM MES-NaOH (pH 5.6) B) 20 mM MES-NaOH (pH 5.6) containing 0.2 M NaCl
Initial gradient conc:	: 35%B (70 mM NaCl)
Gradient slope	: 0.25%B/min (0.5 mM NaCl)
Flow rate	: 180 cm/hr (0.5 mL/min for 100 X 4.6 mm.I.D., 0.378 mL/min for 250 X 4.0 mm.I.D.)
Temperature	: 30°C
Detection	: UV at 280 nm
Injection	: 10 μL
Sample	: Humanized monoclonal IgG1 (1 mg/mL)

The separation of MAB is compared on BioPro IEX SF and competitor's column under the same gradient conditions at pH 5.6.

BioPro IEX SF column provides higher resolution of MAB in a shorter analysis time than the competitor's column.

Excellent batch-to-batch reproducibility

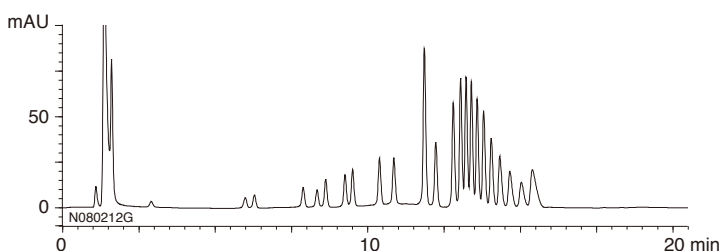


Column	: BioPro IEX SF 5 μm, 100 X 4.6 mm.I.D.
Eluent	: A) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.5) B) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.5) containing 0.2 M NaCl 0-50%B (0.5-30 min)
Flow rate	: 0.5 mL/min (180 cm/hr)
Temperature	: 25°C
Detection	: UV at 215 nm
Injection	: 10 μL
Sample	: Humanized monoclonal IgG1

BioPro IEX SF column exhibits excellent batch-to-batch reproducibility for MAB analysis, including the resolution of small peaks for charge variants. All the medium batches are inspected by various quality control tests and must pass rigorous criteria before release. BioPro IEX columns are the best choice for the quality control of MABs and other biopharmaceuticals.

High-resolution analysis of nucleic acids

BioPro IEX QF 5 μm, 100 X 4.6 mm.I.D.



DNA fragments 1Kb DNA ladder (75-12,216 bp)

Eluent	: A) 20 mM Tris-HCl (pH 8.1) containing 0.7 M NaCl B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl 0-100%B (0-30 min)
Flow rate	: 0.5 mL/min (180 cm/hr)
Temperature	: 25°C
Detection	: UV at 260 nm
Injection	: 20 μL (0.25 mg/mL)

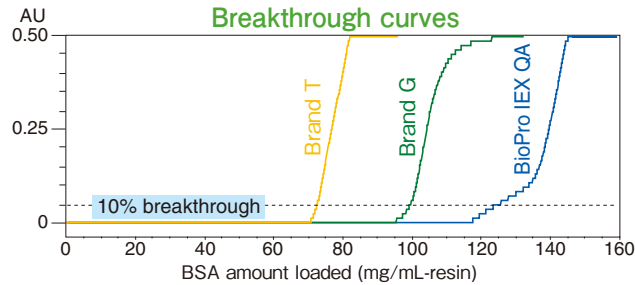
The separation of DNA fragments is shown. BioPro IEX QF of 100 mm length column is a good choice for high-resolution analysis of nucleic acids.

High binding capacity and recovery

Comparison of dynamic binding capacity (DBC) and recovery for BSA

	Dynamic binding capacity (mg/mL-resin, 10% breakthrough)	Eluted amount (mg/mL-resin)	Recovery* (%)
BioPro IEX QA	126	120	95
Brand T (porous Q type)	73	58	79
Brand G (porous Q type)	100	35	35

*Recovery: (Eluted amount/Dynamic binding capacity) X 100

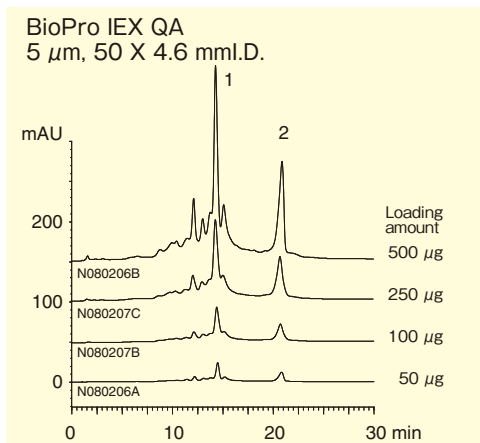


Column : BioPro IEX QA, 50 X 4.6 mmI.D.
Brand T (porous Q type) 50 X 4.6 mmI.D.
Brand G (porous Q type) 50 X 5.0 mmI.D.
Linear velocity : 180 cm/hr
Equilibration buffer : 20 mM Tris-HCl (pH 8.6)
Elution buffer : 20 mM Tris-HCl (pH 8.6) containing 1.0 M NaCl
Detection : UV at 280 nm
Sample : 1 mg/mL Bovine serum albumin (BSA) in equilibration buffer

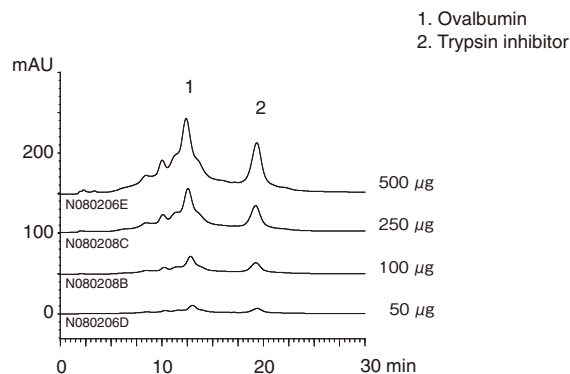
BioPro IEX QA gives the superior DBC and recovery compared with conventional porous polymer anion exchange columns. The surface structure of porous type BioPro IEX, which is designed for maximum interaction with proteins, provides high binding capacity, and the hydrophilic property of polymer beads significantly reduces nonspecific adsorption of proteins.

High loadability

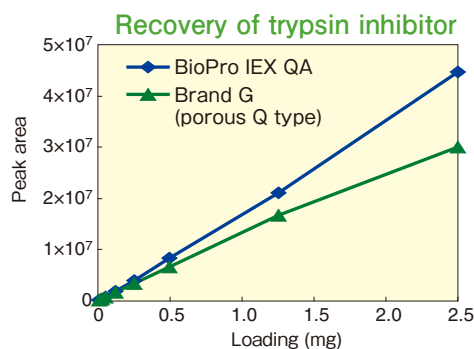
Comparison of the effect of sample load on BioPro IEX QA and commercial Q type column



Brand G (porous Q type)
10 μm, 50 X 5.0 mmI.D.



1. Ovalbumin
2. Trypsin inhibitor



Eluent : A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
10-80%B (0-30 min)
Flow rate : 0.5 mL/min (180 cm/hr for 4.6 mmI.D., 150 cm/hr for 5.0 mmI.D.)
Temperature : 25°C
Detection : UV at 280 nm
Injection : 100 μL

BioPro IEX QA shows excellent resolution and peak shapes even when the loading amount increases. The porous type BioPro IEX columns are suitable for laboratory-scale purification of proteins.

Ordering information

BioPro IEX QF/SF (non-porous type)

Packing material	Particle size (μm)	Column size inner diameter X length (mm)	Product number
BioPro IEX QF	3	4.6 X 30	QF00S03-0346WP
		4.6 X 50	QF00S03-0546WP
		4.6 X 100	QF00S03-1046WP
	5	4.6 X 30	QF00S05-0346WP
		4.6 X 50	QF00S05-0546WP
		4.6 X 100	QF00S05-1046WP
BioPro IEX SF	3	4.6 X 30	SF00S03-0346WP
		4.6 X 50	SF00S03-0546WP
		4.6 X 100	SF00S03-1046WP
	5	4.6 X 30	SF00S05-0346WP
		4.6 X 50	SF00S05-0546WP
		4.6 X 100	SF00S05-1046WP

BioPro IEX QA/SP (porous type)

Packing material	Particle size (μm)	Column size inner diameter X length (mm)	Product number
BioPro IEX QA	5	4.6 X 30	QAA0S05-0346WP
		4.6 X 50	QAA0S05-0546WP
		4.6 X 100	QAA0S05-1046WP
		4.6 X 30	SPA0S05-0346WP
BioPro IEX SP	5	4.6 X 50	SPA0S05-0546WP
		4.6 X 100	SPA0S05-1046WP

High Throughput Ion Exchange Chromatography Media for Purification of Biopharmaceuticals

BioPro IEX SmartSep Q/S

BioPro IEX Q/S

Features

- High productivity on purification
- Hydrophilic polymer beads with low nonspecific adsorption
- High binding capacity and high resolution over a wide range of flow rate
- Suitable for purification of antibodies, proteins and nucleic acids

