

APPLICATION DATA

Antibody IEX/SEC/HPLC

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Analytical Data

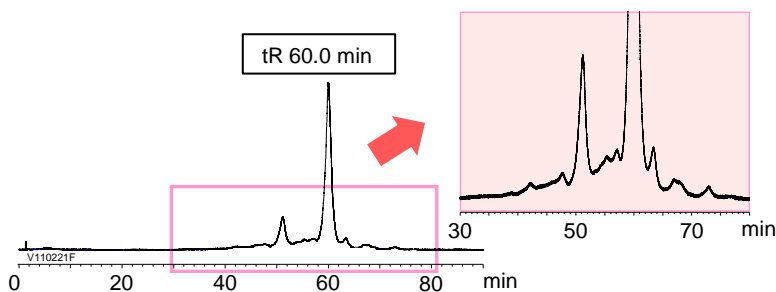
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Analysis of Monoclonal Antibody (MAb) Pharmaceuticals Using Non-Porous Type Ion Exchange Columns

D140827AE

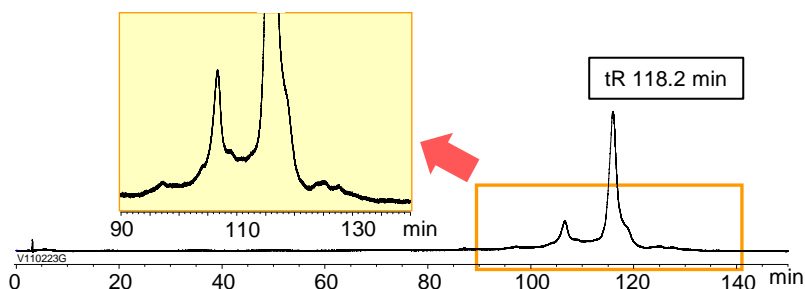
[MAb Analysis on Non-Porous Type Cation Exchange Columns]

BioPro IEX SF 5 μm, 100 X 4.6 mmI.D.



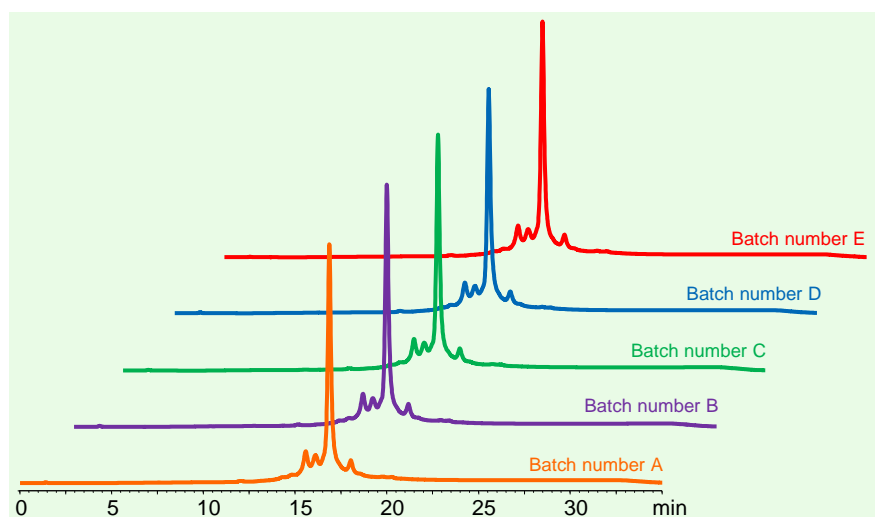
Eluent	: A) 20 mM MES-NaOH (pH 5.6) B) 20 mM MES-NaOH (pH 5.6) containing 0.2 M NaCl
Initial gradient conc.	: 35%B (70 mM NaCl)
Gradient slope	: 0.25%B/min (0.5 mM NaCl)
Flow rate	: 0.5 mL/min for 100 X 4.6 mmI.D., 0.378 mL/min for 250 X 4.0 mmI.D.
Temperature	: 30°C
Detection	: UV at 280 nm
Sample	: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

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



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

[Excellent Batch-to-Batch Reproducibility – Ideal for QC of MAb]



Column	: BioPro IEX SF 5 μm, 100 X 4.6 mmI.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
Flow rate	: 0.5 mL/min
Temperature	: 25°C
Detection	: UV at 215 nm
Injection	: 10 μL
Sample	: monoclonal antibody (IgG1)

BioPro IEX SF column exhibits excellent batch-to-batch reproducibility on MAb analysis, and even on 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. BioPro IEX columns are the best choice for the quality control of MAb and other biopharmaceuticals.

High resolution analysis of monoclonal antibodies utilizing cation exchange column

BioPro IEX SF

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The Universities of Geneva and Lausanne performed a comparative examination of dissimilar ion exchange columns of different manufacturers within an independent study.

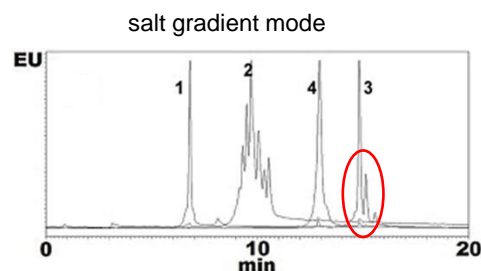
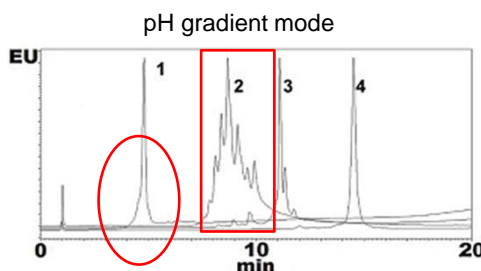
The capacity of ion exchange columns for therapeutic monoclonal antibodies (MAb) was chromatographic analyzed in the pH and salt gradient mode. BioPro IEX SF (former name YMC-BioPro SP-F) scoring highest selectivity and resolution.

BioPro IEX SF column is an outstanding stationary phase for the characterization of charge variants mAbs in cation exchange mode.

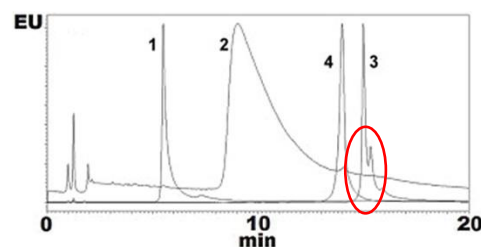
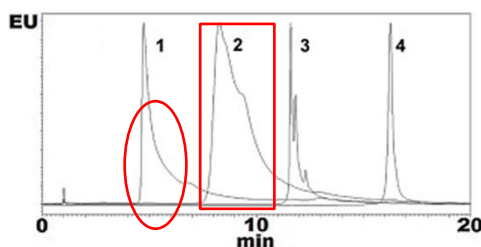
Comparison of monoclonal antibodies separation on pH and salt gradient mode

The chromatograms obtained on BioPro IEX SF, Protein-Pak Hi Res SP (waters), Antibodix WCX NP3 (Sepax) and on MabPac SCX-10RS (Thermo Scientific) columns in pH and salt gradient modes for natalizumab, cetuximab, adalimumab and denosumab is shown below.

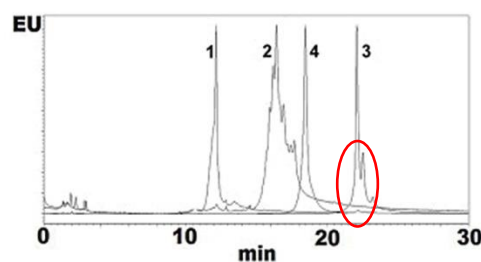
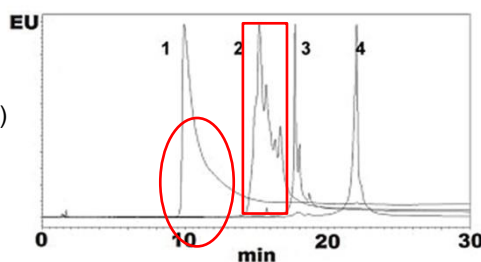
BioPro IEX SF
(S type 5 μ m 100 X 4.6 mmI.D.)



