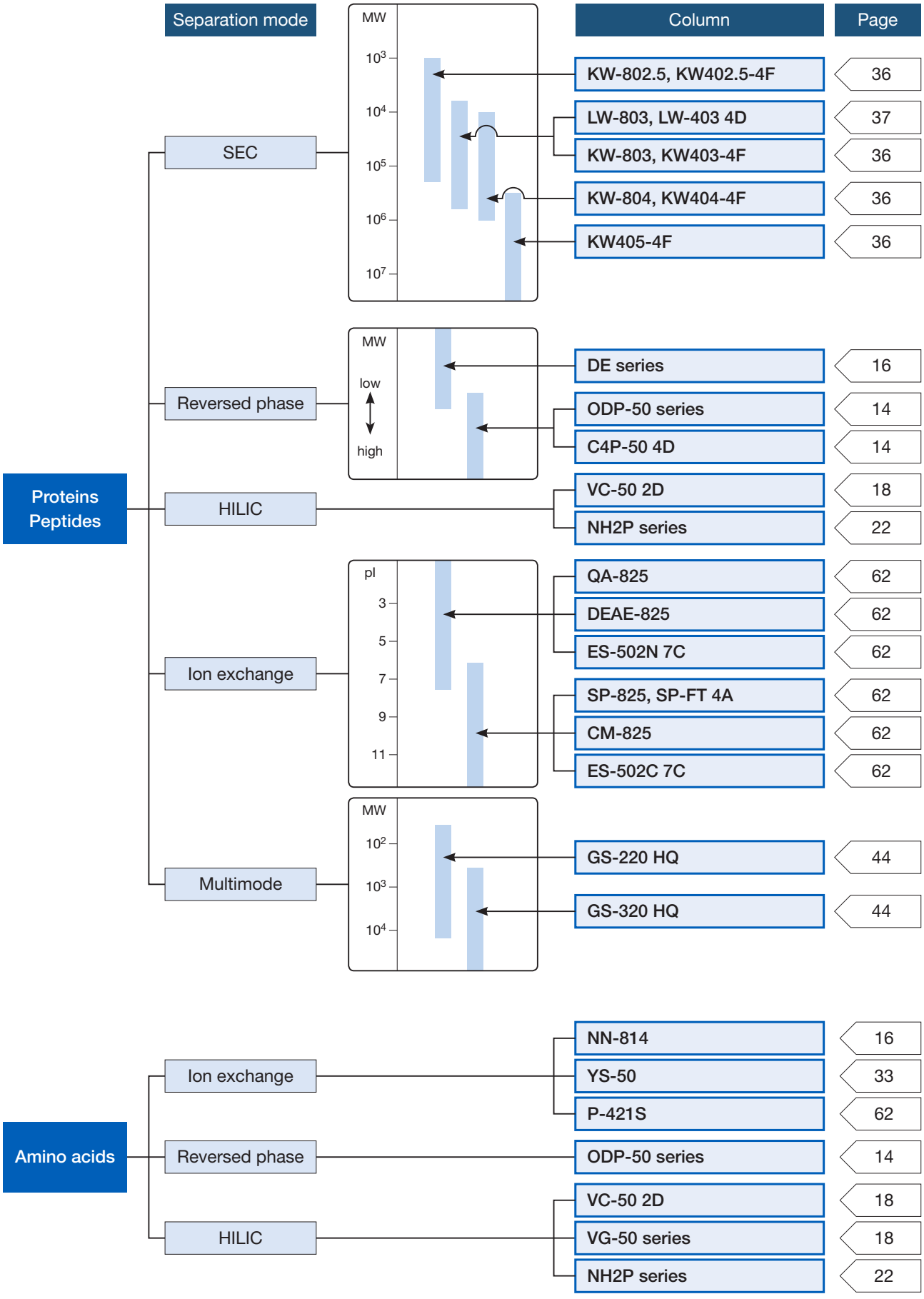
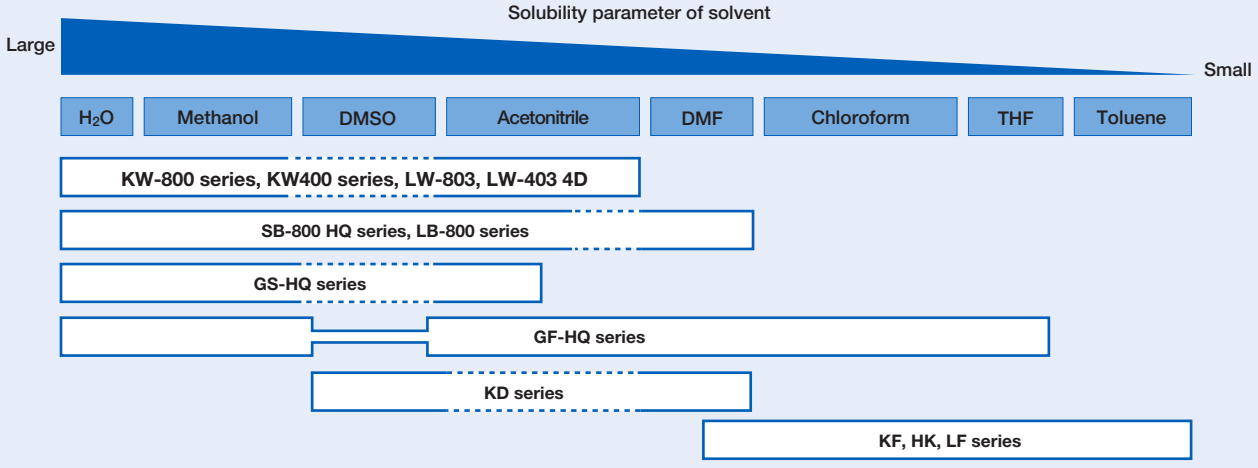
Column Selection (Proteins, Peptides, and Amino Acids)