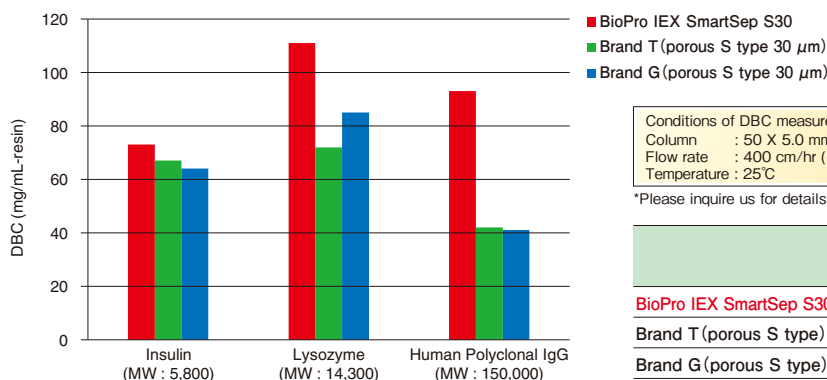


Specifications

	BioPro IEX SmartSep Q	BioPro IEX SmartSep S	BioPro IEX Q	BioPro IEX S
Matrix	Hydrophilic porous polymer			
Particle size (μm)	10, 20, 30		75	
Charged group	-R-N ⁺ (CH ₃) ₃	-R-SO ₃ ⁻	-R-N ⁺ (CH ₃) ₃	-R-SO ₃ ⁻
Ion exchange capacity (meq/mL-resin)	>0.08		>0.10	
Dynamic binding capacity (mg/mL-resin)	>100 (BSA)	>100 (lysozyme)	>160 (BSA)	>160 (lysozyme)
Usable pH range	2-12			

High sample loadability

High dynamic binding capacity (DBC) for various samples

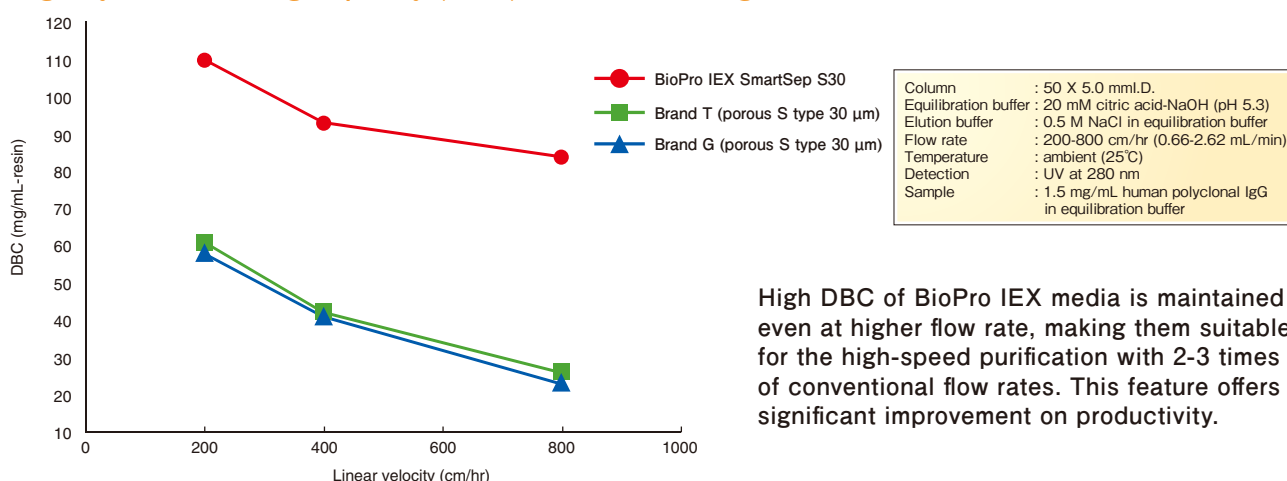


Conditions of DBC measurement*
 Column : 50 X 5.0 mm I.D.
 Flow rate : 400 cm/hr (1.32 mL/min)
 Temperature : 25°C

	Particle size (μm)	DBC (mg/mL-resin, 10% breakthrough)		
		Insulin	Lysozyme	Human Polyclonal IgG
BioPro IEX SmartSep S30	30	73	111	93
Brand T (porous S type)	30	67	72	42
Brand G (porous S type)	30	64	85	41

BioPro IEX media have higher DBC compared to conventional ion exchange media. Especially for IgG, BioPro IEX media have more than twice as high DBC as competitors' media. This feature of BioPro IEX media make purification productivity of IgG per unit time double or more.

High dynamic binding capacity (DBC) over a wide range of flow rate



Column : 50 X 5.0 mm I.D.
 Equilibration buffer : 20 mM citric acid-NaOH (pH 5.3)
 Elution buffer : 0.5 M NaCl in equilibration buffer
 Flow rate : 200-800 cm/hr (0.66-2.62 mL/min)
 Temperature : ambient (25°C)
 Detection : UV at 280 nm
 Sample : 1.5 mg/mL human polyclonal IgG in equilibration buffer

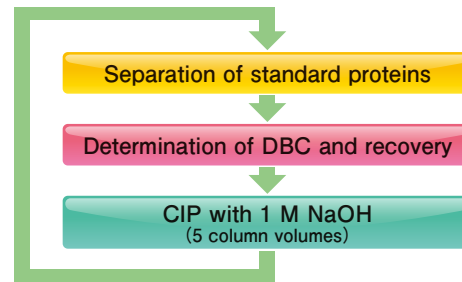
High DBC of BioPro IEX media is maintained even at higher flow rate, making them suitable for the high-speed purification with 2-3 times of conventional flow rates. This feature offers significant improvement on productivity.

■ Excellent durability

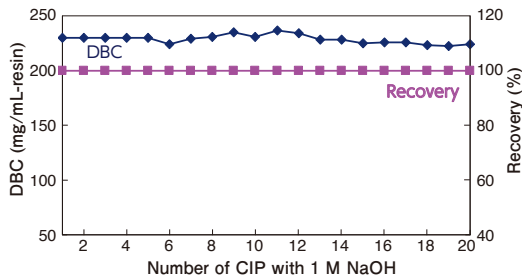
Stability on CIP

Cleaning in place (CIP) is an important procedure for cleaning and sterilization of columns used for protein purification. The DBC and the selectivity of proteins are unaffected following 20 cycles of CIP with 1 M NaOH. The high chemical stability of BioPro IEX media allow effective cleaning with alkaline solution.

Test protocols

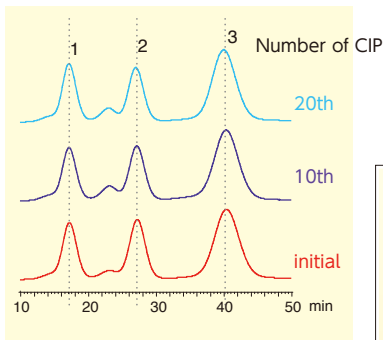


DBC and recovery



Conditions of DBC measurement	
Column	: BioPro IEX S75, 50 X 5.0 mmI.D.
Flow rate	: 800 cm/hr (2.62 mL/min)
Equilibration buffer	: 20 mM Glycine-NaOH (pH 9.0)
Elution buffer	: 0.5 M NaCl in equilibration buffer
Sample	: 1.0 mg/mL Lysozyme in equilibration buffer
Temperature	: ambient
Detection	: UV at 300 nm
*DBC was determined at 10% breakthrough	

Separation of standard proteins



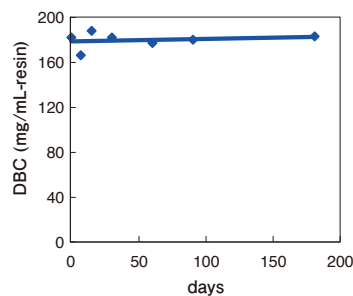
Conditions of separation of standard proteins	
Column	: BioPro IEX S75, 50 X 5.0 mmI.D.
Eluent	: A) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.8) B) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.8) containing 0.5 M NaCl
Gradient	: 0-100%B (0.60 min; Linear)
Flow rate	: 180 cm/hr (0.59 mL/min)
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 24 µL
Sample	: 1. Ribonuclease A, 2. Cytochrome c, 3. Lysozyme (0.5 mg/mL)

Stability on storage in alkaline solution

BioPro IEX media have high stability under alkaline condition. This feature is effective for storing the medium in alkaline solution as well as CIP.

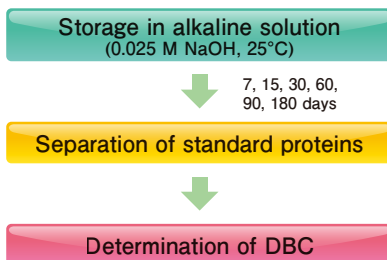
We recommend storing the medium in 20% ethanol aqueous solution in general.

