YMC

From Microanalysis To Plant-Scale Purification

Bioseparation

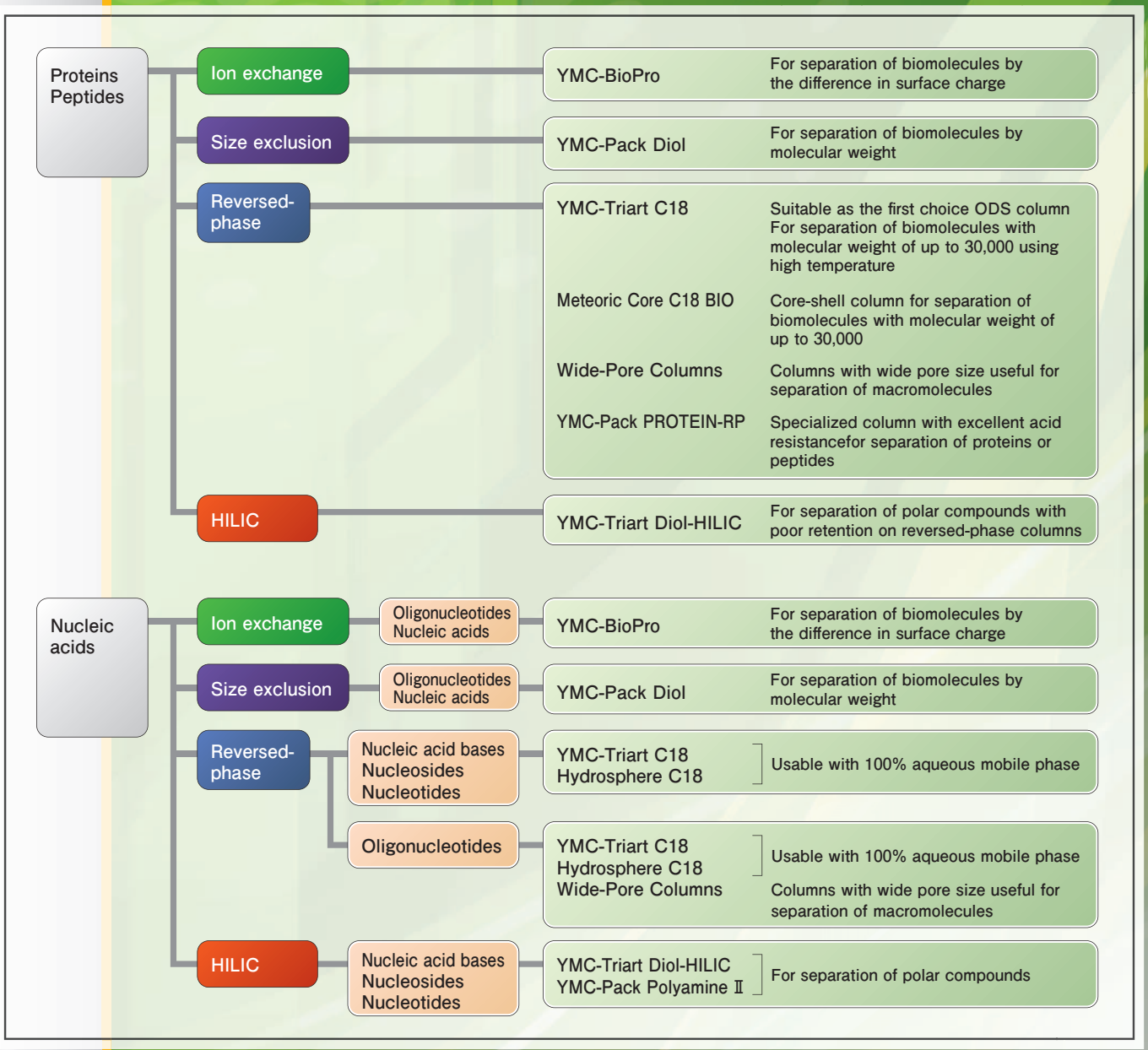
- **Column Selection Guide**
- **Comparison of Separation Mode**
- **Columns/Packing Materials**
  - Ion Exchange Columns/Media
  - Size Exclusion Columns
  - Reversed-phase Columns/Packing Materials
- **Preparative Systems**
  - Biochromatography Devices/Columns
  - Glass Columns

YMC CO., LTD.
http://www.ymc.co.jp

PB-0065E

Bioseparation

Column Selection Guide

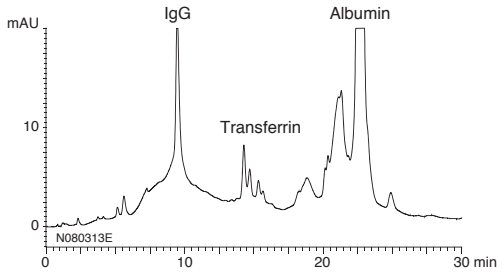


Comparison of Separation Mode

Separation of proteins using different modes

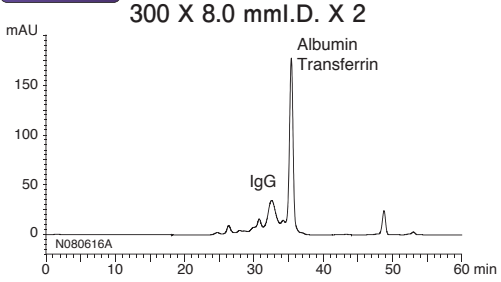
Human serum

Ion exchange YMC-BioPro QA 5 μm, 50 X 4.6 mmI.D



Eluent	: A) 20 mM Tris-HCl (pH 8.6) B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl 0-30%B (0-15 min), 30-100%B (15-30 min)
Flow rate	: 0.5 mL/min
Temperature	: 25°C
Detection	: UV at 280 nm
Injection	: 20 μL (100 μL/mL)

Size exclusion YMC-Pack Diol-300 + Diol-200 5 μm, 300 X 8.0 mmI.D. X 2

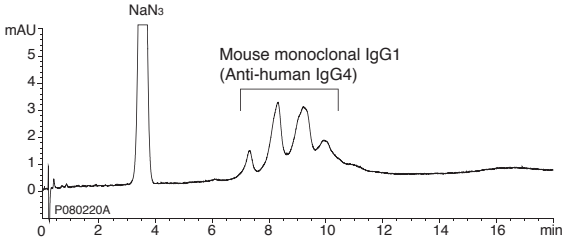


Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.5 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 280 nm
Injection	: 20 μL (100 μL/mL)

Proteins in human serum are separated by the difference in the surface charge on ion exchange chromatography (IEC) and by the difference in the molecular weight on size exclusion chromatography (SEC).

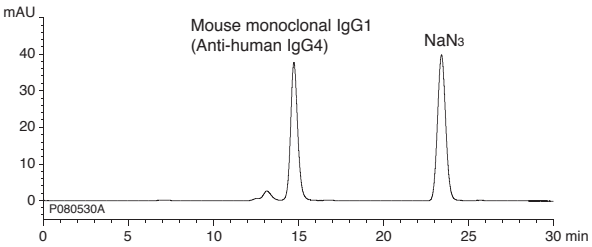
Mouse monoclonal IgG1 anti-human IgG4 (Purified by DEAE chromatography, containing NaN_3)

Ion exchange YMC-BioPro QA-F 5 μm, 30 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 10-25%B (0-18 min)
Flow rate	: 1.0 mL/min
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 10 μL (0.1 mg/mL)

Size exclusion YMC-Pack Diol-200 5 μm, 300 X 4.6 mmI.D.

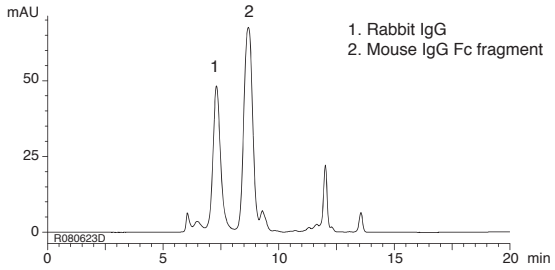


Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0)
Flow rate	: 0.17 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 220 nm
Injection	: 10 μL (0.05 mg/mL)

Mouse monoclonal antibody against human IgG4 is analyzed on ion exchange chromatography (IEC) and size exclusion chromatography (SEC). Several peaks possibly derived from isoform of antibody are observed in ion exchange mode, while a single peak is detected in size exclusion mode.

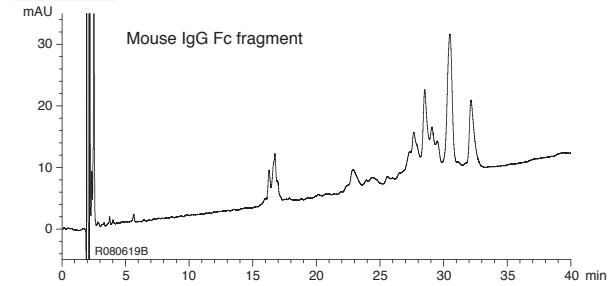
Mouse IgG Fc fragment (Prepared from normal serum)

Size exclusion YMC-Pack Diol-200 5 μm, 300 X 8.0 mmI.D.



Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 6.9) containing 0.2 M NaCl
Flow rate	: 1.0 mL/min
Temperature	: ambient (27°C)
Detection	: UV at 220 nm
Injection	: 5 μL (0.5 mg/mL)

Reversed-phase YMC-Pack C_4 (30 nm) 5 μm, 150 X 4.6 mmI.D.



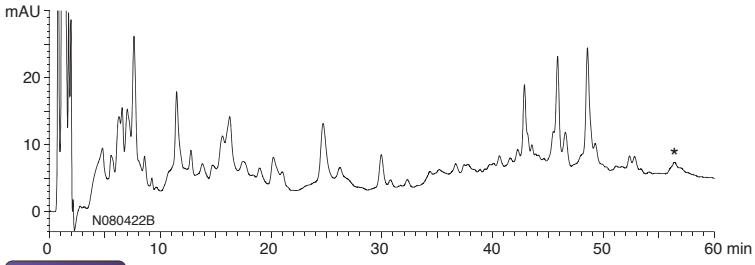
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 25-45%B (0-40 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 5 μL (1.0 mg/mL)

Size exclusion chromatography (SEC) is useful for separation of substances which have distinct differences in molecular weight, such as IgG and its fragments. On the other hand, reversed-phase chromatography (RPC) is suitable for a precise analysis of peptides and proteins with a molecular weight of less than 100 kDa such as IgG Fc fragment.

Separation of proteins using different modes

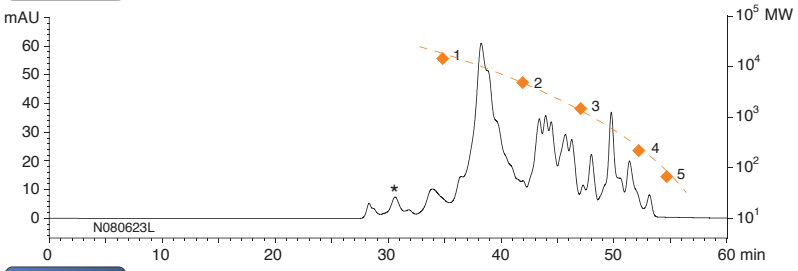
Tryptic digests of BSA

Ion exchange YMC-BioPro QA 5 μm, 50 X 4.6 mmI.D.



Eluent	: A) 20 mM Tris-HCl (pH 8.6) B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl 0-15%B (0-30 min), 15-60%B (30-60 min)
Flow rate	: 0.5 mL/min
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 20 μL

Size exclusion YMC-Pack Diol-120 + Diol-60 5 μm, 500 X 8.0 mmI.D. X 2

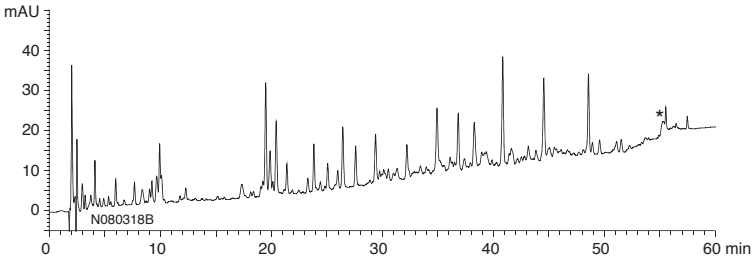


Calibration curve of peptides and proteins

1. Myoglobin (MW 17,000)
2. Insulin (Bovine) (MW 5,700)
3. Neurotensin (MW 1,672)
4. Tetraglycine (MW 246)
5. Glycine (MW 75)

Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl/acetonitrile (70/30)
Flow rate	: 0.7 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 220 nm
Injection	: 5 μL

Reversed-phase YMCbasic 5 μm, 150 X 2.0 mmI.D.



Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 5-35%B (0-50 min), 35-45%B (50-55 min), 45%B (55-60 min)
Flow rate	: 0.2 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 1 μL

* undigested BSA

These chromatograms show separation of tryptic digests of BSA (MW: 66,000) using ion exchange chromatography (IEC), size exclusion chromatography (SEC) and reversed-phase chromatography (RPC). The molecular weight of the digests is estimated to be approximately between 100 and 20,000 by SEC chromatogram. IEC and RPC chromatograms show many peaks of fragments which are separated by the difference in structure, charge and hydrophobicity.

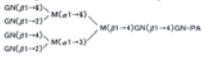
Separation of sugar chains using different modes

Pyridylamino (PA)-Sugar chains

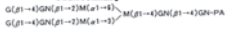
Reversed-phase YMC-Pack ODS-A (12 nm) 5 μm, 150 X 4.6 mmI.D.

G910607A

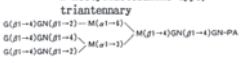
1.PA-Sugar Chain 014
N-Acetyllactosamine type, agalacto tetraantennary



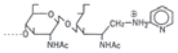
2.PA-Sugar Chain 001
N-Acetyllactosamine type, biantennary



3.PA-Sugar Chain 002
N-Acetyllactosamine type, triantennary

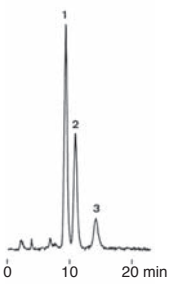


G : galactose
GN : N-acetylglucosamine
M : mannose
F : fucose
PA : pyridylamino(group)



Eluent	: methanol/20 mM $NH_4H_2PO_4$ (5/95)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: FLS at Ex. 320 nm, Em. 400 nm
Injection	: 2 μL (3.3 pmol/mL)
Sample	: PA-Sugar Chain Series, manufactured by TAKARA BIO INC.

Normal-phase YMC-Pack Polyamine Ⅱ 5 μm, 150 X 4.6 mmI.D.



G910620A

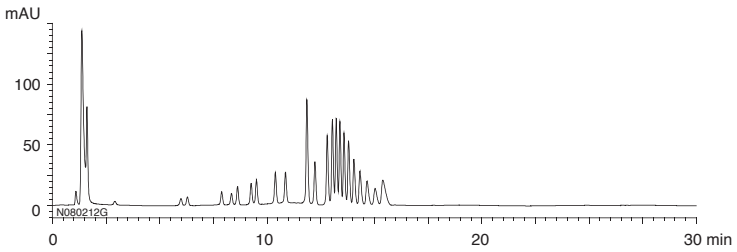
Eluent	: methanol/20 mM $NH_4H_2PO_4$ (80/20)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: FLS at Ex. 320 nm, Em. 400 nm
Injection	: 3 μL (3.3 pmol/mL)
Sample	: PA-Sugar Chain Series, manufactured by TAKARA BIO INC.

Pyridylamino (PA) sugar chains are often analyzed for structural determination of sugar chain in glycoproteins and glycolipids. Separations of PA sugar chains in reversed-phase (RP) mode and normal-phase (NP) mode are shown. Two dimensional HPLC combining two different modes, such as RP mode and NP mode, is a useful tool for structural determination of sugar chain.

Separation of nucleic acids using different modes

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

Ion exchange YMC-BioPro QA-F 5 μm, 100 X 4.6 mmI.D.

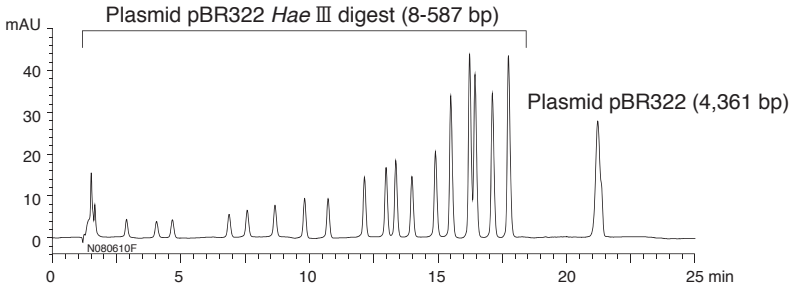


Eluent	: A) 20 mM Tris-HCl (pH 8.1) containing 0.7 M NaCl B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl 0-100%B (0-30 min)
Flow rate	: 0.5 mL/min
Temperature	: 25°C
Detection	: UV at 260 nm
Injection	: 20 μL

DNA fragments are analyzed using a YMC-BioPro QA-F ion exchange column, 100 mm length column. YMC-BioPro QA-F is ideal for high-resolution analysis of nucleic acids.

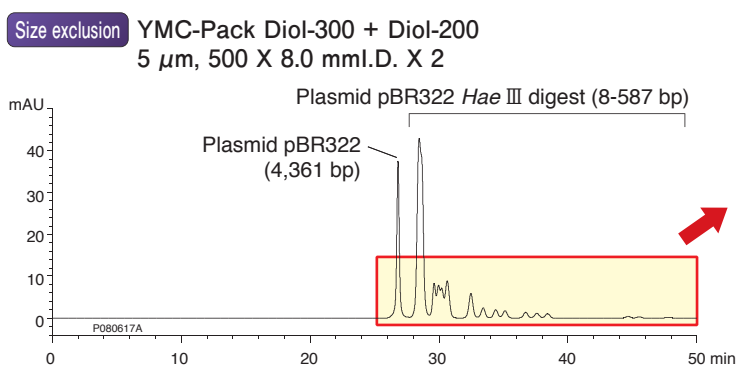
Plasmid pBR322 restriction fragments

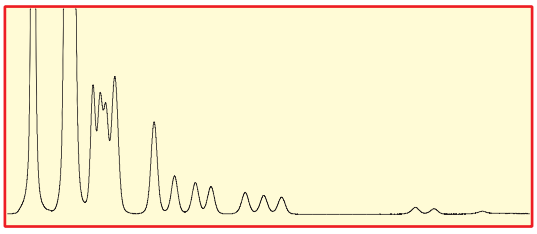
Ion exchange YMC-BioPro QA-F 5 μm, 100 X 4.6 mmI.D.



Eluent	: A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl 70-85%B (0-20 min), 85%B (20-25 min)
Flow rate	: 0.5 mL/min
Temperature	: 35°C
Detection	: UV at 260 nm
Injection	: 10 μL

Size exclusion YMC-Pack Diol-300 + Diol-200
5 μm, 500 X 8.0 mmI.D. X 2





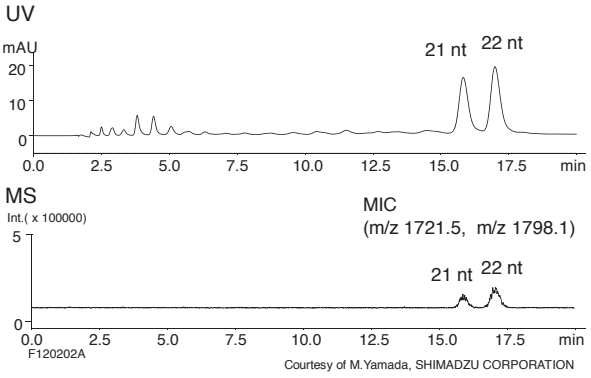
Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.7 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 260 nm
Injection	: 10 μL

The separation of plasmid pBR322 restriction fragments (8-857 bp) is compared for ion exchange mode and size exclusion modes. Ion exchange chromatography (IEC) is applicable to identification of each fragment requiring high resolution, and size exclusion chromatography (SEC) is usable for characterization of molecular weight distribution.

Oligonucleotide (miRNA)

Reversed-phase YMC-Triart C18 3 μm, 150 X 2.0 mmI.D.

5'-pUGG AGU GUG ACA AUG GUG UUG-3' (21 nt, MW 6890.1)
5'-pUGG AGU GUG ACA AUG GUG UUG U-3' (22 nt, MW 7196.3)



Courtesy of M.Yamada, SHIMADZU CORPORATION

Eluent	: A) 10 mM DBAA* (pH 7.5) B) 10 mM DBAA* (pH 7.5)/acetonitrile (50/50) 62-72%B (0-20 min)
Flow rate	: 0.2 mL/min
Temperature	: 30°C
Detection	: UV at 260 nm and ESI-negative mode
Injection	: 4 μL (5 nmol/mL)
Instrument	: LC) Shimadzu Prominence MS) Shimadzu LCMS2020

* di-*n*-butylamin-acetic acid

This figure shows LC/MS analysis of oligonucleotides in reversed-phase mode. YMC-Triart C18 columns are useful for oligonucleotides and they can achieve excellent separation by one nucleotide difference and sufficient intensity in UV and ESI-MS.

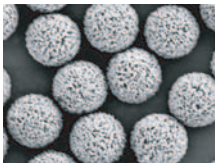
Columns/Packing Materials

Ion exchange columns/media
BioPro

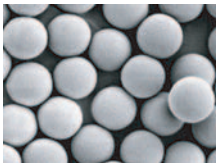
Features

- Ideal for analysis and purification of biopharmaceuticals
- Ion exchange columns designed for analytical and laboratory-scale purification
- High binding capacity/high recovery/high resolution/low backpressure

SEM images of polymer beads



Porous polymer beads



Non-porous polymer beads

Specifications

Ion exchange columns

	YMC-BioPro QA	YMC-BioPro SP	YMC-BioPro QA-F	YMC-BioPro SP-F
Matrix	Hydrophilic porous polymer		Hydrophilic non-porous polymer	
Particle size (μm)	5		3, 5	
Ion exchanger	$-CH_2N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$	$-CH_2N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$
Counter ion	Cl^-	Na^+	Cl^-	Na^+
Ion exchange capacity (meq/mL-resin)	0.075 - 0.100	0.070 - 0.095	0.075 - 0.110	0.230 - 0.290
Binding capacity* (mg/mL-resin)	DBC >110 (BSA)	DBC >70 (human-IgG)	DBC >12 (BSA)	DBC >10 (human-IgG)
Usable temperature	4 - 60℃			
Usable pH range	2.0 - 12.0			
Column material	PEEK			

Ion exchange media ～ Suitable for intermediate purification step and polishing step ～

	BioPro SmartSep Q	BioPro SmartSep S
Matrix	Hydrophilic porous polymer	
Particle size (μm)	10, 30	
Ion exchanger	$-R\text{-}N^+(CH_3)_3$	$-R\text{-}SO_3^-$
Usable pH range	2.0 - 12.0	
Ion exchange capacity (meq/mL-resin)	>0.08	
Binding capacity* (mg/mL-resin)	DBC >100 (BSA)	DBC >100 (lysozyme)

Ion exchange media ～ Suitable for capture step and intermediate purification step ～

	BioPro Q	BioPro S	BioPro DA	BioPro CM
Matrix	Hydrophilic porous polymer			
Particle size (μm)	75		60	
Ion exchanger	$-R\text{-}N^+(CH_3)_3$	$-R\text{-}SO_3^-$	$-R\text{-}N(CH_3)_2$	$-R\text{-}COOH$
Usable pH range	2.0 - 12.0		Regular use:3.0 -12.0	Short term:1.0 - 13.0
Ion exchange capacity (meq/mL-resin)	>0.10		≧0.10	≧0.08
Binding capacity* (mg/mL-resin)	DBC >160 (BSA)	DBC >160 (lysozyme)	SBC ≧77 (human-IgG)	SBC ≧90 (human-IgG)

* DBC : dynamic binding capacity, SBC : static binding capacity

Porous type

YMC-BioPro QA / YMC-BioPro SP

Features

- Ion exchange columns based on porous polymer beads
- Excellent resolution
- High binding capacity and high recovery of biomolecules
- Suitable for laboratory-scale purification

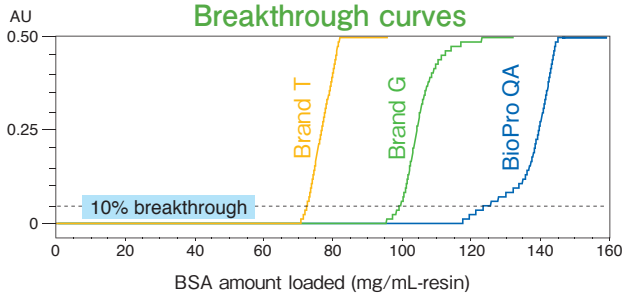
High binding capacity and recovery

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

	Dynamic binding capacity (mg/mL-resin, 10% breakthrough)	Eluted amount (mg/mL-resin)	Recovery* (%)
YMC-BioPro 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

Breakthrough curves



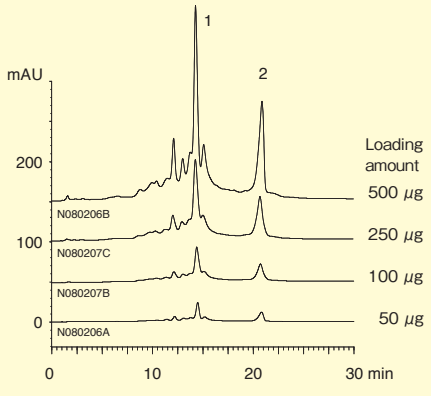
Column	: YMC-BioPro QA 50 X 4.6 mmI.D. Brand T (porous Q type) 50 X 4.6 mmI.D. Brand G (porous Q type) 50 X 5.0 mmI.D.
Linear velocity	: 180 cm/hr
Equilibration buffer	: 20 mM Tris-HCl (pH 8.6)
Elution buffer	: 20 mM Tris-HCl (pH 8.6) containing 1.0 M NaCl
Detection	: UV at 280 nm
Sample	: 1 mg/mL Bovine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

YMC-BioPro QA gives the superior DBC and recovery compared with conventional porous polymer anion exchange columns. The surface structure of YMC-BioPro, 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

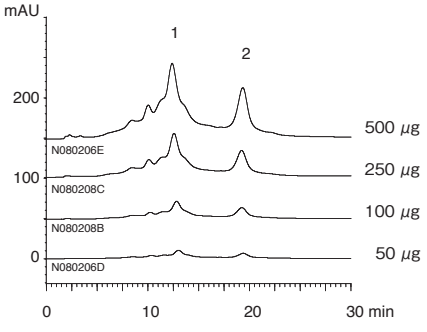
Comparison of the effect of sample load on YMC-BioPro QA and commercial Q type column

YMC-BioPro QA
5 μm, 50 X 4.6 mmI.D.