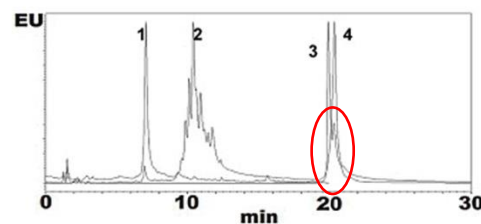
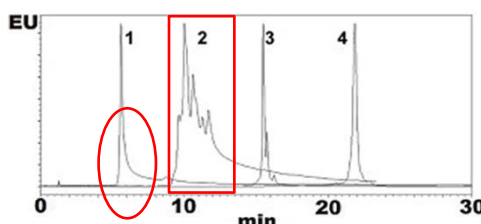
Waters: Protein-Pak Hi Res SP
(S type 7 μ m 100 X 4.6 mmI.D.)



Sepax: Antibodix WCX-NP3
(WCX type 3 μ m 150 X 4.6 mmI.D.)



Thermo Scientific: MabPac SCX-10
(S type 5 μ m 150 X 4.6 mmI.D.)



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)

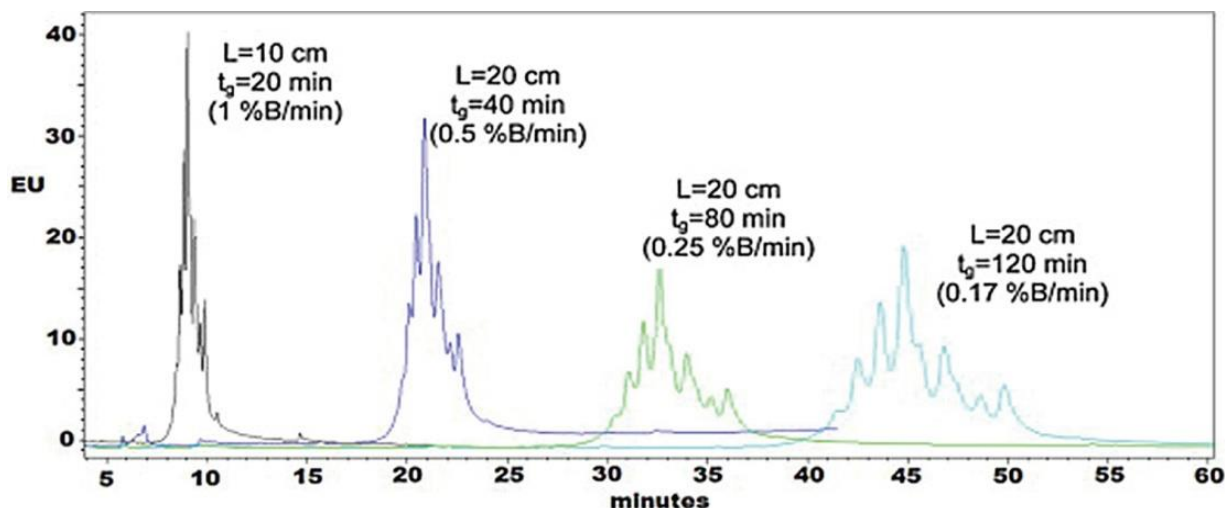
Eluent : A) CX-1 pH Gradient Buffer A* (pH 5.6)
B) CX-1 pH Gradient Buffer B* (pH 10.2)
0-100%B (0-20 min) for 100 X 4.6 mmI.D.
0-100%B (0-30 min) for 150 X 4.6 mmI.D.
Flow rate : 0.6 mL/min

*Purchased Thermo Fisher Scientific Inc.

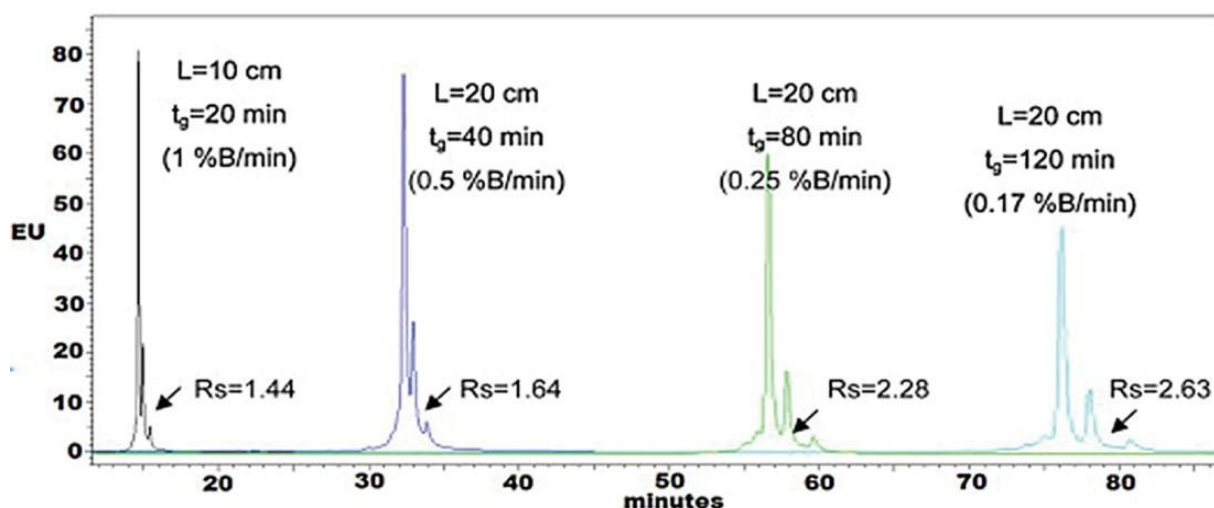
Eluent : A) 10 mM MES-NaOH (pH 5.7)
B) 10 mM MES-NaOH (pH 5.7) containing 1 M NaCl
0-20%B (0-20 min) for 100 X 4.6 mmI.D.
0-20%B (0-30 min) for 150 X 4.6 mmI.D.
Flow rate: 0.6 mL/min

■ BioPro IEX SF is able to provide good peak shapes and achieve higher resolution.

Cetuximab



Adalimumab



salt gradient mode

Column : BioPro IEX SF 5 μ m,
100 X 4.6 mm I.D. X 2
Eluent : A) 10 mM MES-NaOH (pH 5.7)
B) 10 mM MES-NaOH (pH 5.7)
containing 1 M NaCl
Flow rate : 0.6 mL/min

- A column coupling approach could help to improve antibody cation exchange analysis. This allows to compare the chromatographic profiles of different batches of bio-similar products.
- Because BioPro IEX SF showed the highest resolution for the separation of natalizumab, cetuximab, adalimumab and denosumab, it was selected to perform high resolution separation by using a column coupling approach.
- The improved resolution of the different variants through the extension of column length and gradient time is illustrated below.

References

S. Fekete, A. Beck, D. Guillaume, Characterisation of cation exchanger stationary phases applied for the separations of therapeutic monoclonal antibodies, J. Pharm. Biomed. Anal., 2015, 111, 169–176.