Change in DBC

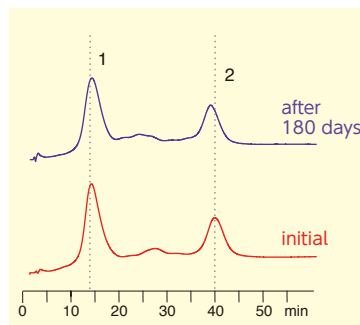


Conditions of DBC measurement	
Column	: BioPro IEX Q75, 50 X 4.6 mmI.D.
Equilibration buffer	: 20 mM Tris-HCl (pH 8.6)
Elution buffer	: 0.5 M NaCl in equilibration buffer
Flow rate	: 180 cm/hr (0.50 mL/min)
Sample	: 1.5 mg/mL BSA in equilibration buffer
Temperature	: 25°C
Detection	: UV at 280 nm
*DBC was determined at 10% breakthrough	

Test protocols

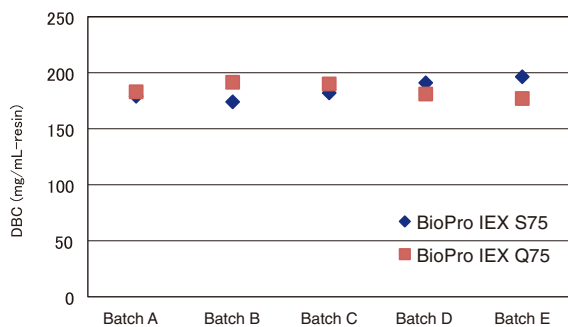


Separation of standard proteins



Conditions of separation of standard proteins	
Column	: BioPro IEX Q75, 50 X 4.6 mmI.D.
Eluent	: A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
Gradient	: 10-80%B (0.60 min; Linear)
Flow rate	: 180 cm/hr (0.50 mL/min)
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 20 µL
Sample	: 1. Transferrin (0.25 mg/mL), 2. Trypsin inhibitor (0.5 mg/mL)

Excellent batch-to-batch reproducibility of DBC



Column	: 50 X 4.6 mm I.D
Flow rate	: 180 cm/hr
for anion-exchange resin	
Equilibration buffer	: 20 mM Tris-HCl (pH 8.6)
Elution buffer	: 0.5 M NaCl in equilibration buffer
Sample	: 1.5 mg/mL BSA in equilibration buffer
Detection	: UV at 280 nm
for cation-exchange resin	
Equilibration buffer	: 20 mM Glycine-NaOH (pH 9.0)
Elution buffer	: 0.5 M NaCl in equilibration buffer
Sample	: 1.5 mg/mL Lysozyme in equilibration buffer
Detection	: UV at 300 nm

BioPro IEX media exhibit excellent batch-to-batch reproducibility of DBC. All the medium batches are inspected by various quality control tests. We supply stable products over a long period of time.

BioPro Ion Exchange Screening Kit



The BioPro Ion Exchange Screening Kit is a set of screening columns packed with BioPro IEX media.

- Two column types (1 mL and 5 mL) ideal for media screening, development of purification methods, and loadability studies
- Easy installation and convenient use

Ordering information

BioPro IEX SmartSep Q/S

Packing material	Particle size (μm)	Charged group	Product number
BioPro IEX SmartSep Q10	10	-R-N ⁺ (CH ₃) ₃	QSA0S10
BioPro IEX SmartSep S10		-R-SO ₃ ⁻	SSA0S10
BioPro IEX SmartSep Q20	20	-R-N ⁺ (CH ₃) ₃	QSA0S20
BioPro IEX SmartSep S20		-R-SO ₃ ⁻	SSA0S20
BioPro IEX SmartSep Q30	30	-R-N ⁺ (CH ₃) ₃	QSA0S30
BioPro IEX SmartSep S30		-R-SO ₃ ⁻	SSA0S30

BioPro IEX Q/S

Packing material	Particle size (μm)	Charged group	Product number
BioPro IEX Q75	75	-R-N ⁺ (CH ₃) ₃	QAA0S75
BioPro IEX S75		-R-SO ₃ ⁻	SPA0S75

BioPro Ion Exchange Screening Kit

Packing material	Particle size (μm)	Specification	Column volume	Product number
BioPro IEX SmartSep Q20	20	5/pack	1 mL	BPQSA0S20-01PK
			5 mL	BPQSA0S20-05PK
BioPro IEX SmartSep S20			1 mL	BPSSA0S20-01PK
			5 mL	BPSSA0S20-05PK
BioPro IEX SmartSep Q30	30	5/pack	1 mL	BPQSA0S30-01PK
			5 mL	BPQSA0S30-05PK
BioPro IEX SmartSep S30			1 mL	BPSSA0S30-01PK
			5 mL	BPSSA0S30-05PK

BioPro IEX Q/S

Packing material	Particle size (μm)	Specification	Column volume	Product number
BioPro IEX Q75	75	5/pack	1 mL	BPQAA0S75-01PK
			5 mL	BPQAA0S75-05PK
BioPro IEX S75			1 mL	BPSPA0S75-01PK
			5 mL	BPSPA0S75-05PK

Size Exclusion Chromatography Column for Separation of Monoclonal Antibody (MAb)

YMC-SEC MAB

Features

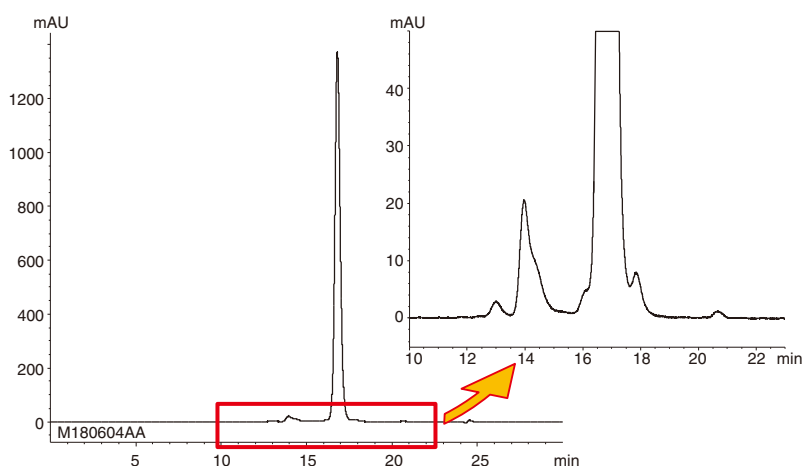
- Silica based SEC column optimized for separation of MAb
- Suitable for separation of aggregates and fragments of MAb
- Excellent resolution and peak shapes

Specifications

Base	Silica gel
Particle size (μm)	3
Pore size (\AA)	250
Functional group	Dihydroxypropyl
Usable pH range	5-7.5
Molecular weight range	10,000-700,000

Suitable for separation of aggregates and fragments of MAb

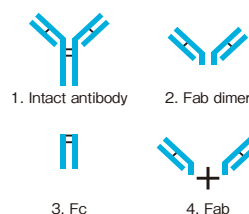
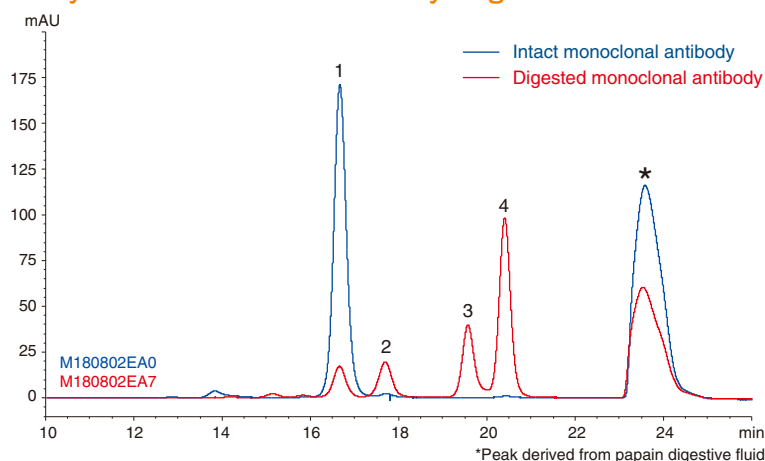
Analysis of monoclonal antibody aggregates



Column : YMC-SEC MAB (3 μm , 250 \AA)
 300 X 4.6 mm I.D.
 Eluent : 0.1 M $\text{KH}_2\text{PO}_4\text{-K}_2\text{HPO}_4$ (pH 7.0)
 containing 0.2 M NaCl
 Flow rate : 0.165 mL/min
 Temperature : 25°C
 Detection : UV at 280 nm
 Injection : 10 μL
 Sample : Humanized monoclonal antibody (5 mg/mL)

Humanized monoclonal antibody was analyzed using YMC-SEC MAB. The chromatogram shows excellent peak shape, and good separation between aggregates and a monomer peak. YMC-SEC MAB is effective for characterization and quality control of antibody therapeutics.