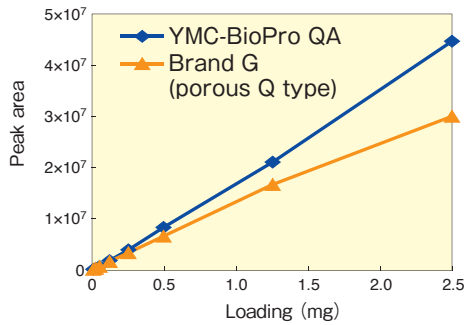


Brand G (porous Q type)
10 μm, 50 X 5.0 mmI.D.

1. Ovalbumin
2. Trypsin inhibitor



Recovery of 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

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

Non-porous type

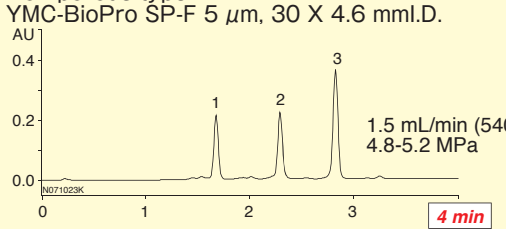
YMC-BioPro QA-F / YMC-BioPro SP-F

Features

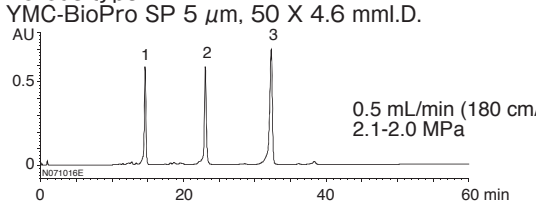
- Ion exchange columns based on non-porous polymer beads
- High efficiency with low operating pressure
- 30 mm length column for ultra high-throughput analysis
- 100 mm length column for high-resolution analysis

Ultra high-throughput analysis of proteins

Non-porous type
YMC-BioPro SP-F 5 μm, 30 X 4.6 mmI.D.



Porous type
YMC-BioPro SP 5 μm, 50 X 4.6 mmI.D.



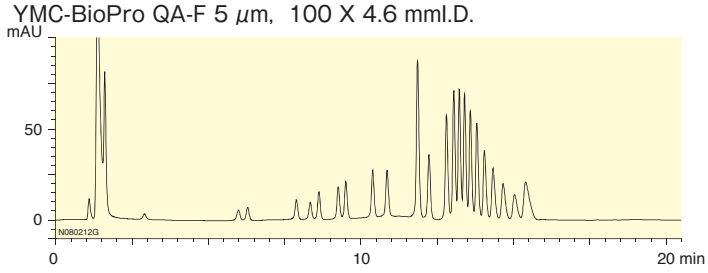
1. Ribonuclease A
2. Cytochrome *c*
3. Lysozyme

Eluent	: A) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) B) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) containing 0.5 M NaCl 0-100%B (0-4 min) for YMC-BioPro SP-F 0-100%B (0-60 min) for YMC-BioPro SP
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 20 μL

The high mechanical stability of non-porous polymer beads and the short column length enable faster elution of proteins at a higher flow rate.

High-resolution analysis of nucleic acids

YMC-BioPro QA-F 5 μm, 100 X 4.6 mmI.D.



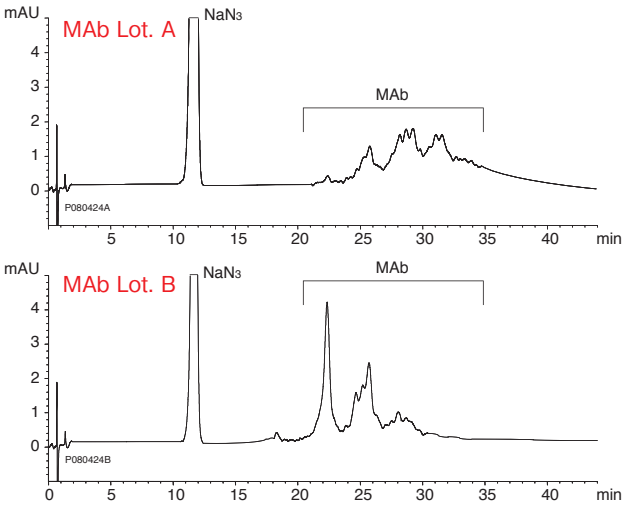
DNA fragments 1Kb DNA ladder (75-12,216 bp)

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. YMC-BioPro QA-F of 100 mm length column is a good choice for high-resolution analysis of nucleic acids.

High-resolution analysis of proteins

YMC-BioPro QA-F 5 μm, 100 X 4.6 mmI.D.



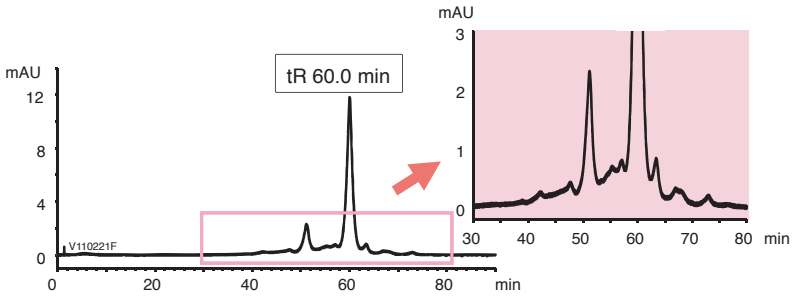
Monoclonal antibody (MAb) anti-human IgG4

Eluent	: A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl 10-25%B (0-60 min)
Flow rate	: 1.0 mL/min (360 cm/hr)
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 14 μL (0.1 mg/mL)
Sample	: Mouse monoclonal IgG1 anti-human IgG4 (Purified by DEAE chromatography, containing NaN_3)

Two different lots of commercially available MAb purified by DEAE chromatography, are analyzed on a 100 mm length column of YMCBioPro QA-F. The MAb is resolved into several peaks, and the lot-to-lot variability is observed. 100 mm length column of YMC-BioPro QA-F/SP-F, which has high efficiency, is ideal for characterization of glycoproteins such as monoclonal antibodies and for quality control assessment of biopharmaceuticals.

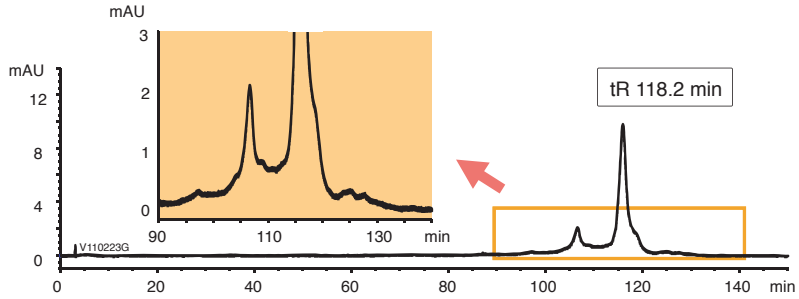
Monoclonal antibody (MAb) analysis on non-porous type cation exchange columns

YMC-BioPro SP-F 5 μm, 100 X 4.6 mmI.D.



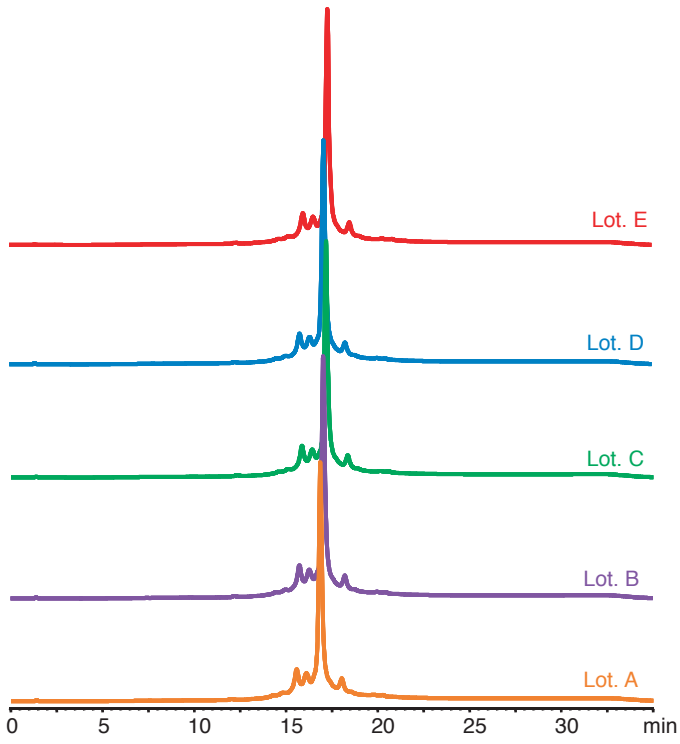
Eluent	: A) 20 mM MES-NaOH (pH 5.6) B) 20 mM MES-NaOH (pH 5.6) containing 0.2 M NaCl
Initial gradient conc.	: 35%B (70 mM NaCl)
Gradient slope	: 0.25%B/min (0.5 mM NaCl)
Flow rate	: 180 cm/hr (0.5 mL/min for 100 X 4.6 mmI.D., 0.378 mL/min for 250 X 4.0 mmI.D.)
Temperature	: 30°C
Detection	: UV at 280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

Competitor WCX column 10 μm, 250 X 4.0 mmI.D.



The separation of MAb is compared on SCX (YMC-BioPro SP-F) and WCX (competitor's) under the same gradient condition at pH 5.6. YMC-BioPro SP-F column provides the higher resolution of MAb in shorter analysis time than the competitor column.

Excellent batch-to-batch reproducibility



Column	: YMC-BioPro SP-F 5 μm, 100 X 4.6 mmI.D.
Eluent	: A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.5) B) 20 mM NaH_2PO_4-Na_2HPO_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	: monoclonal antibody (IgG1)

YMC-BioPro SP-F column exhibits excellent batch-to-batch reproducibility for MAb analysis, including the resolution of peaks for small charge variants. All the gel batches are inspected by various quality control tests including HPLC analysis of MAb, and must pass rigorous criteria before release. YMC-BioPro ion exchange columns are the best choice for the quality control of MAb and other biopharmaceuticals.