High resolution analysis of monoclonal antibodies and fragments using YMC-Pack Diol

R170831AE

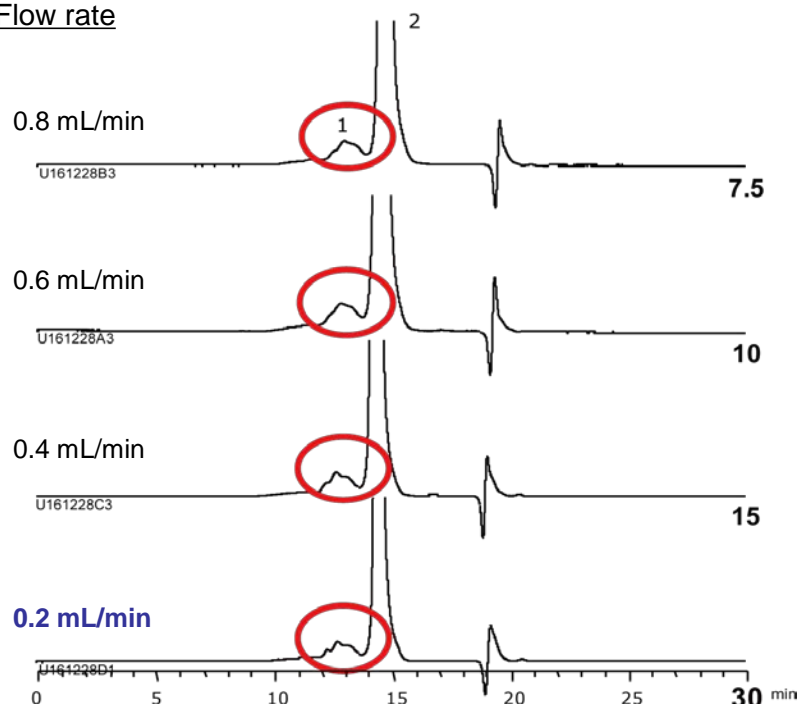
YMC-Pack Diol is a silica gel based size exclusion chromatography (SEC) phase which comes in range of particle sizes and pore sizes for the optimum separation of different biomolecules. Columns packed with 2 μm particles are effective for rapid analysis and high resolution of aggregates and fragments of biopharmaceuticals.

Analysis of monoclonal antibody and its aggregates

● Influence of flow rate on antibody analysis

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

Flow rate

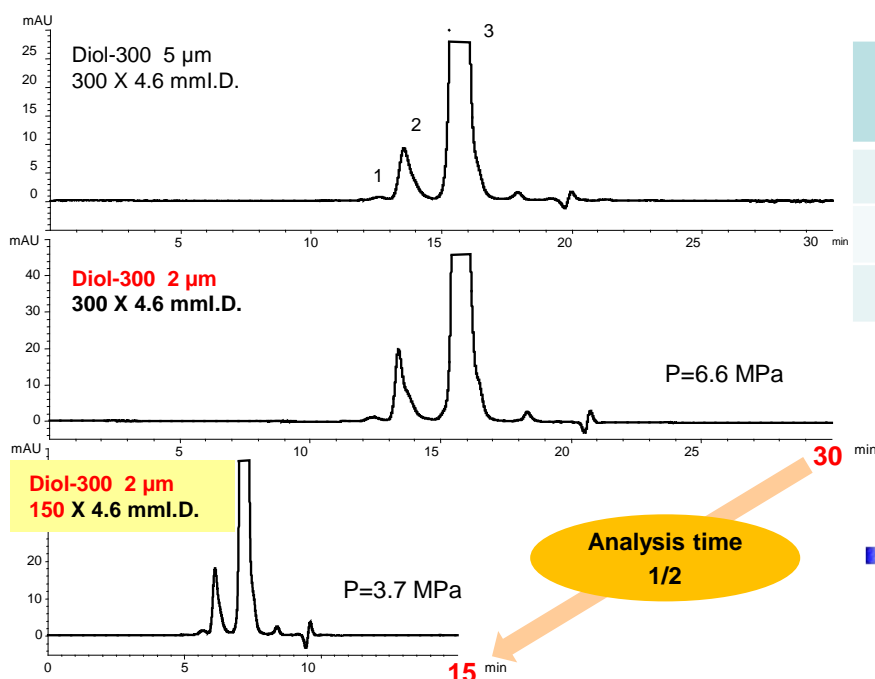


Flow rate (mL/min)	N (2)	P (MPa)
0.8	7,300	28.3
0.6	8,900	20.9
0.4	9,400	14.4
0.2	15,200	7.2

Column : YMC-Pack Diol-300, 2 μm, 300 X 4.6 mmI.D.
 Eluent : 0.1 M KH₂PO₄-K₂HPO₄ (pH 7.0) containing 0.2 M NaCl
 Detection : UV at 280 nm
 Temperature : ambient
 Sample : Humanized monoclonal IgG1

- Diol-300 2 μm column was used for monoclonal antibody analysis. Resolution between aggregates and monomer is improved as the flow rate is decreased.
- Higher flow rates are suitable for increased sample throughput, especially when the resolution is sufficient.

● Increasing throughput by using the 2 μm column

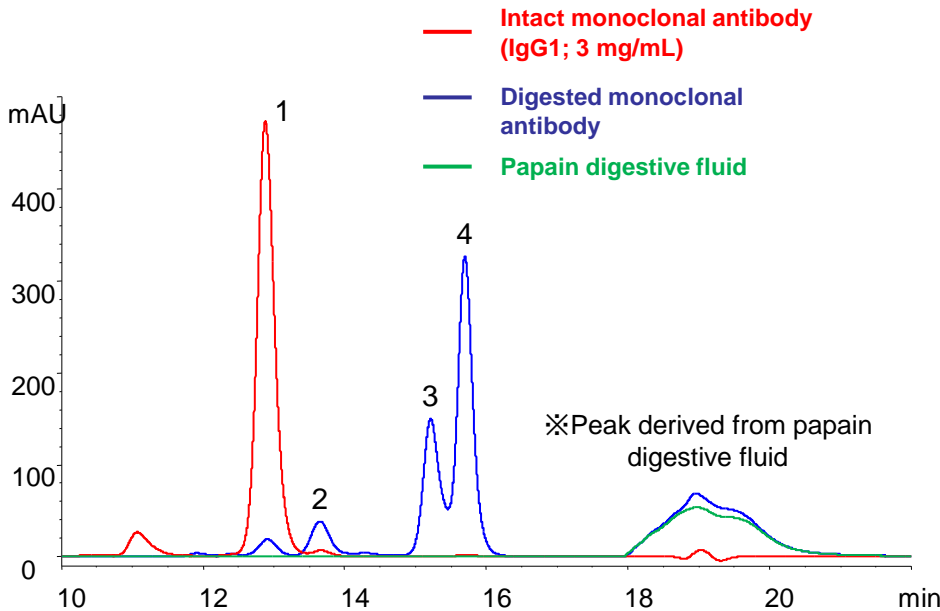


Particle size (μm)	Column size length X I.D. (mm)	N (3)	Rs (1,2)	Rs (2,3)
5	300 X 4.6	8,500	0.88	2.67
2	300 X 4.6	16,200	1.17	4.15
2	150 X 4.6	8,700	0.85	2.75