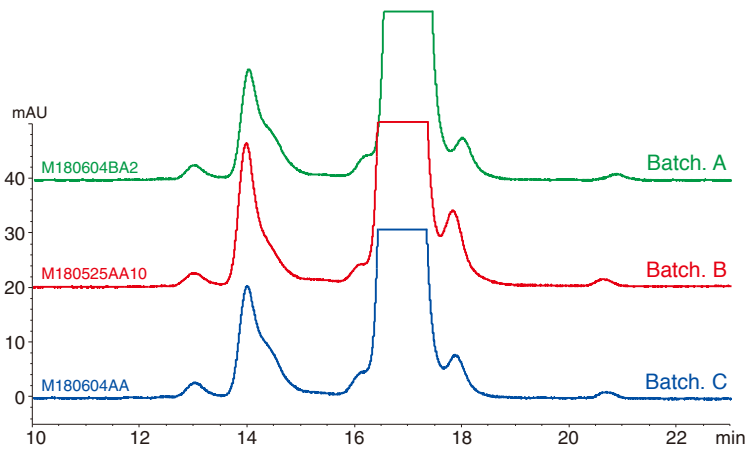
Analysis of monoclonal antibody fragments



Column : YMC-SEC MAB (3 μm , 250 \AA)
 300 X 4.6 mm I.D.
 Eluent : 0.1 M $\text{KH}_2\text{PO}_4\text{-K}_2\text{HPO}_4$ (pH 7.0)
 containing 0.2 M NaCl
 Flow rate : 0.165 mL/min
 Temperature : 25°C
 Detection : UV at 280 nm
 Injection : 2 μL (3 mg/mL)

A monoclonal antibody digested by papain and an intact monoclonal antibody were analyzed using YMC-SEC MAB column. Since the column shows excellent separation between Fc and Fab fragments, which have similar amount of MW, it is effective for separation of antibody fragments.

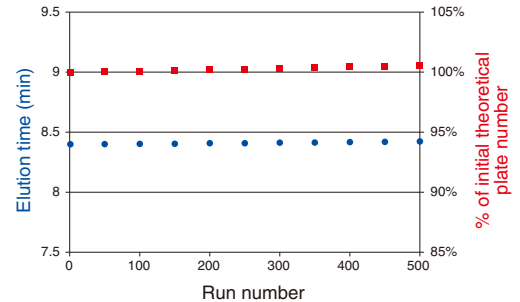
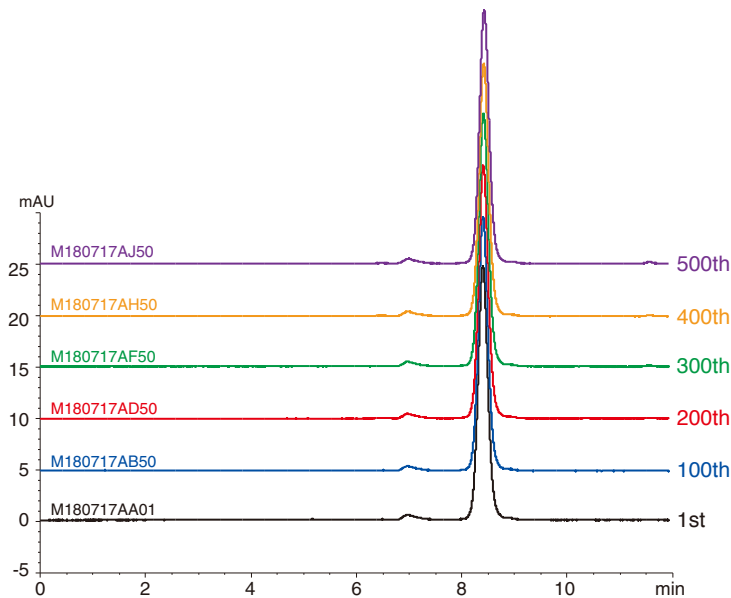
Batch-to-batch reproducibility



Column : YMC-SEC MAB (3 μ m, 250 \AA)
 300 X 4.6 mm.I.D.
 Eluent : 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0)
 containing 0.2 M NaCl
 Flow rate : 0.165 mL/min
 Temperature : 25°C
 Detection : UV at 280 nm
 Injection : 10 μ L
 Sample : Humanized monoclonal antibody (5 mg/mL)

A three-batch comparison is shown on the left. YMC-SEC MAB achieves excellent reproducibility of separation of monomer and aggregates as well as monomer and fragments and is effective for quality control.

Excellent durability



Column : YMC-SEC MAB (3 μ m, 250 \AA)
 300 X 4.6 mm.I.D.
 Eluent : 0.1 M NaH_2PO_4 - Na_2HPO_4 (pH 7.0)
 containing 0.2 M NaCl
 Flow rate : 0.33 mL/min
 Temperature : 25°C
 Detection : UV at 280 nm
 Injection : 2 μ L
 Sample : Humanized monoclonal antibody (1 mg/mL)

YMC-SEC MAB column has excellent repeatability of separation without any change of theoretical plate number and elution time even after repeated separation of monoclonal antibody for more than 500 times because of its superior durability.

Ordering information

Particle size (μ m)	Column size inner diameter X length (mm)	Product number
3	4.6 X 300	DLM25S03-3046WT

Please contact us about the product with other sizes.

Silica-based Size Exclusion Chromatography Columns

YMC-Pack Diol

Features

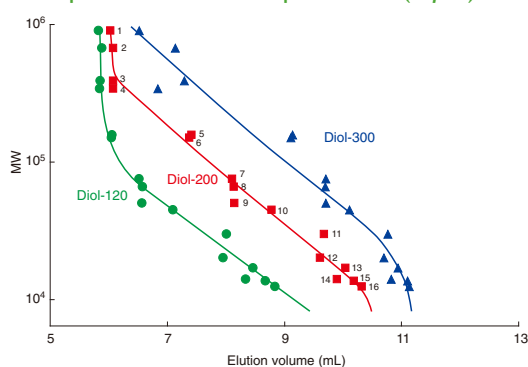
- Silica-based columns with high mechanical stability
- Useful for molecular weight determination of proteins and sugars
- High-resolution and fast analysis using 2 μm , 3 μm particle

Specifications

Column	Base	Functional group	Pore size (Å)	Particle size (μm)	Usable pH range	Characteristics
Diol-60	Silica gel	Dihydroxypropyl	60	3, 5	5-7.5	For molecular weights below 10,000
Diol-120			120			For molecular weights 1,000 to 100,000
Diol-200			200	For molecular weights 5,000 to 300,000		
Diol-300			300	For molecular weights 20,000 to 1,000,000		

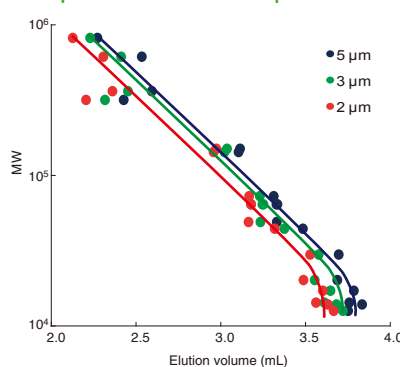
Calibration curves of various proteins

Comparison of different pore sizes (5 μm)



Column : YMC-Pack Diol
300 X 8.0 mmI.D.
Eluent : 0.1 M $\text{KH}_2\text{PO}_4\text{-K}_2\text{HPO}_4$ (pH 7.0)
containing 0.2 M NaCl
Flow rate : 0.5 mL/min
Temperature : 25°C
Detection : UV at 280 nm

Comparison of different particle sizes (Diol-300)

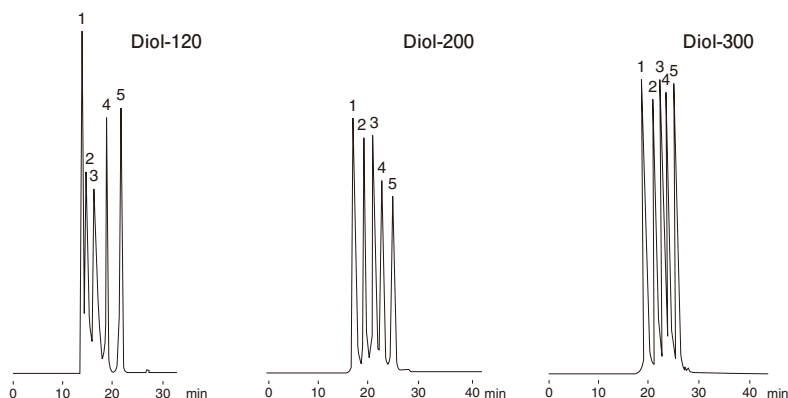


Column : YMC-Pack Diol
300 X 4.6 mmI.D.
Eluent : 0.1 M $\text{KH}_2\text{PO}_4\text{-K}_2\text{HPO}_4$ (pH 7.0)
containing 0.2 M NaCl
Flow rate : 0.165 mL/min
Temperature : 25°C
Detection : UV at 280 nm

	MW
1. IgM	900,000
2. Thyroglobulin	670,000
3. IgA	390,000
4. Fibrinogen	340,000
5. γ -Globulin	158,000
6. IgG	150,000
7. Transferrin	75,000
8. HSA (human serum albumin)	66,000
9. α_1 -Antitrypsin	50,000
10. Ovalbumin	45,000
11. Carbonic anhydrase	30,000
12. Trypsin inhibitor	20,100
13. Myoglobin	17,000
14. α -Lactalbumin	14,100
15. Ribonuclease A	13,700
16. Cytochrome c	12,400

Diol-120, Diol-200, and Diol-300 are suitable for the separation or molecular weight determination of proteins with molecular weights from 1,000 to 1,000,000. Calibration curves of proteins with different particle sizes using YMC-Pack Diol are almost the same and this enables easy method transfer between UHPLC and HPLC.