Ion exchange media for high-throughput purification of biopharmaceuticals

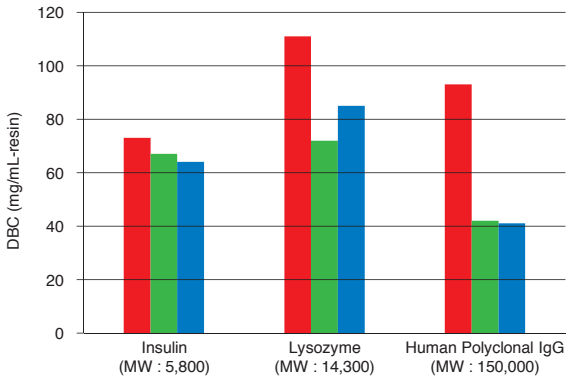
BioPro SmartSep Q/S

Features

- High-throughput purification by utilizing high mechanical strength polymer beads
- High binding capacity and high resolution over a wide range of flow rate
- Suitable for intermediate purification step and polishing step
- Available in strong ion exchangers (Q and S chemistries)
- Particle size 30 μm for industrial processes and 10 μm for high resolution purification

High sample loadability

High dynamic binding capacity (DBC) for various samples



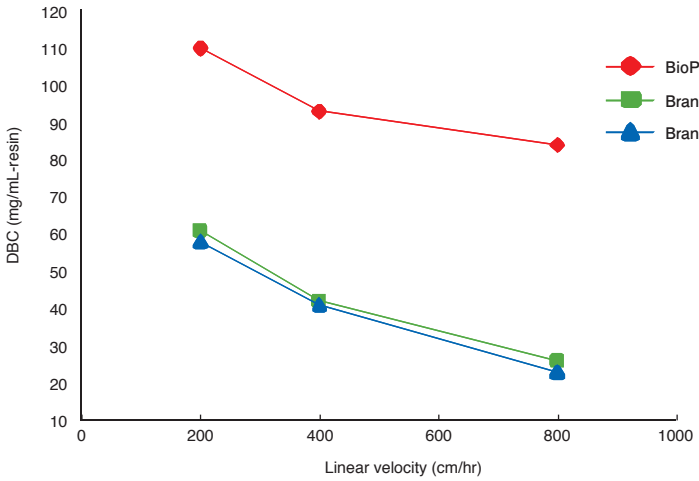
Conditions of DBC measurement*
Column : 50 X 5.0 mmI.D.
Flow rate : 400 cm/hr (1.32 mL/min)
Temperature : 25°C

*Please inquire us for details.

	Particle size (μm)	DBC (mg/mL-resin, 10% breakthrough)		
		Insulin	Lysozyme	Human Polyclonal IgG
BioPro 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 SmartSep ion exchange media have higher DBC compared to conventional ion exchange media. Especially for IgG, BioPro SmartSep has more than twice as high DBC as competitors'. This feature of BioPro SmartSep makes purification productivity of IgG per unit time double or more.

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

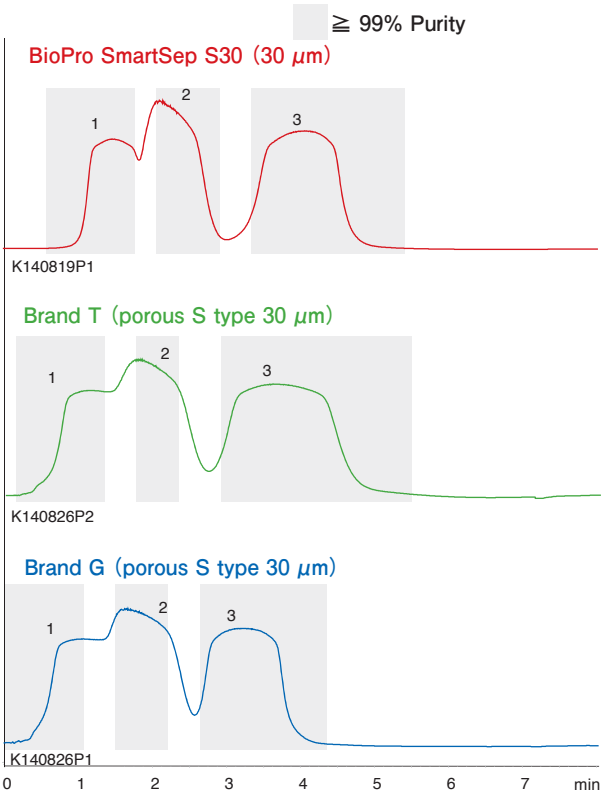


Column : 50 X 5.0 mmI.D.
Equilibration buffer : 20 mM citric acid-NaOH (pH 5.3)
Elution buffer : Equilibration buffer containing 0.5 M NaCl
Flow rate : 200-800 cm/hr (0.66-2.62 mL/min)
Temperature : ambient (25°C)
Detection : UV at 280 nm
Sample : **1.5 mg/mL human polyclonal IgG** in equilibration buffer

High DBC of BioPro SmartSep is maintained even at a higher flow rate, making them suitable for the high-speed purification with 2-4 times of conventional flow rates. This feature offers significant improvement on productivity.

High resolution and excellent recovery

Separation at high flow rate and high loading condition



Comparison of recovery of proteins

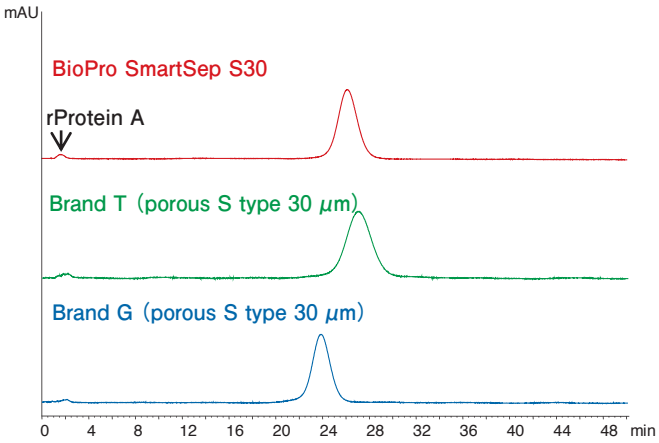
	Recovery (Load: 45 mg, Purity: ≧99%)			
	Ribonuclease A	Cytochrome *c*	Lysozyme	Total
BioPro SmartSep S30	90.9 %	80.3 %	99.2 %	90.6 %
Brand T (porous S type)	80.6 %	59.6 %	98.3 %	80.1 %
Brand G (porous S type)	72.5 %	70.2 %	97.2 %	80.2 %

Column : 50 X 5.0 mmI.D.
Eluent : A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8)
B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 0.5 M NaCl
0-100%B (0-30 column volumes)
Flow rate : 1600 cm/hr (5.23 mL/min)
Temperature : 25°C
Detection : UV at 220 nm
Injection : 30 mL (45 mg Proteins)
Sample : 1. Ribonuclease A (0.5 mg/mL)
2. Cytochrome *c* (0.5 mg/mL)
3. Lysozyme (0.5 mg/mL)

BioPro SmartSep ion exchange media show high resolution and recovery even at a high flow rate and high loading condition. BioPro SmartSep ion exchange media offer high efficiency on intermediate purification step and polishing step requiring high resolution and recovery.

Purification of IgG1 (Anti-hTNF alpha IgG1)

Intermediate purification (cation exchange chromatography)



Column : 50 X 5.0 mmI.D.
Eluent : A) 20 mM citric acid-NaOH (pH 5.3)
B) 20 mM citric acid-NaOH (pH 5.3) containing 0.5 M NaCl
0-100%B, 30 column volumes
Flow rate : 180 cm/hr (0.59 mL/min)
Temperature : ambient
Detection : UV at 280 nm
Sample : Anti-h TNF alpha IgG1 (Purified by Affinity chromatography)
Injection : 0.25 mL (0.1 mg IgG1)

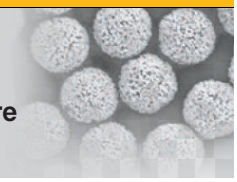
This is an example where an IgG1 monoclonal antibody was purified from cell culture medium by BioPro SmartSep S30. In general, purification of antibody starts from clarification. After clarification, it is subjected to initial purification (capture step) by affinity chromatography (rProtein A), followed by ion exchange chromatography. In the capture step rProtein A derived from affinity media contaminate the elueate. This was separated and removed by ion exchange chromatography.

Ion exchange media for purification of biopharmaceuticals, proteins and nucleotides

BioPro Ion Exchange Media

Features

- High productivity on purification
- Hydrophilic polymer beads with low nonspecific adsorption
- High binding capacity/high recovery/high resolution/low backpressure
- Suitable for capture step and intermediate purification step



High dynamic binding capacity (DBC) for proteins

BioPro ion exchange media have higher DBC of protein than commercial ion exchange media. BioPro ion exchange media are effective in protein purification from capture step requiring high capacity to intermediate step requiring high efficiency.

Anion exchanger	Particle size (μm)	Ion exchange capacity (meq/mL-resin)	DBC*1 (mg/mL-resin)
BioPro Q75	75	0.13	183
Brand G (porous Q type)	90	0.19	102

Cation exchanger	Particle size (μm)	Ion exchange capacity (meq/mL-resin)	DBC*1 (mg/mL-resin)
BioPro S75	75	0.12	192
Brand G (porous S type)	90	0.13	80

*1 Dynamic binding capacities were determined at 10% breakthrough under following conditions:

Column : 50 X 4.6 mmI.D.
Flow rate : 180 cm/hr (3.0 cm/min)

for anion-exchange media

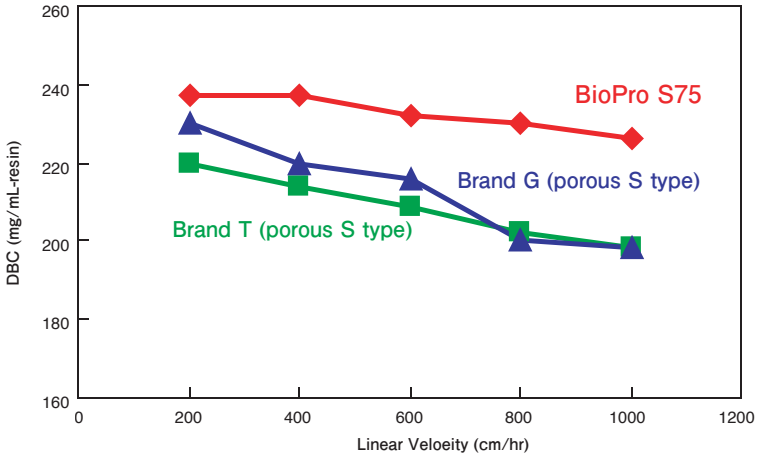
Equilibration buffer : 20 mM Tris-HCl (pH 8.6)
Elution buffer : 0.5 M NaCl in equilibration buffer
Sample : 1.5 mg/mL BSA in equilibration buffer
Detection : UV at 280 nm

for cation-exchange media

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

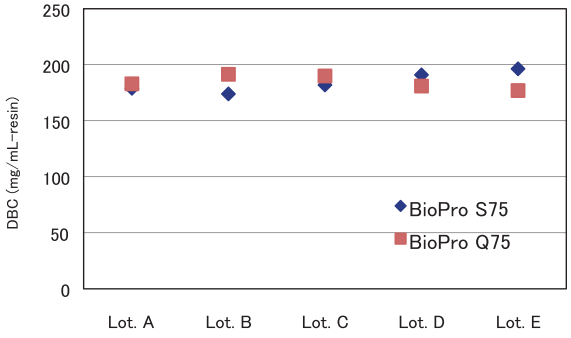
High productivity on purification

BioPro ion exchange media show high DBC over a wide range of flow rate, and the difference in DBC is less than 5% between 200 cm/hr and 1000 cm/hr. BioPro ion exchange media give increased productivity and reduced cost in biopharmaceutical production.



Column : 50 X 5.0 mmI.D.
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
Detection : UV at 300 nm

Excellent batch-to-batch reproducibility of DBC



Column : 50 X 4.6 mmI.D
Flow rate : 180 cm/hr

for anion-exchange media

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 media

Equilibraiton 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

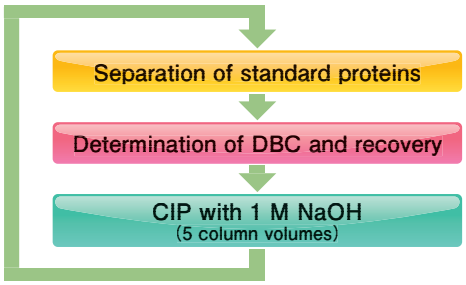
BioPro ion exchange media exhibit excellent batch-to-batch reproducibility of DBC. All the gel batches are inspected by various quality control tests. We supply stable products over a long period of time.

Excellent durability

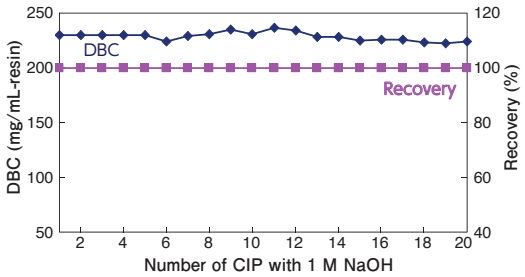
Stability on CIP

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 ion exchange media allow effective cleaning with alkaline solution.