Column Selection (Polymers)

	Application	Eluent	Column	Page
Aqueous SEC (GFC)	Biological macromolecules (Proteins, Peptides, Nucleic acids, etc.)	Buffer etc.	KW-800 series	36
			KW400 series	36
			LW-803	37
			LW-403 4D	37
	Biological macromolecules (High MW range)	Buffer etc.	SB-800 HQ series	40
			LB-800 series	41
	Water-soluble polymers (Polyacrylamide, etc.)	Water, buffer and aqueous salt solution, etc.	SB-800 HQ series	40
			LB-800 series	41
Organic SEC (GPC)	General polymers	THF	KF-800 series	48
			KF-400HQ series	52
			HK-400 series	54
			LF series	56
		Chloroform	KF-800 series	48
			HK-400 series	54
			LF series	56
	Polar polymers (Polyimides etc.)	DMF	KD-800 series	50
			HK-400 series	54
			LF series	56
			SB-800 HQ series	40
			LB-800 series	41
	Engineering plastics (Polyamides etc.)	HFIP	KD-800 series	50
			HK-400 series	54
			LF series	56
Aqueous-Organic SEC			GF-HQ series	46

Guideline for SEC column selection by solvent usability



See page 60 for the solvent replaceability of organic solvent SEC (GPC) packed columns.

Precautions for Polar Polymer Analysis

Unexpected interactions in the column can affect the size exclusion chromatography analysis of polar polymers.
These interactions may change elution patterns and results in an invalid molecular weight calculation.
It is important to reduce these interfering interactions in order to obtain the accurate molecular weight distribution.

~ Interfering interactions likely to be observed ~

Interactions between the analyte and the packing materials

◆ Hydrophobic interaction
→ The analyte is adsorbed on the packing material.
This delays the analyte elution and results in under estimating the analyte's molecular weight. See (B) and (D).

◆ Ionic interaction
(1) Ion Exclusion
→ The analyte is repelled from the packing material.
This accelerates the analyte elution and results in over estimating the analyte's molecular weight. See (A) and (C).
(2) Ion Exchange
→ The analyte is adsorbed onto the packing material.
This delays the analyte elution and results in under estimating the analyte's molecular weight. See (B) and (D).