Separation for standard protein markers

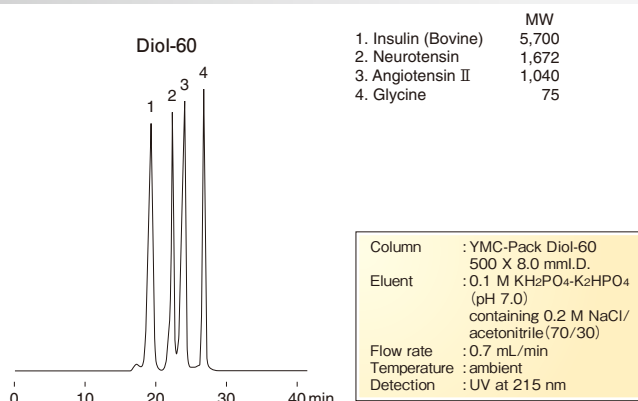


	MW
1. Glutamate dehydrogenase	290,000
2. Lactate dehydrogenase	142,000
3. Enolase	67,000
4. Adenylate kinase	32,000
5. Cytochrome c	12,400

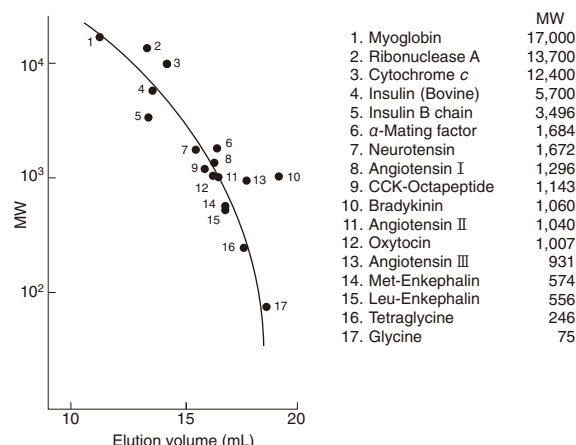
Column : YMC-Pack Diol
500 X 8.0 mmI.D.
Eluent : 0.1 M $\text{KH}_2\text{PO}_4\text{-K}_2\text{HPO}_4$ (pH 7.0)
containing 0.2 M NaCl
Flow rate : 0.7 mL/min
Temperature : ambient
Detection : UV at 280 nm

For molecular weights from 5,000 to 300,000 substances, Diol-200 is suitable for this separation.

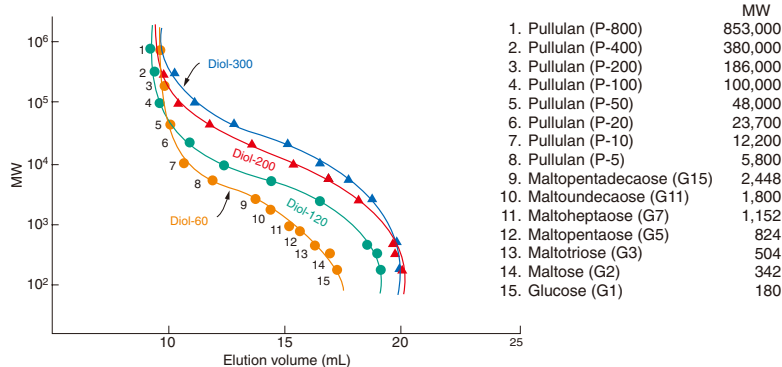
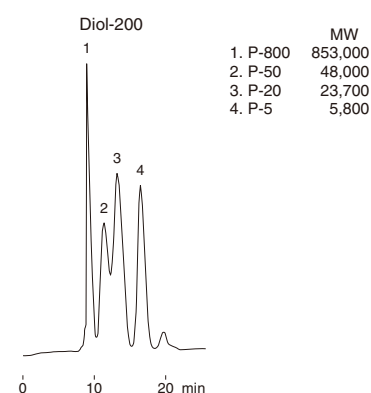
■ Separation of peptides with molecular weights less than 10,000



For peptides with molecular weights less than 10,000, Diol-60 is suitable for the separation.



■ Separation of oligo- and polysaccharides



Column : YMC-Pack Diol, 500 X 8.0 mm.I.D.
Eluent : water
Flow rate : 1.0 mL/min
Temperature : ambient
Detection : RI

For separation or molecular weight determination of water-soluble oligo- and polysaccharides, Diol-60, Diol-120, Diol-200, and Diol-300 are useful individually or in combination.

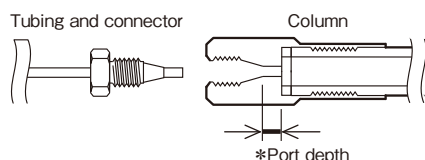
■ Ordering information

YMC-Pack Diol (stainless steel column)

Packing material	Particle size (μm)	Pore Size (Å)	Column size inner diameter X length (mm)	Product number
Diol-60	3	60	4.6 X 300	DL06S03-3046WT
			4.6 X 300	DL06S05-3046WT
	5	60	8.0 X 300	DL06S05-3008WT
			8.0 X 500	DL06S05-5008WT
			20 X 300	DL06S05-3020WT
			20 X 500	DL06S05-5020WT
Diol-120	3	120	4.6 X 300	DL12S03-3046WT
			4.6 X 300	DL12S05-3046WT
	5	120	8.0 X 300	DL12S05-3008WT
			8.0 X 500	DL12S05-5008WT
			20 X 300	DL12S05-3020WT
			20 X 500	DL12S05-5020WT

Packing material	Particle size (μm)	Pore Size (Å)	Column size inner diameter X length (mm)	Product number
Diol-200	2	200	4.6 X 150	DL20S02-1546PTH
			4.6 X 300	DL20S02-3046PTH
	3	200	4.6 X 300	DL20S03-3046WT
			4.6 X 300	DL20S05-3046WT
	5	200	4.6 X 300	DL20S05-3008WT
			8.0 X 300	DL20S05-3008WT
			8.0 X 500	DL20S05-5008WT
			20 X 300	DL20S05-3020WT
			20 X 500	DL20S05-5020WT
			20 X 500	DL20S05-5020WT
Diol-300	2	300	4.6 X 150	DL30S02-1546PTH
			4.6 X 300	DL30S02-3046PTH
	3	300	4.6 X 300	DL30S03-3046WT
			4.6 X 300	DL30S05-3046WT
	5	300	4.6 X 300	DL30S05-3008WT
			8.0 X 300	DL30S05-3008WT
			8.0 X 500	DL30S05-5008WT
			20 X 300	DL30S05-3020WT
			20 X 500	DL30S05-5020WT
			20 X 500	DL30S05-5020WT

Consideration of connector and column fittings



Please see our website for guard columns and glass columns.