Test protocols



DBC and recovery

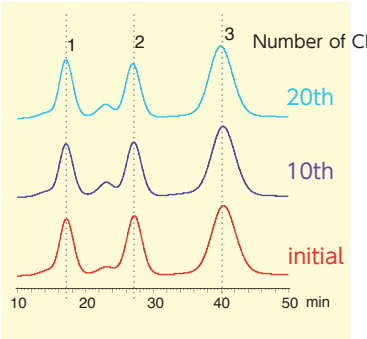


Conditions of DBC measurement

Column	: BioPro 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



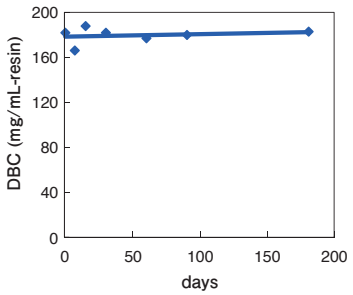
Conditions of separation of standard proteins

Column	: BioPro S75 50 X 5.0 mmI.D.
Eluent	: A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) B) 20 mM NaH_2PO_4-Na_2HPO_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 Q75 has high stability under alkaline condition. This feature is effective for storing the medium in alkaline solution* as well as CIP.

Change in DBC

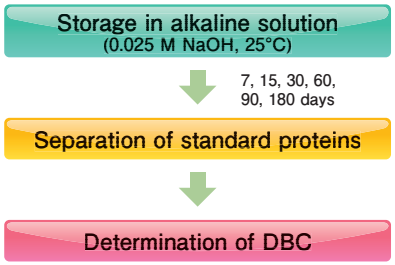


Conditions of DBC measurement

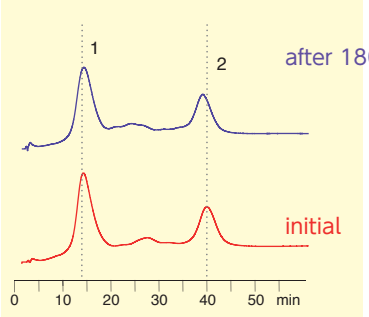
Column	: BioPro Q75 50 X 4.6 mmI.D.
Equilibration buffer	: 20 mM Tris-HCl (pH 8.6)
Elution buffer	: 0.5 M NaCl in equilibration buffer
Flow rate	: 180 cm/hr (0.50 mL/min)
Sample	: 1.5 mg/mL BSA in equilibration buffer
Temperature	: 25°C
Detection	: UV at 280 nm

※DBC was determined at 10% breakthrough

Test protocols



Separation of standard proteins



Conditions of separation of standard proteins

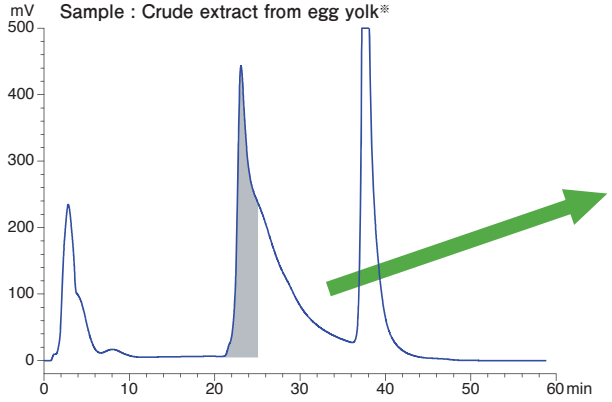
Column	: BioPro Q75 50 X 4.6 mmI.D.
Eluent	: A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
Gradient	: 10-80%B (0-60 min; Linear)
Flow rate	: 180 cm/hr (0.50 mL/min)
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 20 μL
Sample	: 1. Transferrin (0.25 mg/mL), 2. Trypsin inhibitor (0.5 mg/mL)

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

Purification of IgY from egg yolk extract

Capture purification by ion exchange chromatography (IEC)

BioPro Q75 75 μm 50 X 4.6 mmI.D.
Sample : Crude extract from egg yolk※



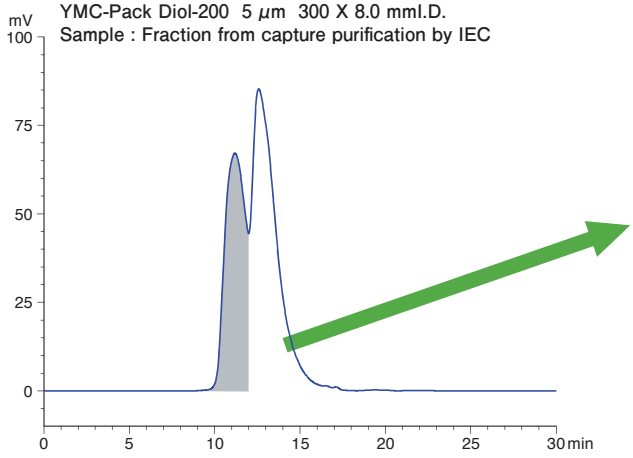
Eluent	: A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl 10%B (0-15 min), 30%B (15-30 min), 90%B (30-40 min)
Flow rate	: 180 cm/hr (0.5 mL/min)
Temperature	: ambient
Detection	: UV at 280 nm
Injection	: 1 mL (about 20 mg Protein)

※ Courtesy of Pharma Foods International Co., Ltd.

polishing step

Polishing by size exclusion chromatography (SEC)

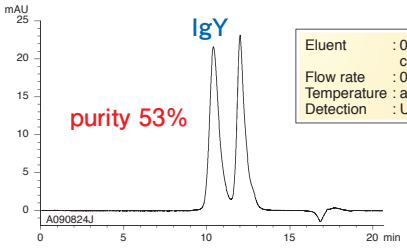
YMC-Pack Diol-200 5 μm 300 X 8.0 mmI.D.
Sample : Fraction from capture purification by IEC



Eluent	: 0.1M KH_2PO_4-K_2HPO_4 (pH 6.9) containing 0.2 M NaCl
Flow rate	: 0.7 mL/min
Temperature	: ambient
Detection	: UV at 280 nm
Injection	: 1 mL (about 0.45 mg IgY)

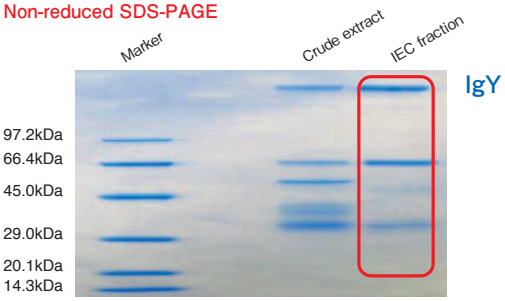
Analysis of captured fraction

SEC
YMC-Pack Diol-200 5 μm, 300 X 4.6 mmI.D.



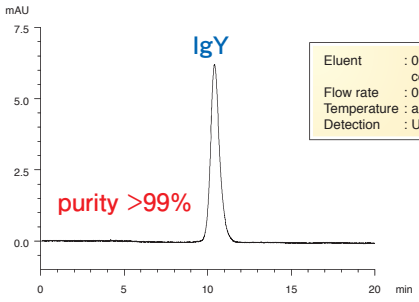
Eluent	: 0.1M KH_2PO_4-K_2HPO_4 (pH 6.9) containing 0.2 M NaCl
Flow rate	: 0.23 mL/min
Temperature	: ambient
Detection	: UV at 280 nm

Non-reduced SDS-PAGE



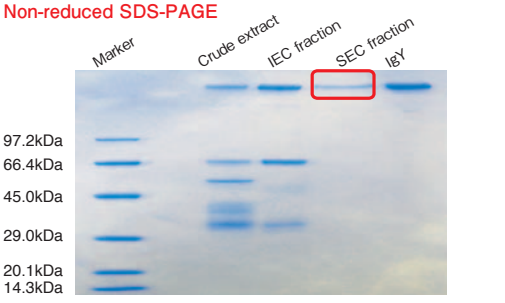
Analysis of purified fraction

SEC
YMC-Pack Diol-200 5 μm, 300 X 4.6 mmI.D.



Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 6.9) containing 0.2 M NaCl
Flow rate	: 0.23 mL/min
Temperature	: ambient
Detection	: UV at 280 nm

Non-reduced SDS-PAGE



Egg yolk antibody (IgY) can be isolated with high purity (greater than 99%) by two chromatographic purification steps, which consist of a capture step by ion exchange chromatography on BioPro Q75 and a polishing step by size exclusion chromatography on YMC-Pack Diol-200.

Screening columns for biopharmaceuticals/proteins

BioPro Ion Exchange Screening Kits

Features

- Available in four chemistries: Strong ion exchangers (Q/S) and weak ion exchangers (DA/CM)
- Two column types (1 mL and 5 mL) that are ideal for media screening, development of purification methods and loadability studies
- Ion Exchange Selection Kit that consists of four different chemistries for fast and easy media screening
- Easy installation and convenient use

Column Size

1 mL Type (26 X 7.0 mmI.D.)

Media screening

Purification method development

5 mL Type (26 X 15.6 mmI.D.)

Purification method development

Loadability study

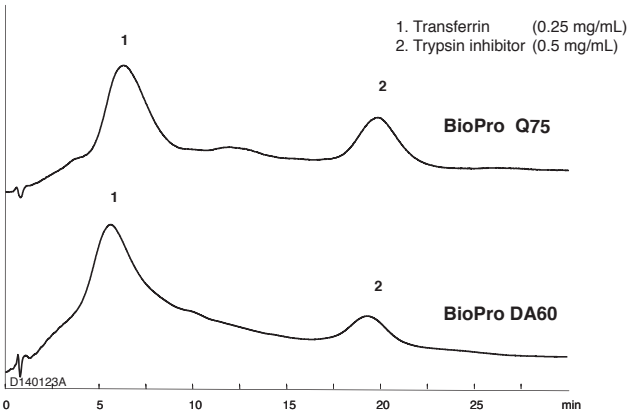
Lab-scale purification

Specifications

	BioPro SmartSep Q	BioPro SmartSep S	BioPro Q	BioPro S	BioPro DA	BioPro CM
Matrix	Hydrophilic porous polymer					
Particle size (μm)	30	30	75	75	60	60
Ion exchanger	-R-N$^+$(CH$_3$)$_3$	-R-SO$_3^-$	-R-N$^+$(CH$_3$)$_3$	-R-SO$_3^-$	-R-N(CH$_3$)$_2$	-R-COOH
Usable pH range	2.0 - 12.0	2.0 - 12.0	2.0 - 12.0	2.0 - 12.0	Regular use:3.0 - 12.0 Short term:1.0 - 13.0	Regular use:3.0 - 12.0 Short term:1.0 - 13.0

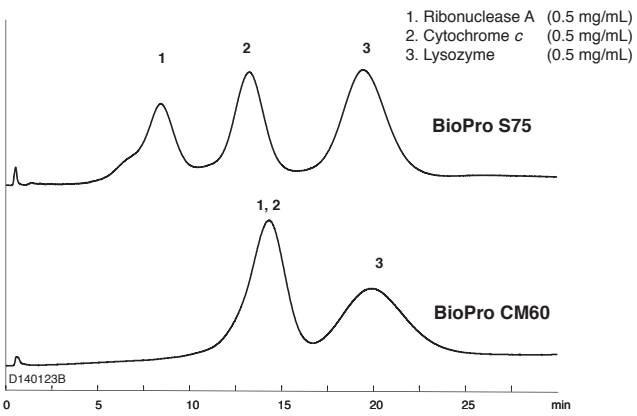
Application

Screening with anion exchange media



Column : 1 mL type (26 X 7.0 mmI.D.)
Eluent : A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 0.5M NaCl
10-80%B (0-30 min)
Flow rate : 180 cm/hr (1.16 mL/min)
Temperature : 25°C
Detection : UV at 220 nm
Injection : 20 μL

Screening with cation exchange media



Column : 1 mL type (26 X 7.0 mmI.D.)
Eluent : A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8)
B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 0.5 M NaCl
0-100%B (0-30 min)
Flow rate : 180 cm/hr (1.16 mL/min)
Temperature : 25°C
Detection : UV at 220 nm
Injection : 20 μL