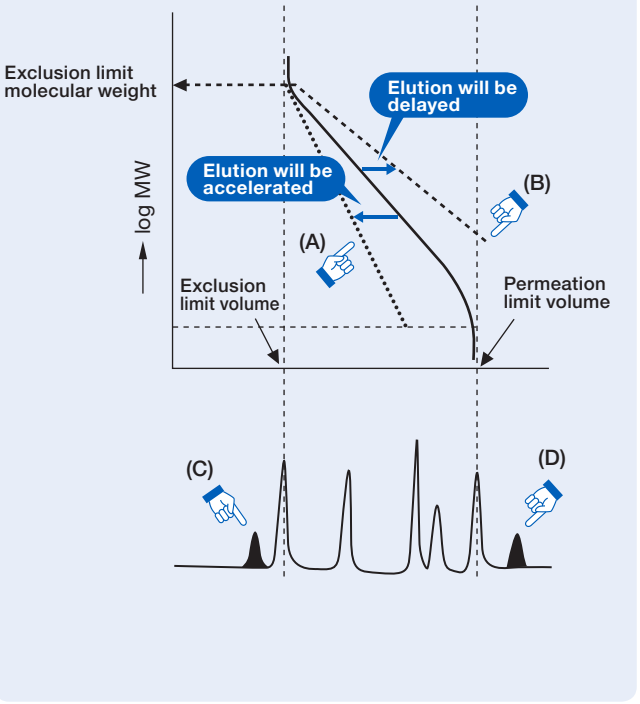
Interaction within and between the analyte

◆ Ionic repulsion effects observed within the multivalent macromolecules causes structure expansion
→ This accelerates the analyte elution and results in over estimating the analyte's molecular weight. See (A).

◆ Association between the molecules
→ This accelerates the analyte elution and results in over estimating the analyte's molecular weight. See (A).

Interactions between the analyte and the solvent

◆ The multivalent ion in the solvent works as a bridge to bind ionic molecules (analyte).



Methods to reduce interactions

Aqueous SEC (GFC)

Ionic interaction

◆ Add salt into the eluent

Hydrophobic interaction

◆ Increase the analyte dissociation
Cationic polymer → Lower the eluent pH
Anionic polymer → Higher the eluent pH

◆ Lower the eluent polarity
e.g. Add acetonitrile or methanol

Organic SEC (GPC)

Ionic interaction

◆ Add salt into the eluent
e.g. Add LiBr to DMF
Add CF_3COONa to HFIP

Hydrophobic interaction

◆ Lower the eluent polarity
e.g. Change the eluent from DMF to THF

Hydrophilic interaction

◆ Increase the eluent polarity
e.g. Change the eluent from THF to DMF

Aqueous SEC (GFC) Columns: Silica-based

Features

KW-800	• Silica-based packed columns for aqueous SEC (GFC) analysis • Suitable for the analysis of proteins and enzymes • Fulfills USP-NF L20, L33, and L59 requirements
KW400	• Reduced packing material particle size enhances column performance • Three to four-fold higher sensitivity than KW-800 series • KW405-4F is applicable analyzing samples with molecular weight above 1,000,000 • Fulfills USP-NF L20, L33, and L59 requirements
LW-803	• Pore size specifically controlled for analyzing proteins with a molecular weight of several hundred of thousand • High performance analysis of antibody drugs and various proteins • High lot-to-lot reproducibility • Fulfills USP-NF L20, L33, and L59 requirements
LW-403 4D	• Rapid analysis column of LW-803 • Achieves approximately halved analysis time compared with standard column • Fulfills USP-NF L20, L33, and L59 requirements