The end of the product number	*Port depth	Style of endfitting
PTH	2 mm	Parker style (UPLC compatible)
WT	3 mm	Waters style

UPLC is registered trademark of Waters Corporation

Hydrophobic Interaction Chromatography Column

BioPro HIC BF

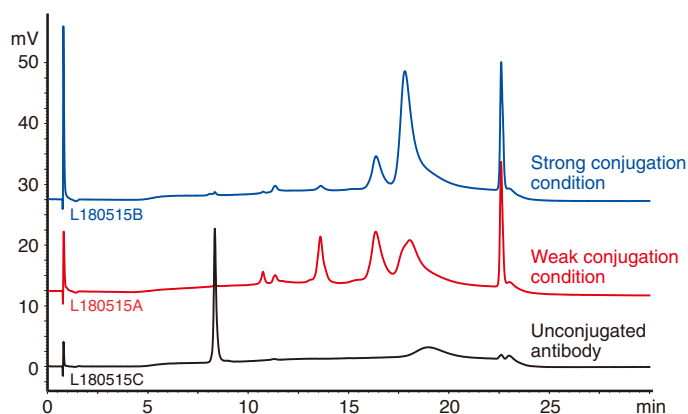
Features

- Effective for separation of proteins and antibodies such as Antibody-Drug Conjugates (ADCs)
- High resolution comparable to sub-3 μm with low operating pressure
- Fast analysis at high flow rates
- Usable for laboratory-scale purification

Specifications

Matrix	Hydrophilic non-porous polymer
Particle size (μm)	4
Functional group	Butyl group
Usable temperature	10-60°C
Usable pH range	2-12

Effective for Drug-to-Antibody Ratio (DAR) analysis of ADCs



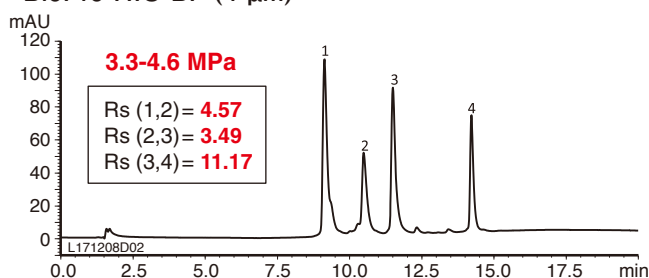
Column : BioPro HIC BF 4 μm , 100 X 4.6 mmI.D.
 Eluent : A) 50 mM NaH₂PO₄-Na₂HPO₄ (pH 7.0) containing 1.5 M (NH₄)₂SO₄/2-propanol (95/5)
 B) 50 mM NaH₂PO₄-Na₂HPO₄ (pH 7.0)/2-propanol (80/20)
 0%B (0-1 min), 0-100%B (1-15 min), 100%B (15-20 min), 0%B (20-30 min)
 Flow rate : 1.0 mL/min
 Temperature : 25°C
 Detection : UV at 280 nm
 Sample : Antibody Drug Conjugate*

*Courtesy by RIKEN.

ADCs with different conjugation condition were analyzed using BioPro HIC BF. These results indicate the increase of ADC with high DAR under the strong condition compared to the weak condition. BioPro HIC BF column provides superior separation for ADCs, therefore it is effective for DAR determination and monitoring of conjugation reactions.

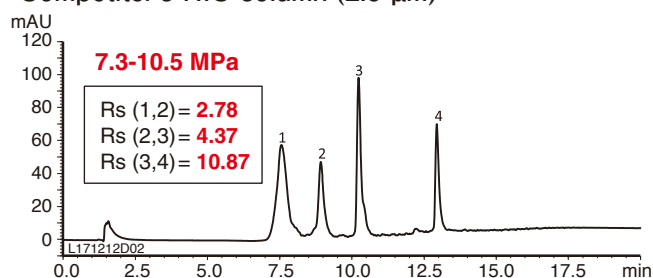
High resolution comparable to sub-3 μm with low operating pressure

BioPro HIC BF (4 μm)



Column : 100 X 4.6 mmI.D.
 Eluent : A) 100 mM NaH₂PO₄-Na₂HPO₄ (pH 7.0) containing 2.0 M (NH₄)₂SO₄
 B) 100 mM NaH₂PO₄-Na₂HPO₄ (pH 7.0)
 0-100%B (0-11 min), 100%B (11-15 min)
 Flow rate : 0.5 mL/min
 Temperature : 25°C
 Detection : UV at 280 nm
 Injection : 15 μL

Competitor's HIC column (2.5 μm)



The separation of proteins is compared on BioPro HIC BF with particle size 4 μm and a competitor's HIC column with particle size 2.5 μm . BioPro HIC BF with optimized media design and packing technology has superior resolution comparable to competitor's column with sub-3 μm .

Ordering information

Particle size (μm)	Column size inner diameter X length (mm)	Product number
4	4.6 X 100	BHB00S04-1046WT

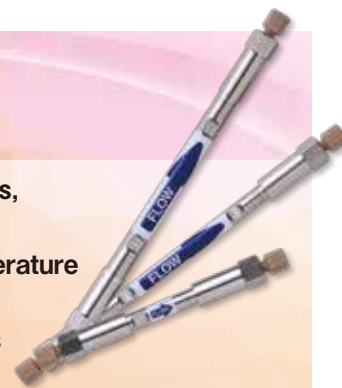
Please contact us about the product with other sizes. Preparative columns are also available.

Reversed-phase Chromatography Columns

YMC-Triart/YMC-Triart Bio

Features

- Useful for separation of biomolecules such as peptides, proteins, antibodies, and oligonucleotides
- Excellent chemical durability enables analysis under high temperature
- Good peak shapes with mobile phase suitable for LC/MS
- Various column sizes are available for separation from analysis to preparative scale



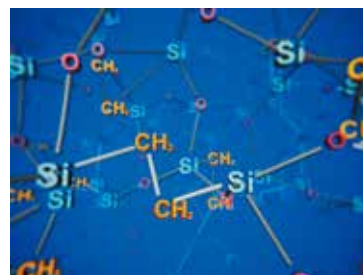
Specifications

	Triart C18	Triart C8	Triart Bio C4
Base	Organic/inorganic hybrid silica		
Particle size (μm)	1.9, 3, 5		
Pore size (\AA)	120		300
Bonding	Trifunctional		
Endcapping	Yes		
Usable pH range	1-12		1-10
Max. temperature	90°C for pH 1-7 50°C for pH 7-12		90°C for pH 1-7 50°C for pH 7-10

Please see our website for the columns with other functional groups.

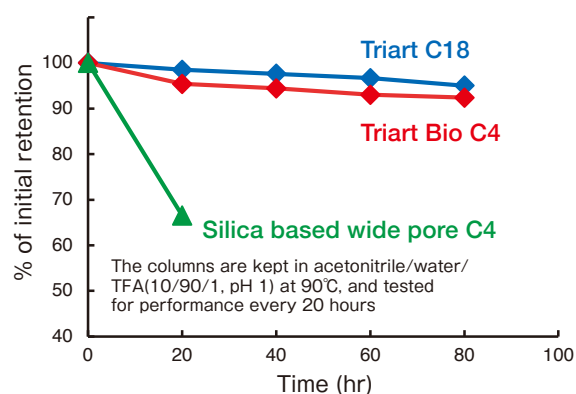
Versatile hybrid base material

YMC-Triart is based on novel organic/inorganic hybrid particles. The particle combines high mechanical stability and high efficiency derived from silica based packing material and high chemical stability derived from polymer based packing material. The granulation process utilizing microreactor technology enables continuous and highly controlled production of hybrid particles. The particle has uniform pore size distribution and smooth surface as well as uniform particle size. This feature greatly contributes to excellent peak shape and separation reproducibility.



Excellent chemical durability

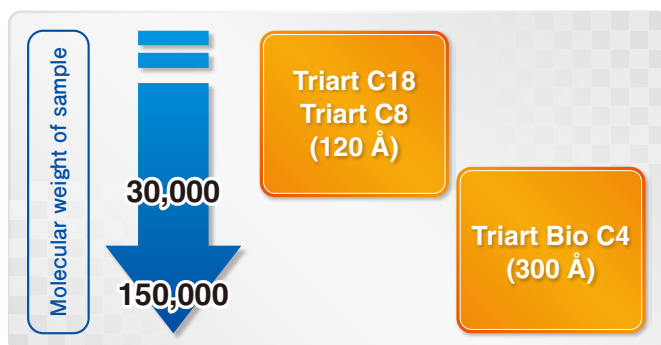
pH 1 (1% TFA), 90°C



Test conditions
 Column : 5 μm , 150 X 3.0 mm I.D. for C4
 5 μm , 50 X 2.0 mm I.D. for C18
 Eluent : acetonitrile/water (60/40)
 Flow rate : 0.4 mL/min for 3.0 mm I.D.
 0.2 mL/min for 2.0 mm I.D.
 Temperature : 37°C
 Sample : butyl benzoate

Triart columns are effective in improving chromatographic outcomes for stabilizing retention time (and also peak shape) due to the use of innovative surface modification of the organic/inorganic hybrid silica. When separating proteins and peptides, Triart offers excellent durability even when using mobile phases containing up to 1% TFA or column temperatures of 90°C are utilized.