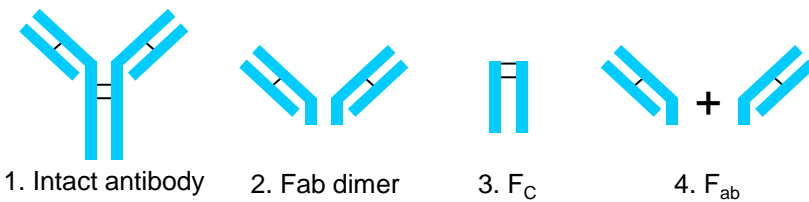
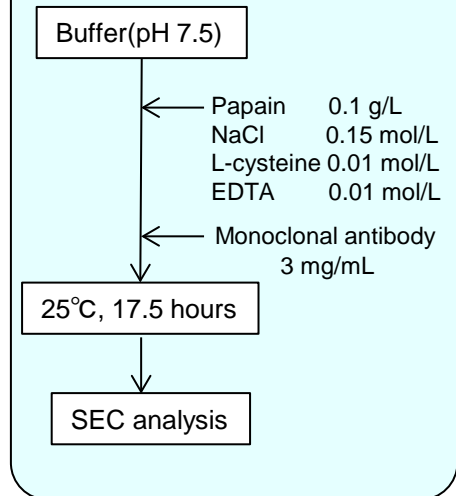
Column : YMC-Pack Diol-300, 2 μm,
 Eluent : 0.1 M KH₂PO₄-K₂HPO₄ (pH 7.0) containing 0.2 M NaCl
 Flow rate : 0.2 mL/min
 Detection : UV at 280 nm
 Temperature : ambient
 Sample : Humanized monoclonal IgG1

- The 2 μm, 150 mm length column offers the same resolution as the 5 μm, 300 mm length column. This means the analysis time can be reduced by half by changing the particle size from 5 μm to 2 μm.

Analysis of monoclonal antibody fragments by using YMC-Pack Diol-200 column



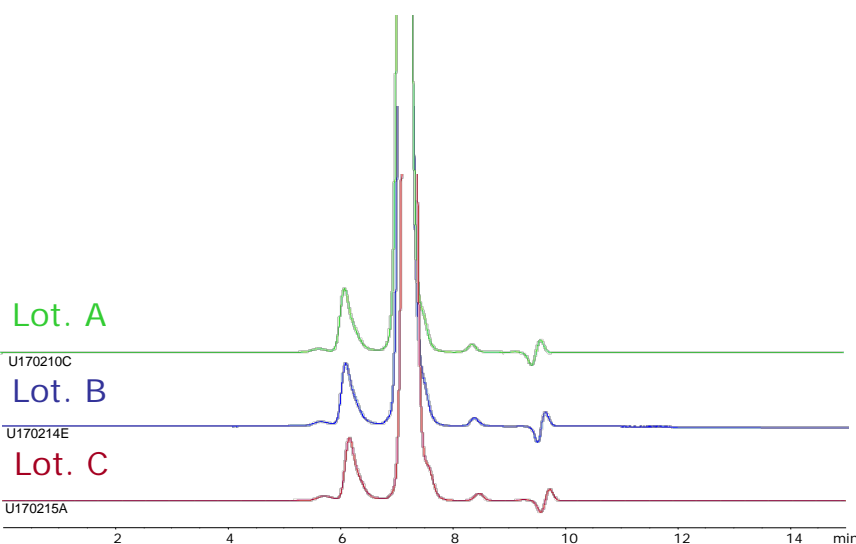
Method of papain digestion



Column	: YMC-Pack Diol-200, 2 μm 300 X 4.6 mm I.D.
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.2 mL/min
Detection	: UV at 280 nm
Temperature	: ambient

- A monoclonal antibody digested by papain, a proteolytic enzyme, as well as the intact monoclonal antibody were analyzed using a YMC-Pack Diol-200 column.
- By using a high resolution 2 μm column, intact antibody and fragments were well separated.

Lot-to-lot reproducibility of YMC-Pack Diol 2 μm



Column	: YMC-Pack Diol-300, 2 μm 150 X 4.6 mm I.D.
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.2 mL/min
Detection	: UV at 280 nm
Temperature	: 25°C
Sample	: Humanized monoclonal antibody

- YMC-Pack Diol 2 μm columns offer high lot-to-lot separation reproducibility. This feature greatly contributes to characterization of antibodies in areas of research as well as quality control of biopharmaceuticals.

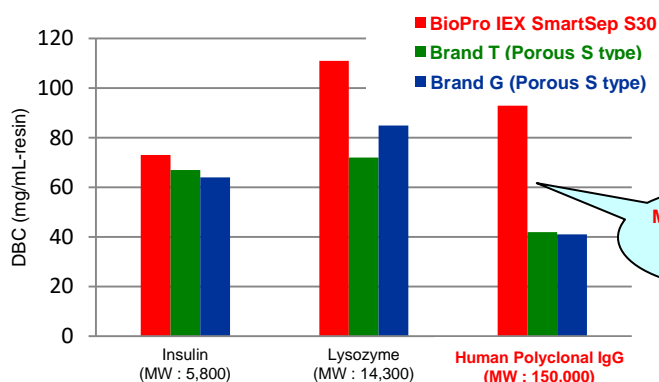
Rapid and effective purification of IgG by using Ion Exchange Media BioPro IEX SmartSep S30

R150521AE

BioPro IEX SmartSep Q/S are ion exchange media dedicated to high-throughput intermediate purification step and polishing step of biopharmaceuticals. BioPro IEX SmartSep media are available in strong ion exchangers of hydrophilic porous polymer beads with low nonspecific adsorption and high binding capacity over a wide range of flow rate. BioPro IEX SmartSep media show both high resolution and recovery even at a high flow rate and high loading condition. DBC is influenced by such as pH, linear velocity and salt concentration. BioPro IEX SmartSep Q/S shows the high DBC in any conditions. BioPro IEX SmartSep Q/S make a big improvement in productivity of biologics, especially, antibody therapeutics.

High Dynamic Binding Capacity (DBC) for IgG

Comparison of DBC of various proteins



	DBC (mg/mL-resin, 10% breakthrough)		
	Insulin (MW : 5,800)	Lysozyme (MW : 14,300)	Human Polyclonal IgG (MW : 150,000)
BioPro IEX SmartSep S30	73	111	93
Brand T (Porous S type, 30 μm)	67	72	42
Brand G (Porous S type, 30 μm)	64	85	41

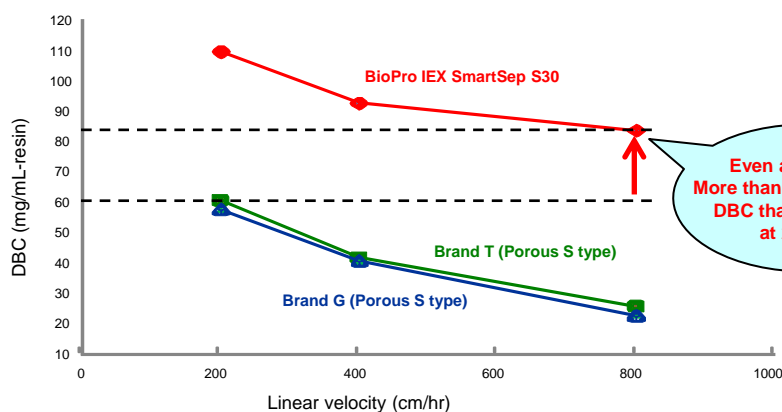
More than 2 times higher than competitors

Conditions of DBC measurement *

Column size : 50 X 5.0 mm I.D.
Sample : 1.5 mg/mL in equilibration buffer
Flow rate : 400 cm/hr (1.32 mL/min)

*Please inquire us for details.