Silica-based SEC

YMC-Pack Diol

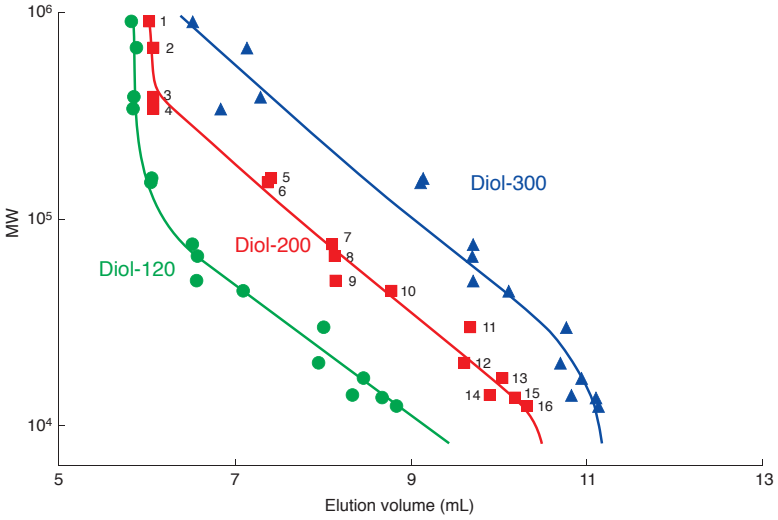
Features

- 5 μm silica-based columns with high mechanical stability
- Low-cost size exclusion chromatography (SEC) columns
- Useful for molecular weight determination of proteins and sugars

Specifications

Column	Base	Functional group	Pore size (nm)	Particle size (μm)	Usable pH range	Characteristics
Diol-60	Silica gel	Dihydroxypropyl	6	5	5.0 - 7.5	For molecular weights below 10,000
Diol-120			12			For molecular weights 5,000 to 100,000
Diol-200			20			For molecular weights 10,000 to ca. 500,000
Diol-300			30			For molecular weights ca. 50,000 to 1,000,000

Calibration curves of various proteins for three different pore sizes

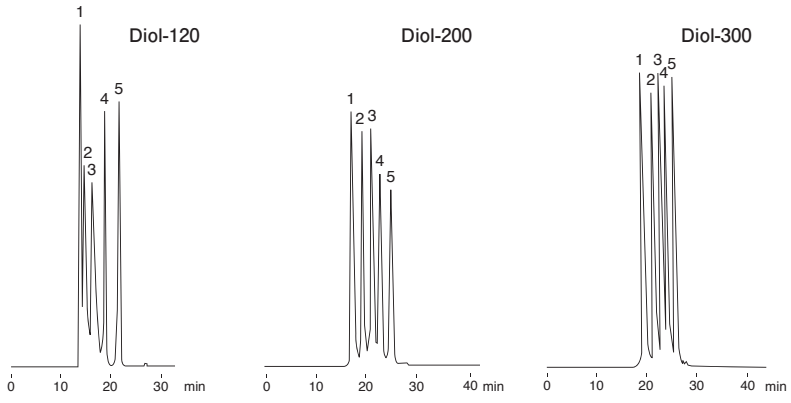


	MW
1. IgM	900,000
2. Thyroglobulin	670,000
3. IgA	390,000
4. Fibrinogen	340,000
5. γ-Globulin	158,000
6. IgG	150,000
7. Transferrin	75,000
8. HSA (human serum albumin)	66,000
9. α1-Antitrypsin	50,000
10. Ovalbumin	45,000
11. Carbonic anhydrase	30,000
12. Trypsin inhibitor	20,100
13. Myoglobin	17,000
14. α-Lactalbumin	14,100
15. Ribonuclease A	13,700
16. Cytochrome *c*	12,400

Column : YMC-Pack Diol 300 X 8.0 mmI.D.
Eluent : 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate : 0.5 mL/min
Temperature : 25˚C
Detection : UV at 280 nm

Diol-120, Diol-200 and Diol-300 are suitable for the separation or molecular weight determination of proteins with molecular weights from 5,000 to several hundred thousand.

Separation for standard protein markers



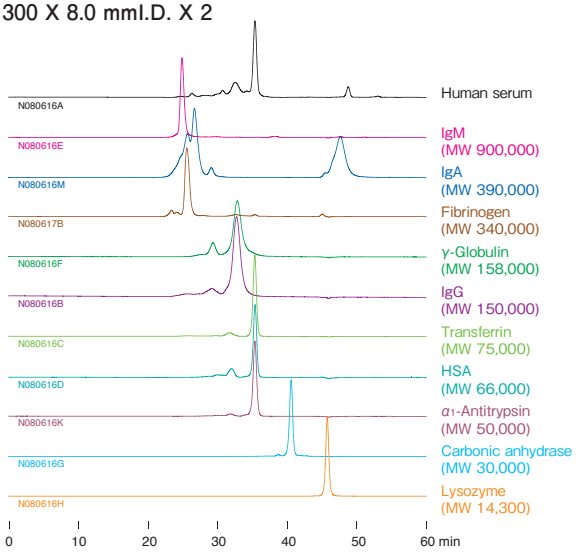
	MW
1. Glutamate dehydrogenase	290,000
2. Lactate dehydrogenase	142,000
3. Enolase	67,000
4. Adenylate kinase	32,000
5. Cytochrome *c*	12,400

Column : YMC-Pack Diol 500 X 8.0 mmI.D.
Eluent : 0.1 M KH_2PO_4-K_2HPO_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 10,000 to 500,000 compounds, Diol-200 is suitable for this separation.

Plasma constituents

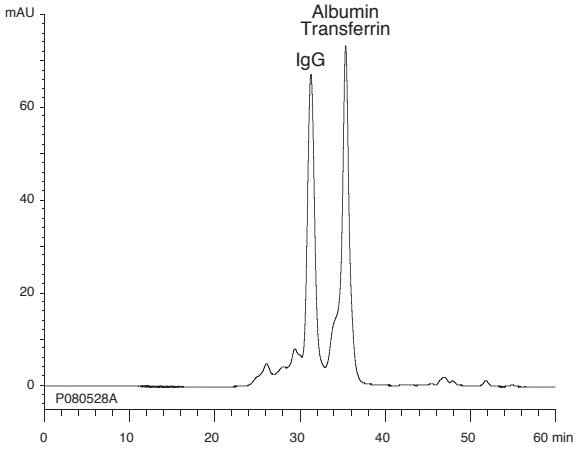
YMC-Pack Diol-300 + Diol-200 5 μm,
300 X 8.0 mmI.D. X 2



Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.5 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 280 nm

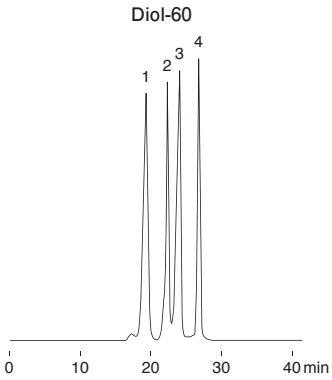
Proteins in mouse ascites fluid

YMC-Pack Diol-300 + Diol-200 5 μm,
300 X 4.6 mmI.D. X 2



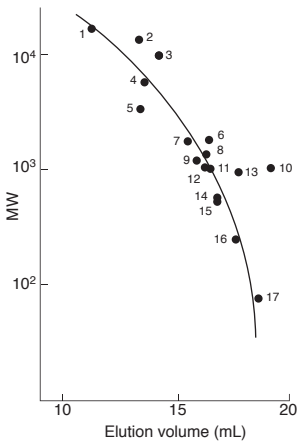
Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0)
Flow rate	: 0.17 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 220 nm
Injection	: 10 μL
Sample	: Mouse ascites fluid (60 times dilution with water)

Separation of peptides with molecular weights less than 10,000



	MW
1. Insulin (Bovine)	5,700
2. Neurotensin	1,672
3. Angiotensin Ⅱ	1,040
4. Glycine	75

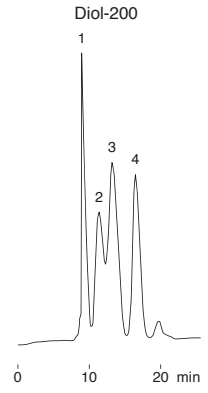
Column	: YMC-Pack Diol-60 500 X 8.0 mmI.D.
Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl/acetonitrile (70/30)
Flow rate	: 0.7 mL/min
Temperature	: ambient
Detection	: UV at 215 nm



	MW
1. Myoglobin	17,000
2. Ribonuclease A	13,700
3. Cytochrome *c*	12,400
4. Insulin (Bovine)	5,700
5. Insulin B chain	3,496
6. α-Mating factor	1,684
7. Neurotensin	1,672
8. Angiotensin Ⅰ	1,296
9. CCK-Octapeptide	1,143
10. Bradykinin	1,060
11. Angiotensin Ⅱ	1,040
12. Oxytocin	1,007
13. Angiotensin Ⅲ	931
14. Met-Enkephalin	574
15. Leu-Enkephalin	556
16. Tetraglycine	246
17. Glycine	75

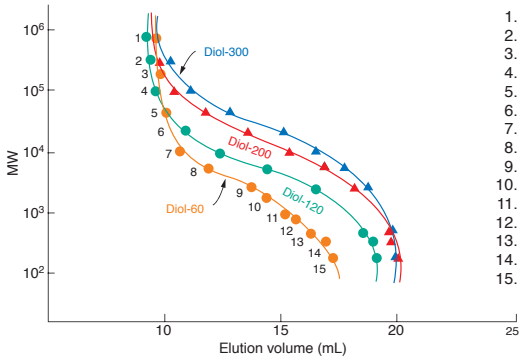
For peptides with molecular weights less than 10,000, Diol-60 is suitable for the separation.

Separation of oligo- and polysaccharides



	MW
1. P-800	853,000
2. P-50	48,000
3. P-20	23,700
4. P-5	5,800

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



	MW
1. Pullulan (P-800)	853,000
2. Pullulan (P-400)	380,000
3. Pullulan (P-200)	186,000
4. Pullulan (P-100)	100,000
5. Pullulan (P-50)	48,000
6. Pullulan (P-20)	23,700
7. Pullulan (P-10)	12,200
8. Pullulan (P-5)	5,800
9. Maltopentadecaose (G15)	2,448
10. Maltoundecaose (G11)	1,800
11. Maltoheptaose (G7)	1,152
12. Maltopentaose (G5)	824
13. Maltotriose (G3)	504
14. Maltose (G2)	342
15. Glucose (G1)	180

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.

Reversed-phase columns and packing materials

Features

- YMC packing materials of various chemistries
- Excellent peak shapes
- High resolution

Specifications

Product name	Base	Functional group	Pore size (nm)	Particle size (μm)	C (%)	Usable pH range	Characteristics
Triart C18	Hybrid silica	C18	12	1.9, 3, 5	20	1.0-12.0	·Suitable as a first choice ODS with excellent durability ·Superior peak shape ·Usable with 100% aqueous mobile phase
Triart C18 ExRS			8		25		·C18 phase with high density bonding on organic/inorganic hybrid silica gel ·Superior chemical durability
Triart C8		C8	12		17		·C8 phase bonding on organic/inorganic hybrid silica gel ·Superior chemical durability
Triart Phenyl		Phenyl			17	1.0-10.0	·Unique selectivity due to π-π interaction
Triart Prep C18-S		C18		10, 15, 20	20	2.0-10.0	·Preparative ODS packing allows the effective cleaning of the gel with alkaline solution
Triart Prep C8-S		C8	20		13		·Preparative C8 packing allows the effective cleaning of the gel with alkaline solution
Meteoric Core C18	Core-Shell type silica	C18	8	2.7	7	1.5-10.0	·Core-Shell type ODS
Meteoric Core C18 BIO			16		5		·Core-Shell type ODS with wide pore size
Pro C18	Silica	C18	12	2, 3, 5, 10	16	2.0-8.0	·Standard ODS with high versatility
Hydrosphere C18				2, 3, 5	12		·Usable with 100% aqueous mobile phase
Pro C18 RS			8	3, 5	22	1.0-10.0	·High carbon ODS packing material
ODS-A			12	3, 5, 10, 15, 20, 50	17	2.0-7.5	·Standard ODS from analytical to preparative ·ODS with wide pore size available, useful for separation of proteins and peptides
			20		12		
			30		7		
ODS-AQ			12		14		·Usable with 100% aqueous mobile phase ·Superior separation of hydrophilic compounds
			20		10		
C_8		C8	20	5, 10, 15, 20	7		·Useful for separation of relatively highly hydrophobic compounds, useful for separation of proteins and peptides
			30		4		
C_4		C4	20		5		·C4 with wide pore size available, useful for separation of proteins and peptides
			30		3		
CN		Cyanopropyl	30	5	3		·CN with wide pore size ·Unique selectivity due to cyano group
YMCbasic		C8	20	3, 5, 10	7		·Superior separation of proteins and peptides, especially of insulin
PROTEIN-RP		C4	20	5	4	1.5-7.5	·Useful for separation of proteins and peptides

Ordering Information

The previous product listing represents commonly used standard column dimension.
In order to identify any specific product version and order number, please see the example and the table below.