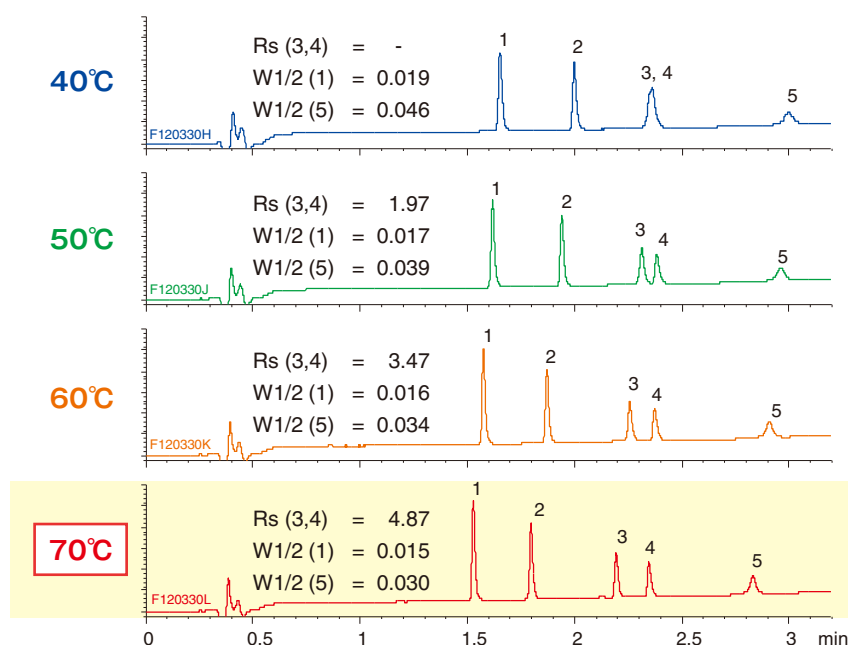
Column selection guide for proteins and peptides



Columns are selected based on molecular weights of the target substances to separate proteins and peptides. Triart C18/C8 with pore size 120 Å provide the good separation of substances with molecular weight of up to 30,000 under high temperature. For separation of substances with larger molecules, wide pore columns are effective. Triart Bio C4 with pore size 300 Å can perform separation of substances with molecular weight of up to 150,000 under high temperature.

An elevated temperature can improve efficiency and peak shape by lowering the viscosity of the mobile phase and improving mass transfer. The appropriate molecular weight range for a given pore size of Triart can be expanded compared to using the same pore size at lower temperature.

Effect of column temperature on separation

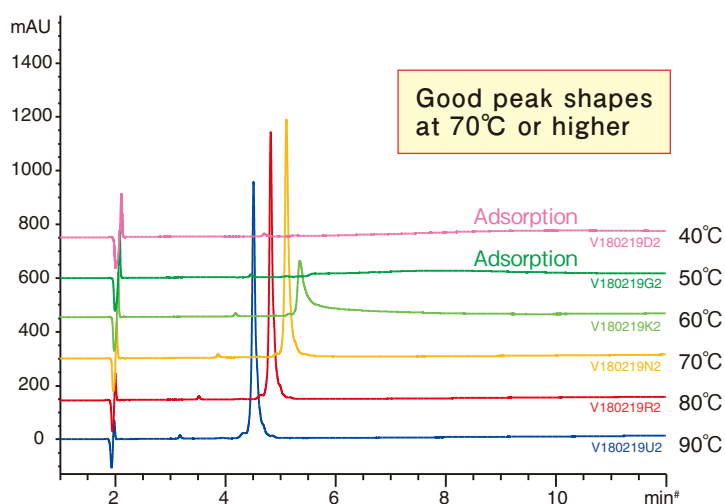


1. Oxytocin (MW 1,007)
2. Leu-Enkephalin (MW 556)
3. β -Endorphin (MW 3,465)
4. Insulin (MW 5,733)
5. β -Lactoglobulin A (MW 18,400)

Column : YMC-Triart C18 (1.9 μ m, 120 Å)
50 X 2.0 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
10-80%B (0-5 min)
Flow rate : 0.4 mL/min
Detection : UV at 220 nm

Increasing column temperature to 70°C provides selectivity change, sharper peaks, and therefore improved resolution especially for larger molecules.

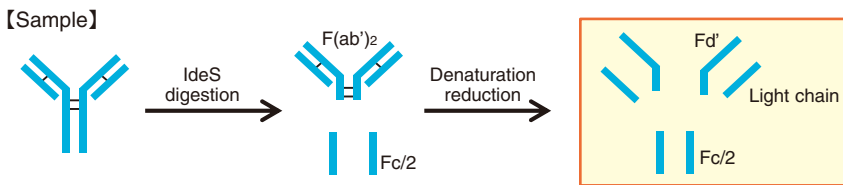
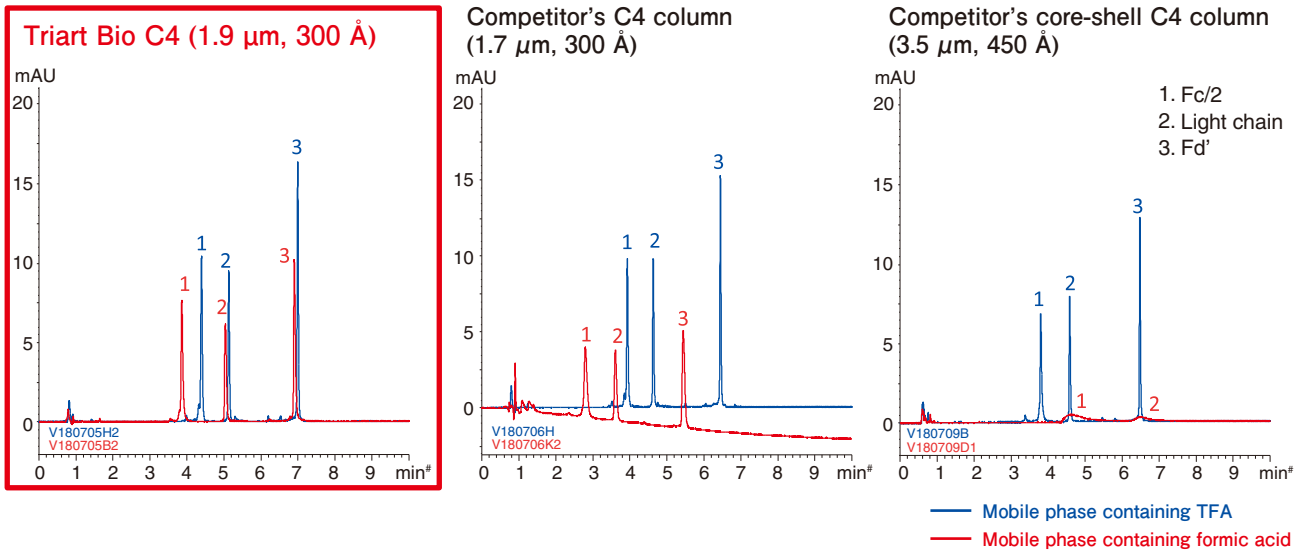
Useful for separation of macromolecules such as antibodies



Column : YMC-Triart Bio C4 (3 μ m, 300 Å)
150 X 3.0 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
30-60%B (0-15 min), 90%B (15-30 min)
Flow rate : 0.4 mL/min
Detection : UV at 220 nm
Injection : 4 μ L
Sample : Humanized monoclonal IgG1

Intact monoclonal antibody was analyzed at temperatures between 40°C and 90°C using Triart Bio C4. Good peak shapes were acquired at 70°C and higher while there was no elution at 50°C and lower. Highly durable Triart Bio C4 can perform stable analysis even at 90°C and is suitable for the reversed-phase analysis of antibodies at high temperatures.

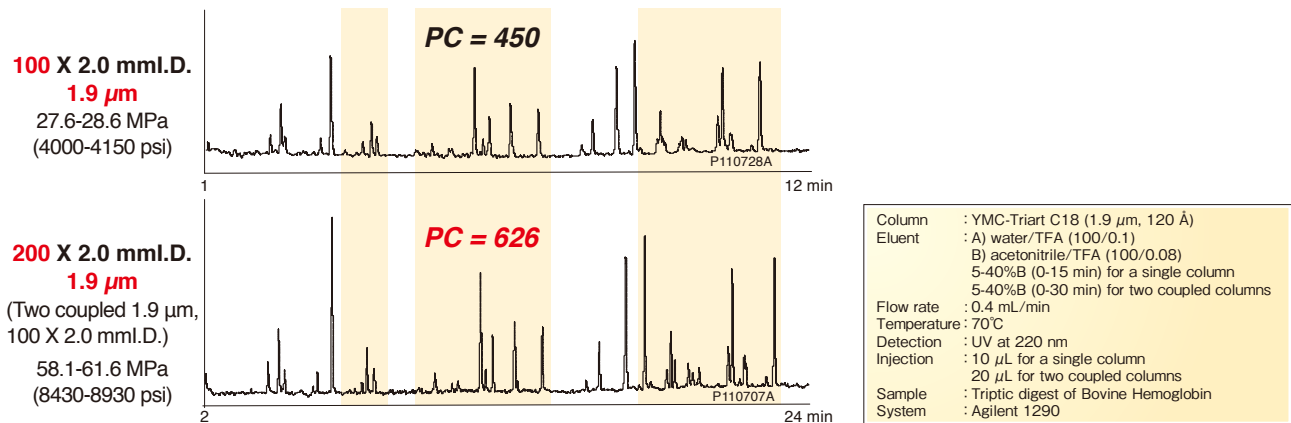
Good peak shapes with mobile phase suitable for LC/MS



Column size	: 150 X 2.1 mm I.D.
Eluent <TFA>	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 25-50%B (0-10 min), 90%B (10-12.5 min)
Eluent <Formic acid>	: A) water/formic acid (100/0.1) B) acetonitrile/formic acid (100/0.1) 20-45%B (0-10 min), 90%B (10-12.5 min)
Detection	: UV at 280 nm
Flow rate	: 0.4 mL/min
Temperature	: 80°C
Injection	: 4 μL (0.25 mg/mL)
Sample	: mAb Subunit Standard (Waters Corporation)

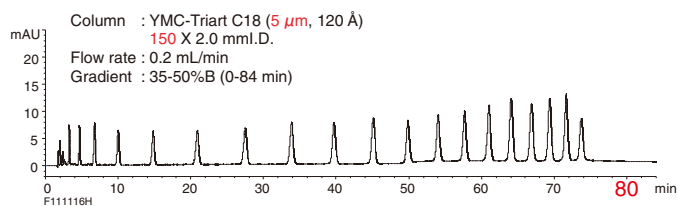
Above are the chromatograms of monoclonal antibody fragments analysis with mobile phase containing either TFA or formic acid. Triart Bio C4 achieves excellent peak shape even when using mobile phase containing formic acid. Therefore it is ideal for the high sensitive LC/MS analysis.