DBC of human polyclonal IgG at various flow rates

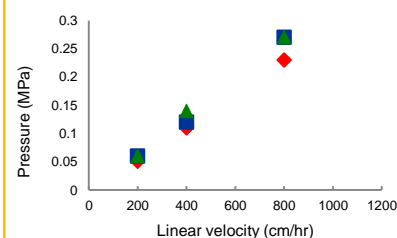


Conditions of DBC measurement

Column : 50 X 5.0 mm I.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

Even at 800 cm/hr, More than 1.5 times higher DBC than competitors at 200 cm/hr

Change of Pressure at various flow rate

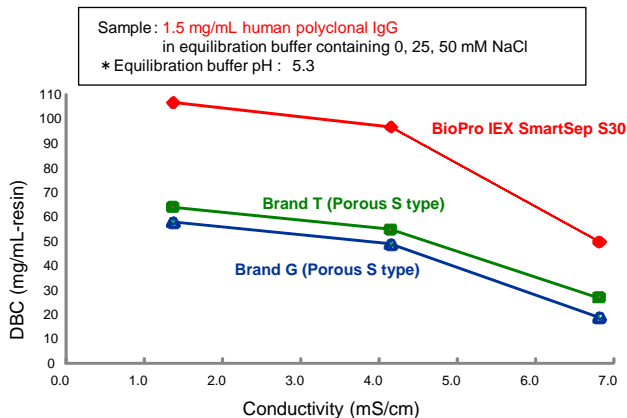


Linear velocity	DBC (mg/mL-resin, 10% breakthrough)		
	200 cm/hr	400 cm/hr	800 cm/hr
BioPro IEX SmartSep S30	110	93	84
Brand T (Porous S type, 30 μm)	61	42	26
Brand G (Porous S type, 30 μm)	58	41	23

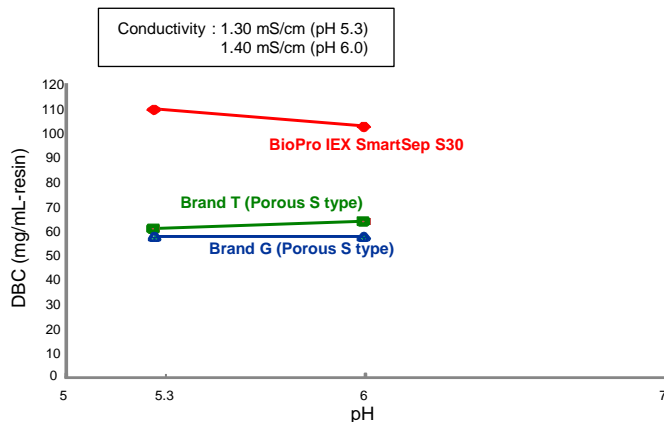
- BioPro IEX SmartSep S30 provides higher DBC on various proteins.
For IgG, BioPro IEX SmartSep has more than twice as high DBC as competitors'
 - ➔ Enabling column downsizing (gel cost reduction) during antibody purification
- Larger difference of DBC at high flow rate
 - Expect improvement of productivity by increasing flow rate.
 - ➔ BioPro IEX SmartSep S30 makes purification productivity per unit time double or more.

High DBC under various conditions

Effect of sample buffers/salts concentration



Effect of equilibration buffer pH



Column : 50 X 5.0 mmI.D.
Equilibration buffer : 20 mM citric acid-NaOH (pH 5.3 or 6.0)
Elution buffer : Equilibration buffer containing 0.5 M NaCl
Flow rate : 200 cm/hr (0.66 mL/min)
Temperature : ambient (25°C)
Detection : UV at 280 nm
Sample : 1.5 mg/mL human polyclonal IgG in equilibration buffer

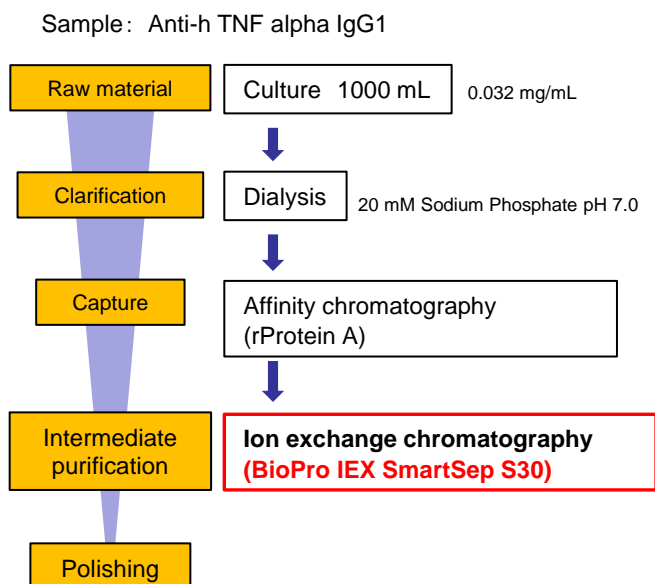
pH	DBC (mg/mL-resin, 10% breakthrough)				
	5.3			5.3	6.0
NaCl concentration	0 mM	25 mM	50 mM	-	-
Conductivity	1.36 mS/cm	4.14 mS/cm	6.8 mS/cm	-	-
BioPro IEX SmartSep S30	107	97	50	110	103
Brand T (Porous S type, 30 μm)	64	55	27	61	64
Brand G (Porous S type, 30 μm)	58	49	19	58	58

- High DBC than competitors even in the presence of 50 mM NaCl.
- ➔ Eluate from Protein A column chromatography could be directly subjected
- High DBC at buffer pH range commonly used in antibody purification by cation exchange chromatography

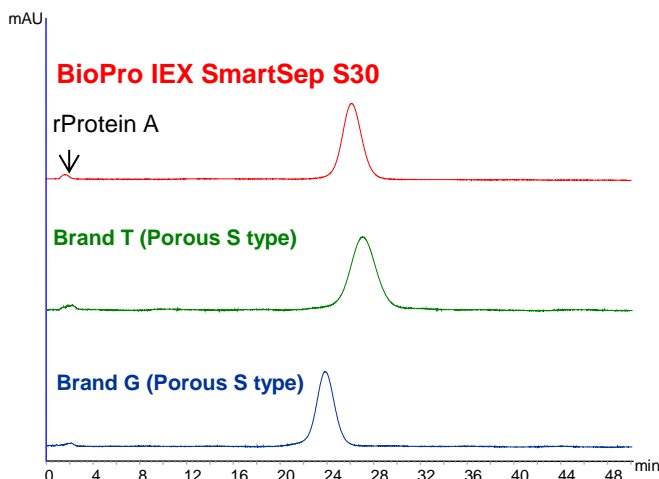
Purification of IgG1 (Anti-hTNFalpha IgG)

This is an example that an IgG1 monoclonal antibody was purified from cell culture medium. After clarified, it was 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 contaminated the eluate, then they were separated and removed by following ion exchange chromatography.

Purification Scheme of 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-hTNFalpha IgG1 (Purified by Affinity chromatography)
Injection : 0.25 mL (0.1 mg IgG1)

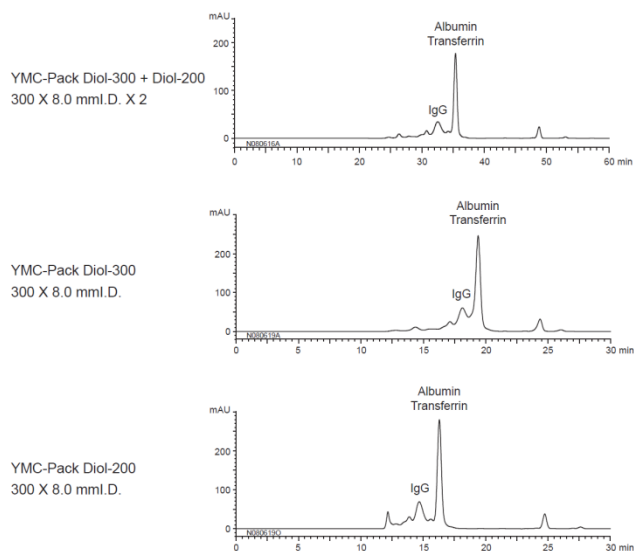
- BioPro IEX SmartSep S30 ion exchange media is effective to remove desorbed rProtein A ligand in the capture step.