Functional group	Code
Triart C18	TA
Triart C18 ExRS	TAR
Triart C8	TO
Triart Phenyl	TPH
Triart Prep C18-S	TAS
Triart Prep C8-S	TOS
Meteoric Core C18	CAS
Meteoric Core C18 BIO	CAW
Pro C18	AS
Hydrosphere C18	HS
Pro C18 RS	RS
ODS-A	AA
ODS-AQ	AQ
C_8	OC
C_4	BU
CN	CN
YMCbasic	BA
PROTEIN-RP	PR

Pore size (nm)	Code
8	8
12	12
16	16
20	20
30	30
YMCbasic	99
PROTEIN-RP	99

Particle size (μm)	Code
1.9	SP9
2	S02
2.7	SQ7
3	S03
5	S05
10	S11
15	S16
20	S21
50	S50

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

Inner diameter (mm)	Code
1.0	01
2.0	02
2.1	Q1
3.0	03
4.0	04
4.6	46
6.0	06
10	10

Column Type	Code
Waters type	WT
Triart 1.9 μm	PT
Triart (high pressure)	PTH

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

Functional group	Pore size	Particle size		Column length	Inner diameter	Column Type
TA	12	SP9	-	10	02	PT

Product number : TA12SP9-1002PT

Reversed-phase separation of biomolecules

How to select reversed-phase columns

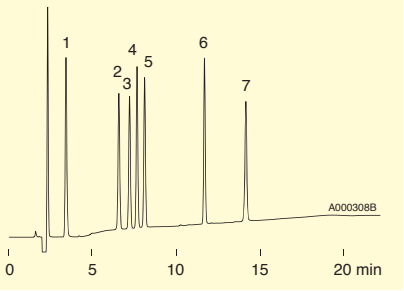
Typical column selection guide for biomolecules is shown in the right. It is good to select a functional group and pore size of packing material by molecular weight of compound(s) to be separated. Generally, a packing material with small pore size and long alkyl chain (e. g. C18, 12 nm) is good for relatively small molecules, and a packing material with large pore size and short alkyl chain (e. g. C8/C4, 20/30 nm) is suitable for macro molecules. Separation may also be influenced by hydrophobicity, type of the functional group(s) and higher-order structure of analyte(s) as well as molecular weight. Optimize the combination of bonded chemistry and pore size if good separation is not obtained. Other chemistries, such as PROTEIN-RP or CN will also be useful.

Molecular weight of sample	Functional group / Pore size	C18	C8	C4
thousands	12 nm	◎	○	△
tens of thousands	20 nm	○	◎	○
hundreds of thousands	30 nm	△	○	◎

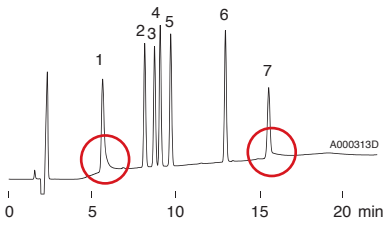
Separation of peptides (MW 574 - 3,465)

Excellent peak shapes for basic peptides

Hydrosphere C18 (12 nm) 5 μm,
150 X 4.6 mmI.D.



Brand E2 (10 nm) 5 μm,
150 X 4.6 mmI.D.
(ODS column for hydrophilic compounds)



1. BAM-12P (MW 1,425)
2. [D-Ala^2,Met^5]-Enkephalinamide (MW 587)
3. α-Endorphin (MW 1,746)
4. Met-Enkephalin (MW 574)
5. [D-Ala^2,Met^5]-Enkephalin (MW 588)
6. γ-Endorphin (MW 1,899)
7. β-Endorphin (MW 3,465)

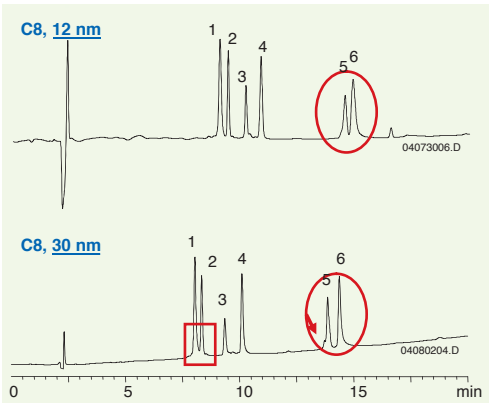
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
20-40%B (0-15 min),
40%B (15-20 min)
Flow rate : 1.0 mL/min
Temperature : 37°C
Detection : UV at 220 nm

Generally, the conventional C18 column with 12 nm pore size is suitable for analysis of small peptides up to 5,000 in molecular weight. Especially Triart and Pro series ODS columns, which are processed with advanced endcapping technology, are ideal for separation of basic peptides. As shown in the above, Hydrosphere C18, a Pro series column, exhibits excellent separations and superior peak shapes of basic peptides (peak 1 and 7), in contrast to the commercial ODS column for hydrophilic compounds, Brand E2.

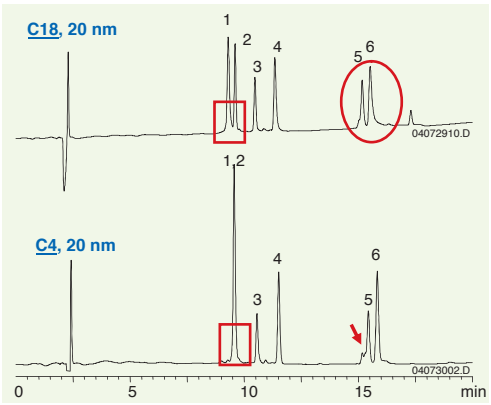
Separation of peptides and proteins (MW 4,300 - 17,000)

Comparison of separation on columns with different pore size and functional group

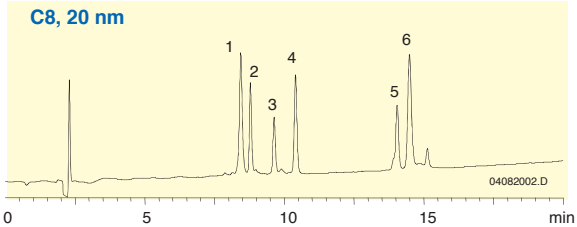
Pore size



Functional group



Optimized combination of pore size and functional group

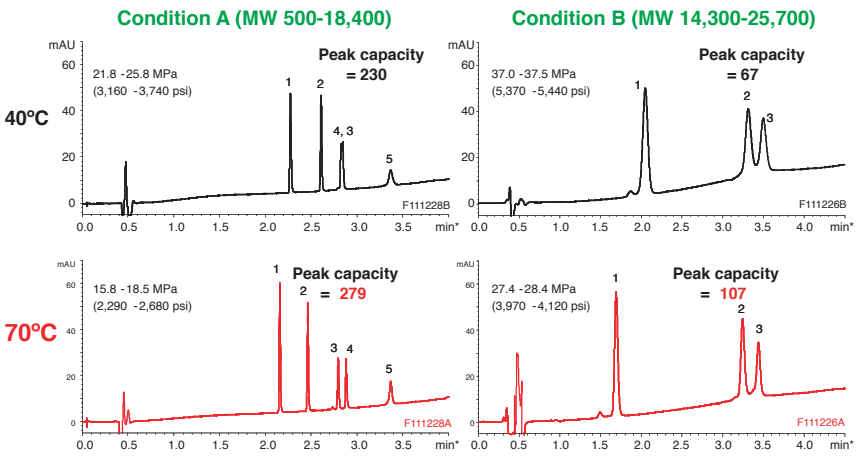


1. Cytochrome *c* (MW 12,400)
2. Insulin (Bovine) (MW 5,700)
3. Amyloid β-protein (MW 4,300)
4. Lysozyme (MW 14,300)
5. α-Lactalbumin (MW 14,100)
6. Myoglobin (MW 17,000)

Column : YMC-Pack 5 μm, 150 X 4.6 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
25-60%B (0-20 min)
Flow rate : 1.0 mL/min
Temperature : 37°C
Detection : UV at 220 nm

For proteins and peptides with molecular weight of 4,300 to 17,000, separation characteristics are compared using columns with different pore size and functional group. In accordance with the table above, the suitable column is C8, 20 nm for groups of compounds with a molecular weight within this range. If either pore size or functional group of the packing material is not optimized, peak broadening and poor resolution are observed. By using the most suitable column (C8, 20 nm) for the target compounds, sharp peak shapes and excellent separation are achieved.

Effect of column temperature on separation of peptides and proteins



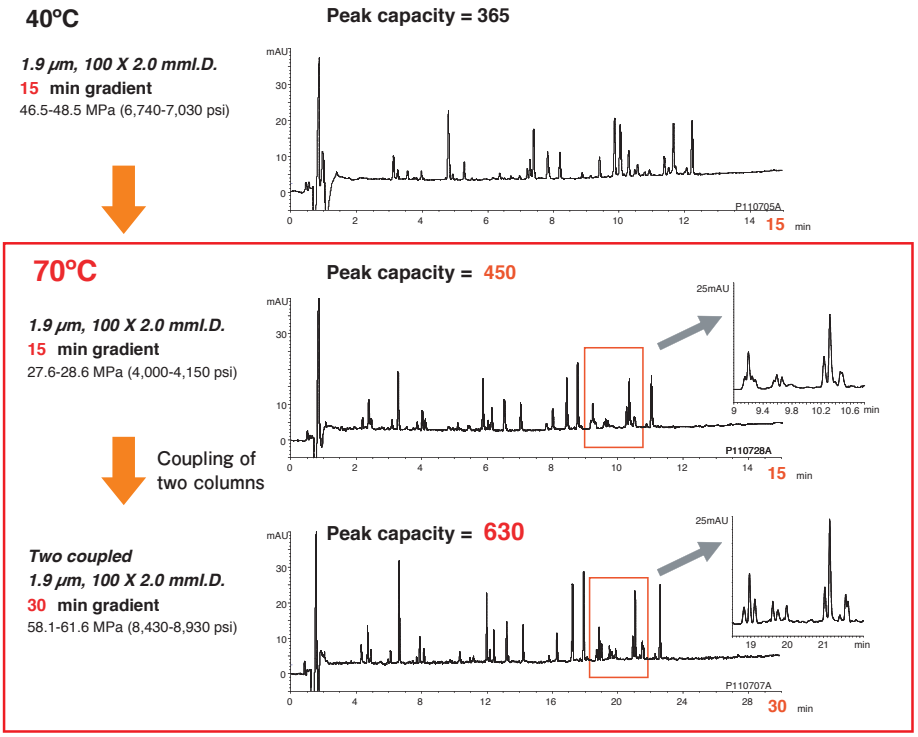
Analytes	MW	Peak width ½ (min)	
		40℃	70℃
Condition A			
1. Oxytocin	1,007	0.017	0.014
2. Leu-Enkephalin	556	0.015	0.015
3. β-Endorphin	3,465	-	0.016
4. Insulin	5,733	-	0.015
5. β-Lactoglobulin A	18,400	0.043	0.030
Condition B			
1. Lysozyme	14,300	0.069	0.044
2. α-Chymotrypsinogen	25,700	0.080	0.049
3. β-Lactoglobulin A	18,400	0.080	0.048

Column : YMC-Triart C18 (1.9 μm, 12 nm), 50 X 2.0 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1) - condition A
B) acetonitrile/2-propanol/TFA (50/50/0.1) - condition B
Gradient : 10-80%B (0-5 min) - condition A
30-60%B (0-5 min) - condition B
Flow rate : 0.4 mL/min
Detection : UV at 220 nm
Injection : 1 μL (50 μg/mL) - condition A
1 μL (250 μg/mL) - condition B
System : Agilent 1200SL

PC (peak capacity) = 1 + (gradient time/peak width*)
*peak width = $2W_{0.5h}$ average

The effect of temperature on separation of peptides and proteins with a variety of molecular weights (MW) is estimated.
The separations at 40°C and 70°C are compared.
By increasing column temperature to 70°C, selectivity change is observed, and peaks become sharper, and improved resolution especially for larger molecules is obtained. Generally, larger molecules diffuse very slowly compared to small molecules.
An elevated temperature can improve efficiency and peak shape by lowering mobile phase viscosity and improving mass transfer.
Temperature is a simple and effective tool to increase resolution in separation of proteins and peptides.

Improvement of resolution by increasing column temperature and coupling of 1.9 μm columns

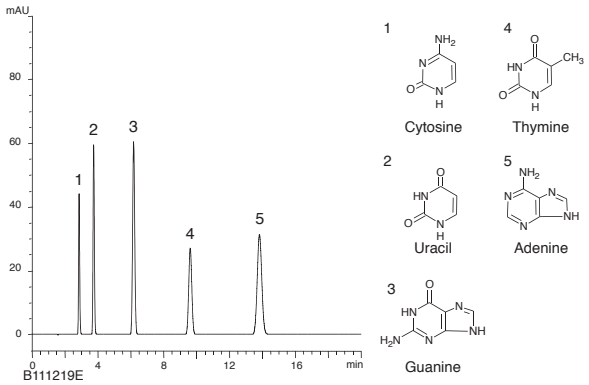


Column : YMC-Triart C18 (1.9 μm, 12 nm)
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.08)
5-40%B (0-15 min) for a single column
5-40%B (0-30 min) for two coupled columns
Flow rate : 0.4 mL/min
Detection : UV at 220 nm
Injection : 10 μL for a single column
20 μL for two coupled columns
Sample : Tryptic digest of Bovine Hemoglobin
System : Agilent 1290

23% more peaks can be resolved by increasing the column temperature to 70°C in the separation of tryptic digest of Hemoglobin.
The outstanding efficiency obtained by a coupling of two 100 mm length of Triart 1.9 μm columns reduces co-elution of peaks and allows the precise separation in an analysis of complicated samples, such as peptide mapping.