Peptide mapping



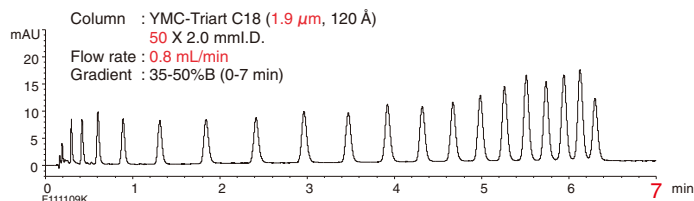
Triart 1.9 μm column has superior column efficiency, and a coupling of two 100 mm length of Triart 1.9 μm columns offers outstanding separation ability. This allows the precise separation in an analysis of complicated samples, such as peptide mapping.

Separation of oligonucleotides



Oligonucleotides d(T)₂₋₂₀

Eluent	: A) 10 mM di- <i>n</i> -butylamine-acetic acid (pH 6.0) B) methanol
Temperature	: 35°C
Detection	: UV at 269 nm
Injection	: 1 μL (5 nmol/mL)



In the separation of oligonucleotides, 19 peaks are completely resolved within 7 minutes using a Triart C18 1.9 μm UHPLC column. The separation is achieved within one tenth of the analysis time of the conventional HPLC method.

Ordering information

Packing material	Code
Triart C18	TA
Triart C8	TO
Triart Bio C4	TB

Pore size (\AA)	Code
120	12
300	30

Particle size (μm)	Code
1.9	SP9
3	S03
5	S05

Column length (mm)	Code
20	02
30	03
33	H3
35	H5
50	05
75	L5
100	10
125	R5
150	15
250	25

Inner diameter (mm)	Code
2.0	02
2.1	Q1
3.0	03
4.0	04
4.6	46
6.0	06
10	10
20	20
30	30

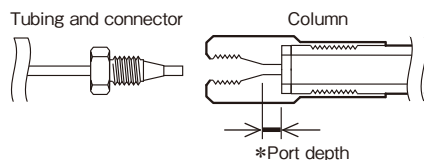
Column Type	Code
Waters style	WT/WX
Parker style	PT/PTH
Metal free column	PTP

Example) YMC-Triart C18 1.9 μm , 100 X 2.0 mmI.D.

Packing material	Pore size	Particle size	Column length	Inner diameter	Column Type
TA	12	SP9	100	2.0	PT

Product number : TA12SP9-1002PT

Consideration of connector and column fittings



The end of the product number	*Port depth	Style of endfitting
PT / PTH / PTP	2 mm	Parker style (UPLC compatible)
WT / WX	3 mm	Waters style

UPLC is registered trademark of Waters Corporation

Please note that combinations of features cannot be selected at random, but only from the possible specifications for a chosen stationary phase. These can be determined from our website.

Preparative Systems/Columns

Glass Columns ECOPLUS

Features

- Bio compatible
- Universal application
- Aqueous buffer (AB) versions and solvent resistant (SR) versions are available
- Low temperature versions available with polyethylene plunger and EPDM sealing ring
- Height adjustable plungers at both ends
- Easy to use
- Compatible with any LC systems



Versatile high-performance glass columns

ECO^{PLUS} glass columns are multi-purpose columns for almost all types of liquid chromatography applications (pressure limit 1.5 to 8.0 MPa). They are available in two forms; AB (Aqueous Buffer) versions for aqueous buffer and cold room applications, and SR (Solvent Resistant) versions for separations involving organic solvents.

With a choice of adjustable length plungers at each ends and a totally inert triple chevron sealing system, the SR versions support the complete spectrum of applications from classical normal phase and reversed-phase chromatography through to biochromatography.

The “Quick-Lock” connection system makes it possible to open and seal the column simply and quickly.



“Quick-Lock” Fitting

Easy self packing with optional packing adaptors

Packing adapters are used if the packing methods requires additional column volume during the packing process. More volume than given by the column is required when the column is packed with the slurry method. The ECO^{PLUS} series of packing adapters consist of a column coupler and an empty glass body. These must be of the same diameter as the column to be packed and must be used as packing adapters, not for extending the length of a column body during use.



Specifications

Model		TAC05	TAC10	TAC15	TAC25	TAC35	TAC50
Inner diameter (mm)		5	10	15	25	35	50
Pressure limit (MPa)	AB	8.0	8.0	7.0	5.0	4.0	3.0
	SR	8.0	5.0	5.0	5.0	4.0	1.5
Column lengths (mm)		125, 250, 500					
Usable temperature range	AB	4–40°C					
	SR	16–40°C					
Connection		1/4”–28G fittings (1/16” tubing)			1/4”–28G fittings (1/8” tubing)		
Frit	AB	Polyethylene					
	SR	Sintered glass			Stainless steel		
Options		Short plungers, short/long plungers, long plungers					

Please see our website for more details

Biochromatography Systems/Columns

Features

- Suitable for downstream purification process for biopharmaceuticals

Preparative LC systems BioStream

- Compliance with cGMP
- Sanitary design superior in cleaning
- Maximum flow rate: 30 L/min
- Customization is available upon request

Glass columns YMC Pilot

- All wetted parts are made of non-metal
- Safe and easy handling for packing
- Wide selection of inner diameter
- Various options available

Glass columns YMC Pilot^{PLUS}

- Upgraded columns of YMC Pilot
- Significant improvement for handling



Specifications

BioStream

Model	BSTP-800	BSTP-03K BSTS-03K	BSTS-10K	BSTS-30K
Max. flow rate (mL/min)	800	3000	10000	30000
System pressure limit (MPa)	0.5 (Max. 0.6)			
Dimensions (mm) (W x D x H)	800 × 900 × 1300	900 × 1100 × 1800	1300 × 1200 × 1800	1800 × 1500 × 1800
Control software	BioStream software			
GMP/CSV support	Yes			
Explosion-proof	No			
Features	Sanitary design, 3 wavelengths measurable simultaneously			

YMC Pilot

Model	PI100/500 PI100/850	PI140/500 PI140/850	PI200/500 PI200/850	PI300/500 PI300/850
Inner diameter (mm)	100	140	200	300
Column lengths (mm)	500, 850		500, 850	
Pressure limit (MPa)	1.0	0.7	0.5	0.3

Please contact us for the information of YMC Pilot^{PLUS}

Contract Purification

From Laboratory to Plant-scale Separation/Purification

Features

- Chromatographic purification with preparative LC systems manufactured in-house
- Purification method development to meet various requirements
- Seamless scale-up depending on the development stages
- Smooth transfer of the purification method and system to clients



Advantages of contract purification

Problems for customers

- Difficulty in purification method optimization
- Expensive columns and packing materials for purification
- No systems and facilities for preparative purification
- Shortage of time and labor

The solutions by outsourcing to YMC

- Accumulated experience and know-how of separation and purification
- Low-cost columns and packing materials manufactured in-house
- Large columns and preparative systems are available

High Purity Compounds

Quick Service

High Reliability

Low-Cost

Applicable Scale

GMP : grams to tons
non-GMP : milligrams to tons

Target Substances

Oligonucleotides Proteins
Peptides Chiral compounds

Before use (installation, operation, maintenance or check-up) of our products, instruction manuals should be carefully read and understood, and safety rules and precautions followed as outlined in the manuals.

Worldwide Availability

YMC America, Inc.
www.ymcamerica.com

YMC Europe GmbH
www.ymc.de

YMC Switzerland LLC
www.ymc-schweiz.ch

YMC Shanghai Rep. Office
www.ymcchina.com

YMC India Pvt. Ltd.
www.ymcindia.com

YMC Korea Co., Ltd.
www.ymckorea.com

YMC Taiwan Co., Ltd.
www.ymctaiwan.com

YMC Singapore Tradelinks Pte. Ltd.
www.ymc.sg

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