Analytical Data

ヒト血清中のタンパク質

Proteins in human serum

N080702B

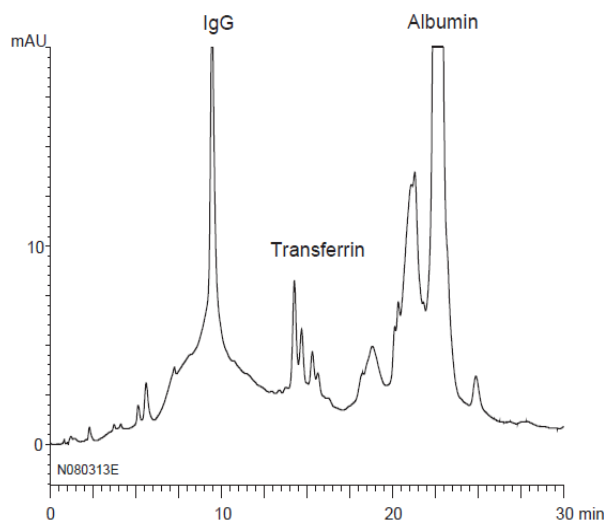


Column : YMC-Pack Diol (5 μ m)
Eluent : 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate : 0.5 mL/min
Temperature : ambient (25°C)
Detection : UV at 280 nm
Injection : 20 μ L
Sample : Human serum (100 μ L/mL)

ヒト血清中のタンパク質

Proteins in human serum

N080313E

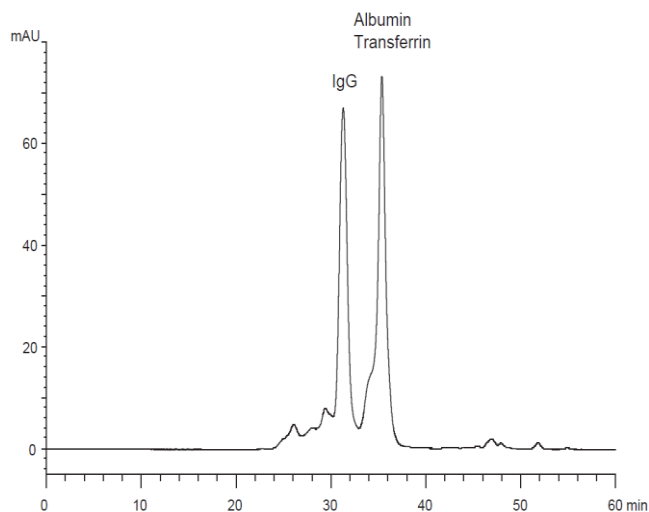


Column : BioPro IEX QA (5 μ m)
50 X 4.6 mmI.D.
Eluent : A) 20 mM Tris-HCl (pH 8.6)
B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl
0-30%B (0-15 min), 30-100%B (15-30 min)
Flow rate : 0.5 mL/min
Temperature : 25°C
Detection : UV at 280 nm
Injection : 20 μ L
Sample : Human serum (100 μ L/mL)

マウス腹水中のタンパク質

Proteins in mouse ascites fluid

P080528A

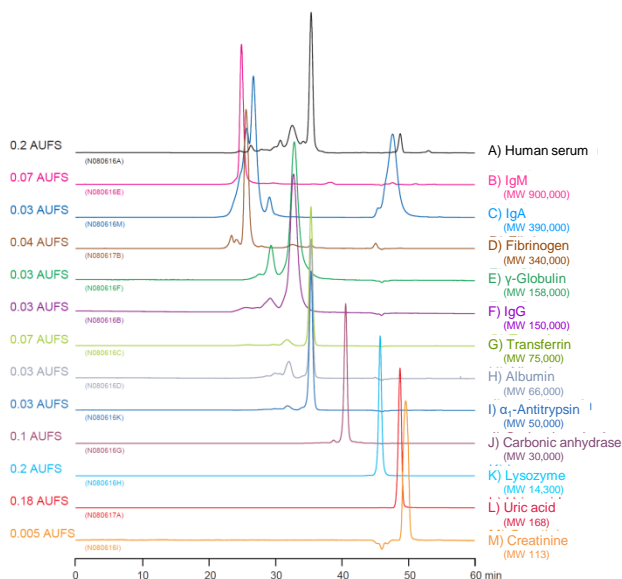


Column : YMC-Pack Diol-300 + Diol-200 (5 μ m)
300 X 4.6 mmI.D. X 2
Eluent : 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0)
Flow rate : 0.17 mL/min
Temperature : ambient (25°C)
Detection : UV at 220 nm
Injection : 10 μ L (60 times dilution with water)

血漿成分

Plasma constituents

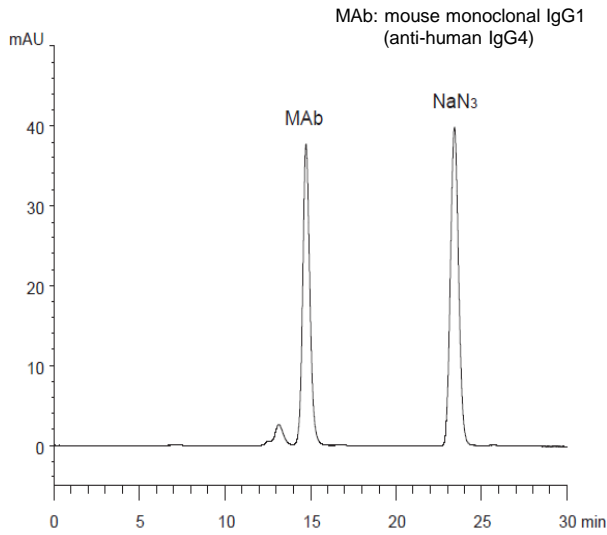
N080702A



Column : YMC-Pack Diol-300 + Diol-200 (5 μ m)
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 μ L (L : 1 μ L)
Sample : A) 100 μ L/mL, B-M) 1.0 mg/mL

モノクローナル抗体 Monoclonal antibody (MAb)

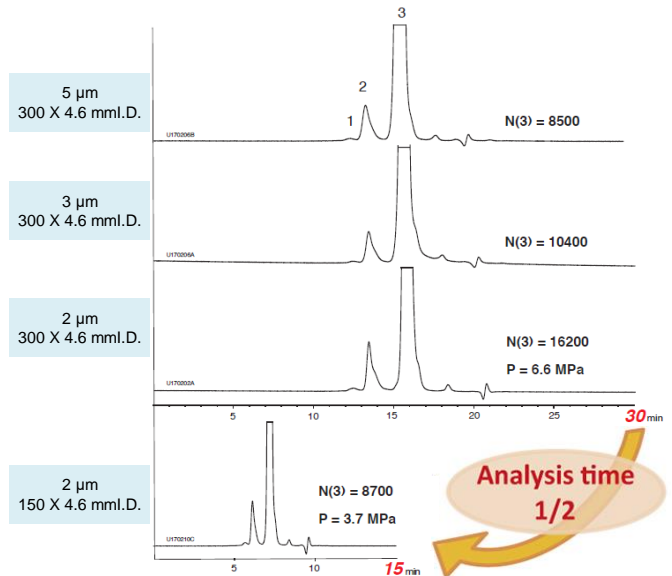
P080530A



Column	: YMC-Pack Diol-200 (5 μm) 300 X 4.6 mmI.D.
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO 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)
Sample	: a commercially available mouse monoclonal IgG1 (purified by DEAE chromatography, containing NaN ₃)

モノクローナル抗体 Monoclonal antibody (MAb)