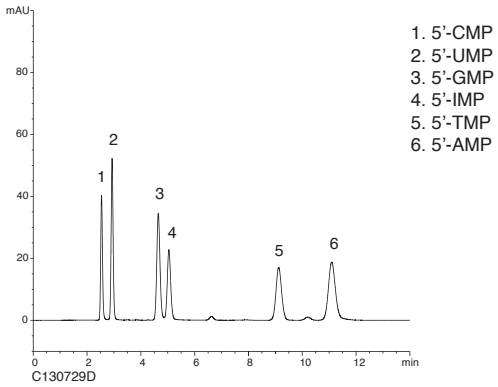
Separation of nucleic acid bases and nucleotides

Nucleic acid bases



Column	: YMC-Triart C18 (5 μm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: 20 mM KH_2PO_4-K_2HPO_4 (pH 6.9)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 254 nm
Injection	: 8 μL (0.01 ~ 0.02 mg/mL)

Nucleotides

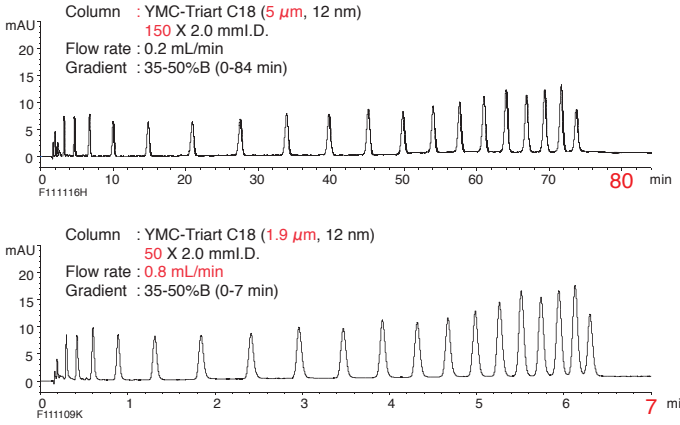


1. 5'-CMP
2. 5'-UMP
3. 5'-GMP
4. 5'-IMP
5. 5'-TMP
6. 5'-AMP

Column	: YMC-Triart C18 (5 μm, 12 nm) 150 X 3.0 mmI.D.
Eluent	: 20 mM CH_3COOH-CH_3COONH_4 (pH 4.7)
Flow rate	: 0.425 mL/min
Temperature	: 25°C
Detection	: UV at 260 nm
Injection	: 2 μL (50 μg/mL)

YMC-Triart C18 is suitable for the separation of hydrophilic compounds and is usable with 100% aqueous mobile phase.

Separation of oligonucleotides



Oligonucleotides d(T)2-20

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

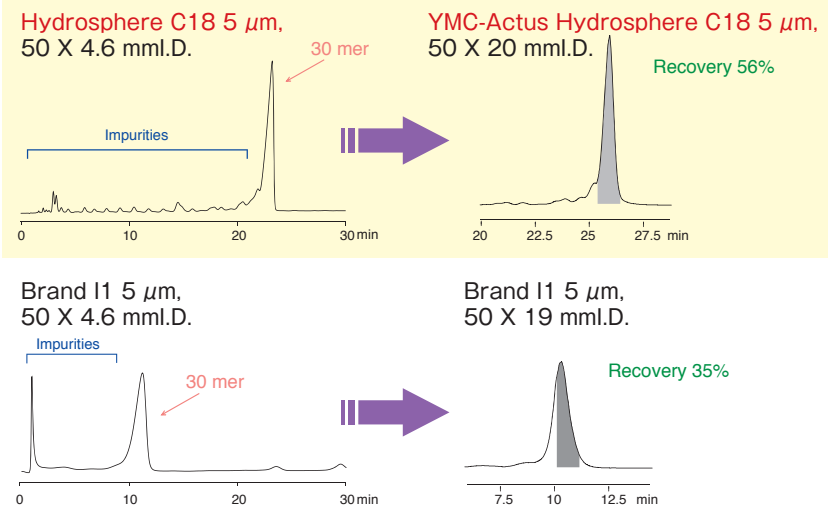
In the separation of oligonucleotides, 19 peaks are completely resolved within 7 minutes using a Triart C18 1.9 μm UHPLC column. The separation is achieved within one tenth of the analysis time of the conventional HPLC method.

Purification of oligonucleotides

Crude synthetic 30 mer oligonucleotides

Analysis 1.0 mL/min, 5 μL injection

Purification 19 mL/min, 100 μL injection



5'-CCGCTCGAGCTAAAA
AAAGCCTGTGTTACC-3'

purity>99%

Eluent	: A) 10 mM DBAA* (pH6.0)/methanol (60/40) B) 10 mM DBAA* (pH6.0)/methanol (20/80) 10-35%B (0-30 min)
Temperature	: ambient
Detection	: UV at 269 nm
Sample	: synthetic oligonucleotide (100 μmol/mL)

* di-*n*-butylamine-acetic acid

In analytical scale, many impurities could be separated from the target compound differing by one nucleotide on Hydrosphere C18. Even in purification scale, YMC-Actus gave superior separation and recovery.
YMC's preparative column has identical performance to analytical column. This enables direct scale up from analytical condition to preparative condition.

Preparative Systems

Biochromatography devices
BioStream

Features

- Suitable for downstream processing for biopharmaceutical manufacturing
- Compliance with cGMP
- Sanitary design superior in cleaning
- Excellent operability provided by the largest 21.5-inch touch panel screen in this industry
- Low flow pumping provided by the quintuplex diaphragm pump
- Compliance with IQ/OQ validation and CSV

* The pump for BSTP-800 is a triple diaphragm pump.

Specifications

Model	BSTP-800	BSTP-03K	BSTS-03K	BSTS-10K	BSTS-30K
Max. flow rate	800 mL/min	3000 mL/min	3000 mL/min	10 L/min	30 L/min
Device pressure limit	0.5 MPa (Max. 0.6 MPa)				
Ambient temperature	5 - 30℃				
Wetted materials	PFA, PTFE, Quartz, Glass, EPDM		SUS316L, PTFE, Quartz, Glass, EPDM		
Sensor	pH sensor, Conductivity sensor, Pressure sensor, Flowmeter sensor, UV sensor (3 variable-wavelengths measurable)				
Other functions	Air trap, Air sensor, Column bypass and Column switching				
Control software	BioStream Software				
Dimensions (mm) (W × D × H)	800 × 900 × 1360	900 × 1100 × 1800	900 × 1100 × 1800	1200 × 1200 × 1800	2000 × 1500 × 1800
Weight	200 kg	250 kg	300 kg	400 kg	600 kg
Utility	Single -phase 100V (15A)	Three-phase 200V (20A)		Three-phase 200V (30A)	Three-phase 200V (40A)
	Instrument air, Dry air				

Software

The large 21.5-inch touch panel screen provides high visibiltiy and operability at production sites. The operation screen has been designed for intuitive and visual operation. Its main control screen provides operation status for control operation and monitoring information of each sensor instantly.

Biochromatography columns
YMC Pilot columns

Features

- Biocompatible and ideal for use in purification of biopharmaceuticals such as proteins and peptides, etc.
- Unique frit design enables reduced losses in diffusion and uniform performance
- Easy scale-up, having the same structure and operability across different column sizes
- Packing bed height easily adjustable by hand wheeled adjusters
- Compliance with IQ/OQ validation and FDA regulations
- Various options available

Specifications

Model		PI100/500	PI100/850	PI140/500	PI140/850	PI200/500	PI200/850
Inner diameter		100 mm	100 mm	140 mm	140 mm	200 mm	200 mm
Packing bed height		50-430 mm	400-780 mm	55-420 mm	405-770 mm	70-435 mm	420-785 mm
Volume	min	0.39 L	3.14 L	0.85 L	6.23 L	2.20 L	13.2 L
	max	3.38 L	6.13 L	6.47 L	11.9 L	13.7 L	24.7 L
Cross-section		78.5 cm^2	78.5 cm^2	154 cm^2	154 cm^2	314 cm^2	314 cm^2
Pressure limit		1.0 MPa	1.0 MPa	0.7 MPa	0.7 MPa	0.5 MPa	0.5 MPa

Other sizes (more than 300 mmI.D.) are available upon request.

Glass columns
ECO PLUS

Features

- Biocompatible
- 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 system

Specifications

Model		TAC05	TAC10	TAC15	TAC25	TAC35	TAC50
Inner diameter		5 mm	10 mm	15 mm	25 mm	35 mm	50 mm
Pressure limit	AB	8.0 MPa	8.0 MPa	7.0 MPa	5.0 MPa	4.0 MPa	3.0 MPa
	SR	8.0 MPa	5.0 MPa	5.0 MPa	5.0 MPa	4.0 MPa	2.5 MPa
Column lengths		125 mm, 250 mm, 500 mm					
Usable temperature range	AB	4 – 40 ℃					
	SR	16 – 40 ℃					
Connection		1/4" – 28G fittings (1/16" tubing)			1/4" – 28G fittings (1/8" tubing)		
Frit	AB	Polyethylene					
	SR	Sintered glass			SUS 316		
Options		short plunger, short/long plunger, long plunger					

Ordering Information

Columns

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

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

Packing material	Particle size (μm)	Pore size (nm)	Column size inner diameter X length (mm)	Product number
BioPro QA-F	3	non-porous	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

Packing material	Particle size (μm)	Pore size (nm)	Column size inner diameter X length (mm)	Product number
BioPro SP-F	3	non-porous	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

Packing material	Particle size (μm)	Pore size (nm)	Column size inner diameter X length (mm)	Product number
Diol-60	5	6	4.6 X 300	DL06S05-3046WT
			8.0 X 300	DL06S05-3008WT
			8.0 X 500	DL06S05-5008WT
			20 X 300	DL06S05-3020WT
			20 X 500	DL06S05-5020WT
Diol-120	5	12	4.6 X 300	DL12S05-3046WT
			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 (nm)	Column size inner diameter X length (mm)	Product number
Diol-200	5	20	4.6 X 300	DL20S05-3046WT
			8.0 X 300	DL20S05-3008WT
			8.0 X 500	DL20S05-5008WT
			20 X 300	DL20S05-3020WT
			20 X 500	DL20S05-5020WT
Diol-300	5	30	4.6 X 300	DL30S05-3046WT
			8.0 X 300	DL30S05-3008WT
			8.0 X 500	DL30S05-5008WT
			20 X 300	DL30S05-3020WT
			20 X 500	DL30S05-5020WT

Bulk media

Product name	Particle size (μm)	Product number
BioPro SmartSep Q10	10	QSA0S10
BioPro SmartSep S10		SSA0S10
BioPro SmartSep Q30	30	QSA0S30
BioPro SmartSep S30		SSA0S30
BioPro Q75	75	QAA0S75
BioPro S75		SPA0S75
BioPro DA60	60	DAM99S60
BioPro CM60		CMM99S60

BioPro Ion Exchange Screening Kits

Product name	Particle size (μm)	Specification	Column volume (mL)	Product number
Ion Exchange Selection Kit (BioPro Q75/S75/DA60/CM60)	75/60	1 each X 4 types	1	BPIESKS99-01PK
BioPro SmartSep Q30	30	5/pack	1	BPQSA0S30-01PK
			5	BPQSA0S30-05PK
BioPro SmartSep S30			1	BPSSA0S30-01PK
			5	BPSSA0S30-05PK
BioPro Q75	75		1	BPQAA0S75-01PK
			5	BPQAA0S75-05PK
BioPro S75			1	BPSPA0S75-01PK
			5	BPSPA0S75-05PK
BioPro DA60	60		1	BPDAM99S60-01PK
			5	BPDAM99S60-05PK
BioPro CM60			1	BPCMM99S60-01PK
			5	BPCMM99S60-05PK

Before use (installation, operation, maintenance or check-up), of our product, an, instruction manual should be carefully read and undeerstood and the safety rules and precautions followed as outlined in a manual.

Worldwide Availability

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www.ymcamerica.com

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