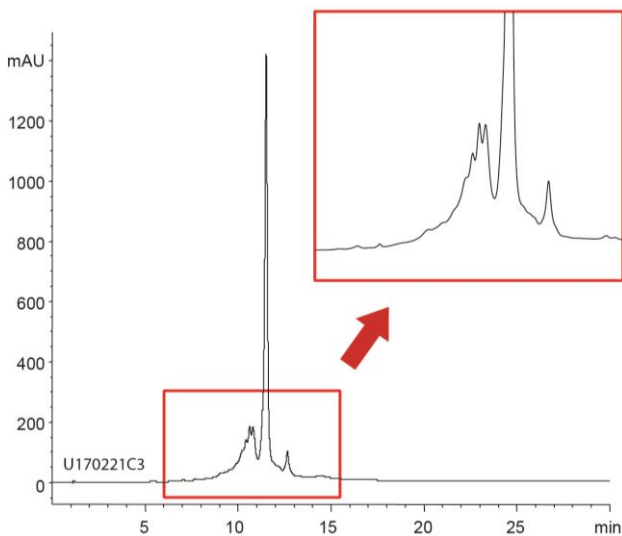
U170210



Column	: YMC-Pack Diol-300
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.2 mL/min
Temperature	: ambient
Detection	: UV at 280 nm
Sample	: Humanized monoclonal IgG1

モノクローナル抗体 Monoclonal antibody (MAb)

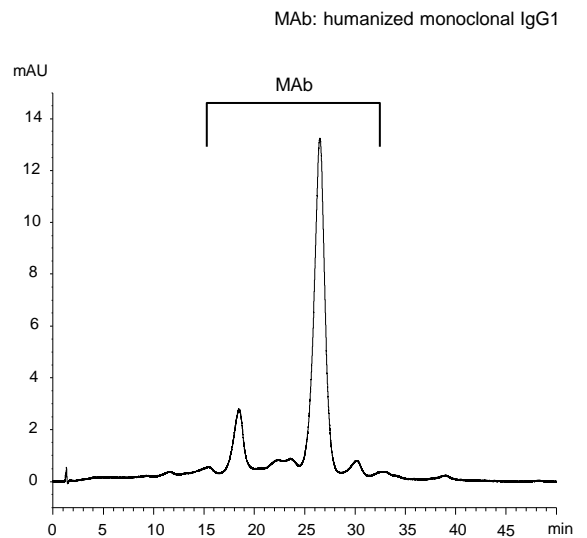
U170221C3



Column	: BioPro IEX QF (3 μm) 100 X 4.6 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 0.2 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 (2.5 mg/mL Humanized monoclonal IgG1)

モノクローナル抗体 Monoclonal antibody (MAb)

V110221B

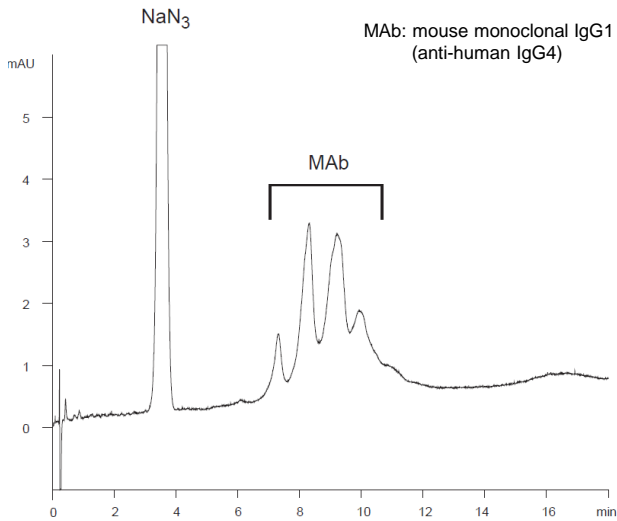


Column	: BioPro IEX SF (5 μm) 100 X 4.6 mmI.D.
Eluent	: A) 20 mM MES-NaOH (pH 6.2) B) 20 mM MES-NaOH (pH 6.2) containing 200 mM NaCl 35-47.5%B (0-50 min)
Flow rate	: 0.5 mL/min (180 cm/hr)
Temperature	: 30°C
Detection	: UV at 280 nm
Injection	: 10 μL
Sample	: Humanized monoclonal IgG1 (1 mg/mL)

モノクローナル抗体

Monoclonal antibody (MAb)

P080220A

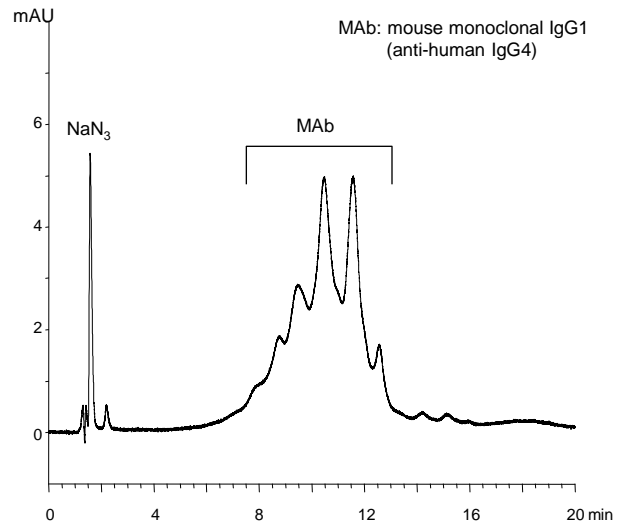


Column : BioPro IEX QF (5 μ m)
30 X 4.6 mmI.D.
Eluent : A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
10-25%B (0-18 min)
Flow rate : 1.0 mL/min
Temperature : 25°C
Detection : UV at 220 nm
Injection : 10 μ L (0.1 mg/mL)
Sample : a commercially available mouse monoclonal IgG1 (purified by DEAE chromatography, containing NaN_3)

モノクローナル抗体

Monoclonal antibody (MAb)

V110203B

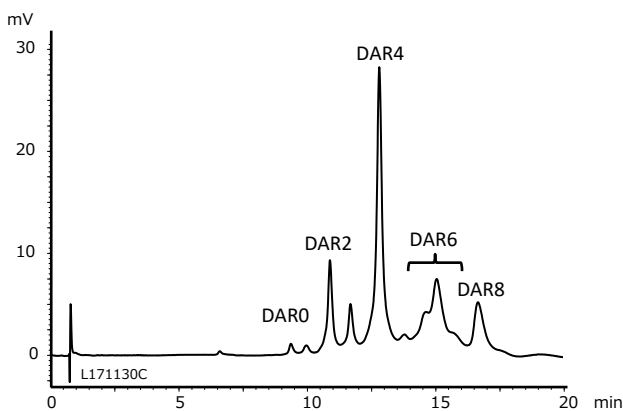


Column : BioPro IEX SF (5 μ m)
100 X 4.6 mmI.D.
Eluent : A) 20 mM MES-NaOH (pH 5.6) containing 30 mM NaCl
B) 20 mM MES-NaOH (pH 5.6) containing 180 mM NaCl
20-40%B (0-20 min)
Flow rate : 0.5 mL/min (180 cm/hr)
Temperature : 30°C
Detection : UV at 280 nm
Injection : 10 μ L (1 mg/mL)
Sample : a commercially available mouse monoclonal IgG1 (purified by DEAE chromatography, containing NaN_3)

抗体薬物複合体(ADC)の薬物抗体比(DAR)分析 Drug-to-Antibody Ratio (DAR) analysis of ADCs*

L171130C

*ADCs : Antibody-Drug Conjugates

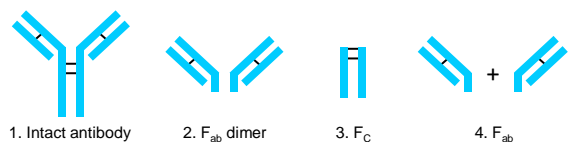
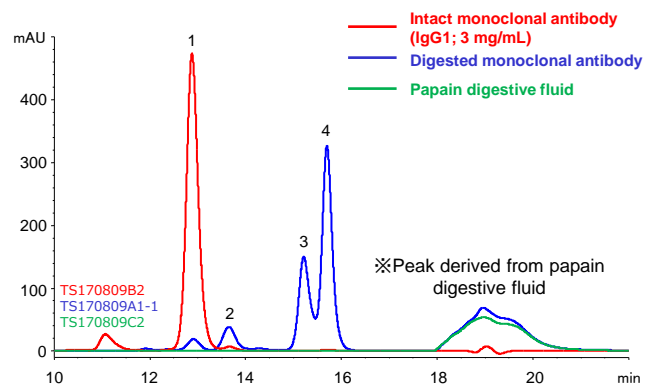


Column : BioPro HIC BF (4 μ m), 100 X 4.6 mmI.D.
Eluent : A) 50 mM NaH_2PO_4 - Na_2HPO_4 (pH 7.0) containing 1.5 M $(\text{NH}_4)_2\text{SO}_4$ /2-propanol (95/5)
B) 50 mM NaH_2PO_4 - Na_2HPO_4 (pH 7.0)/2-propanol (80/20)
0%B (0-1 min), 0-100%B (1-15 min), 100%B (15-20 min)
Flow rate : 1.0 mL/min
Temperature : 25°C
Detection : UV at 280 nm
Injection : 5 μ L
Sample : Cysteine-conjugated ADC mimic (5 mg/mL)

モノクローナル抗体のパパイン消化断片

Monoclonal antibody fragments

TS170809

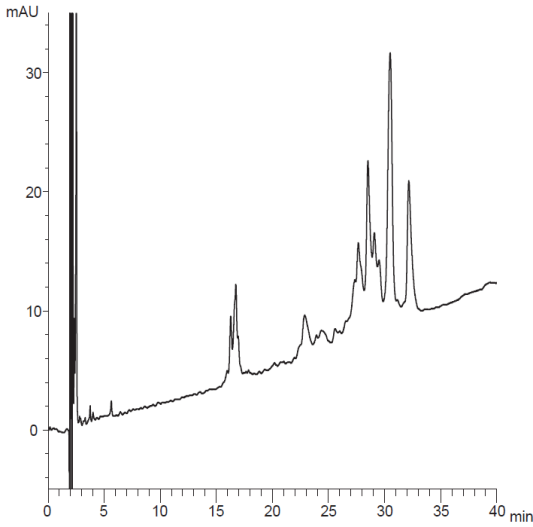


Column : YMC-Pack Diol-200 (2 μ m)
300 X 4.6 mmI.D.
Eluent : 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate : 0.2 mL/min
Temperature : ambient
Detection : UV at 280 nm

マウスIgG Fcフラグメント

Mouse IgG Fc fragment

R080619B

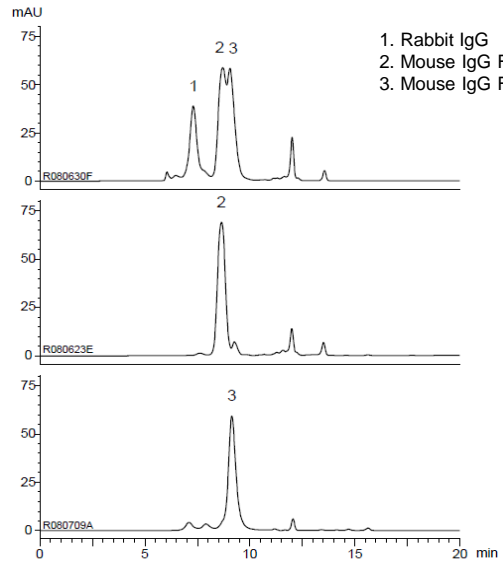


Column	: YMC-Pack C ₄ (5 μm, 30 nm), 150 X 4.6 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 25-45%B (0-40 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 5 μL (1.0 mg/mL)

*Prepared from normal serum by a multi-step process which includes delipidation, salt fractionation, ion exchange chromatography and papain digestion followed by chromatographic separation and extensive dialysis against the buffer stated above.

免疫グロブリン (IgG) およびIgGフラグメント IgG, Fb and Fc fragment

R080708A

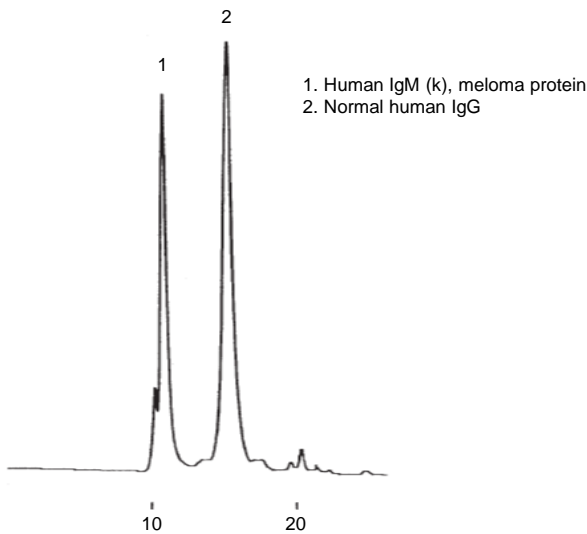


Column	: YMC-Pack Diol-200 (5 μm) 300 X 8.0 mmI.D.
Eluent	: 0.1M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 6.9) containing 0.2 M NaCl
Flow rate	: 1.0 mL/min
Temperature	: ambient (27°C)
Detection	: UV at 220 nm
Injection	: 5 μL (0.4, 0.5 mg/mL)

ヒト免疫グロブリン

Human immunoglobulins

G920108F

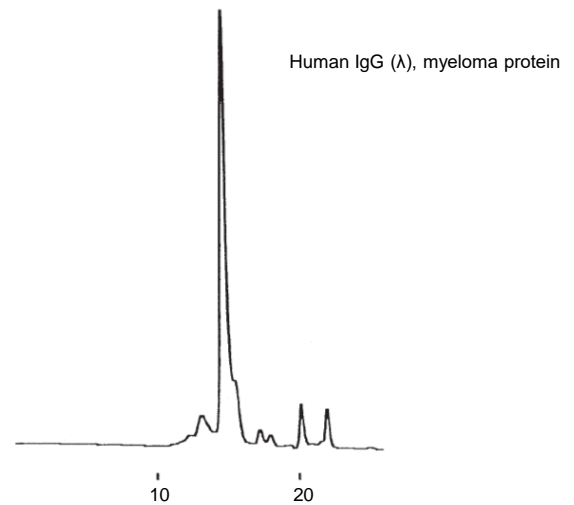


Column	: YMC-Pack Diol-300 (5 μm) 500 X 8.0 mmI.D.
Eluent	: 0.1 M NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.8) containing 0.1 M Na ₂ SO ₄
Flow rate	: 1.0 mL/min
Temperature	: ambient (24°C)
Detection	: UV at 280 nm
Injection	: 40 μL (0.5 mg/mL)

ヒト免疫グロブリン (IgG λ鎖, ミエローマタンパク質)

Human IgG1 (λ), myeloma protein

G920108B



Column	: YMC-Pack Diol-300 (5 μm) 500 X 8.0 mmI.D.
Eluent	: 0.1 M NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.8) containing 0.1 M Na ₂ SO ₄
Flow rate	: 1.0 mL/min
Temperature	: ambient (24°C)
Detection	: UV at 280 nm
Injection	: 20 μL (1.0 mg/mL)

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