• Standard columns

Product Code	Product Name	* Plate Number (TP/column)	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D. x Length	Shipping Solvent
F6989000	**PROTEIN KW-802.5**	≥ 21,000	5	150 (max. 400)	**8.0 x 300**	H_2O
F6989103	**PROTEIN KW-803**	≥ 21,000	5	300 (max. 1,000)	**8.0 x 300**	H_2O
F6989104	**PROTEIN KW-804**	≥ 16,000	7	500 (max. 1,500)	**8.0 x 300**	H_2O
F6700131	**PROTEIN KW-G 6B**	(guard column)	7	—	**6.0 x 50**	H_2O

* Measured with ethylene glycol

Base Material: Silica
Usable pH Range: pH3.0 - 7.5

• High performance semi-micro columns

* KW400 series is recommended to be used with semi-micro type devices.

Product Code	Product Name	* Plate Number (TP/column)	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D. x Length	Shipping Solvent
F6989201	**PROTEIN KW402.5-4F**	≥ 35,000	3	150 (max. 400)	**4.6 x 300**	H_2O
F6989202	**PROTEIN KW403-4F**	≥ 35,000	3	250 (max. 800)	**4.6 x 300**	H_2O
F6989203	**PROTEIN KW404-4F**	≥ 25,000	5	500 (max. 1,500)	**4.6 x 300**	H_2O
F6989204	**PROTEIN KW405-4F**	≥ 25,000	5	1,000 (max. 2,000)	**4.6 x 300**	H_2O
F6700132	**PROTEIN KW400G-4A**	(guard column)	5	—	**4.6 x 10**	H_2O

* Measured with uridine

Base Material: Silica
Usable pH Range: pH3.0 - 7.5

For antibody drugs analysis

● Standard columns

Product Code	Product Name	* Plate Number (TP/column)	Particle Size (μm)	Pore Size (Å)	Column Size (mm) I.D. x Length	Shipping Solvent
F6989303	**PROTEIN LW-803**	≥ 12,000	3	300 (max. 1,000)	**8.0 x 300**	H_2O
F6700133	**PROTEIN LW-G 6B**	(guard column)	3	—	**6.0 x 50**	H_2O

* Measured with bovine serum albumin

Base Material: Silica
Usable pH Range: pH3.0 - 7.5

● Semi-micro columns

* LW-403 4D is recommended to be used with semi-micro type devices.

Product Code	Product Name	* Plate Number (TP/column)	Particle Size (μm)	Pore Size (Å)	Column Size (mm) I.D. x Length	Shipping Solvent
F6989403	**PROTEIN LW-403 4D**	≥ 11,000	1.9	300 (max. 1,000)	**4.6 x 150**	H_2O
F6700134	**PROTEIN LS-G 4J**	(guard column)	1.9	—	**4.6 x 20**	H_2O

* Measured with bovine serum albumin

Base Material: Silica
Usable pH Range: pH3.0 - 7.5

Usable solvents

Product Name	Solvent			
	Acetonitrile	Methanol	Ethanol	2-Propanol (IPA)
KW-802.5, KW-803, KW-804	○	○	○	○
KW402.5-4F	○	○	○	△
KW403-4F	○	○	○	×
KW404-4F, KW405-4F	○	○	○	○
LW-803	○	○	○	○
LW-403 4D	○	○	○	×

○: Solvent replacement possible △: Solvent replacement possible up to 50 % ×: Solvent replacement not possible

Target molecular weight range and exclusion limit

● Measured with protein (eluent: phosphate buffer)

Product Name	Target Molecular Weight Range	Exclusion Limit
KW-802.5	5,000 - 100,000	150,000
KW-803	10,000 - 700,000	* (1,000,000)
KW-804	30,000 - * (4,000,000)	* (4,000,000)
KW402.5-4F	5,000 - 70,000	150,000
KW403-4F	10,000 - 500,000	600,000
KW404-4F	30,000 - * (4,000,000)	* (4,000,000)
KW405-4F	200,000 - * (20,000,000)	* (20,000,000)
LW-803, LW-403 4D	10,000 - 700,000	* (1,000,000)

Please use the above table for reference purposes only when selecting columns.

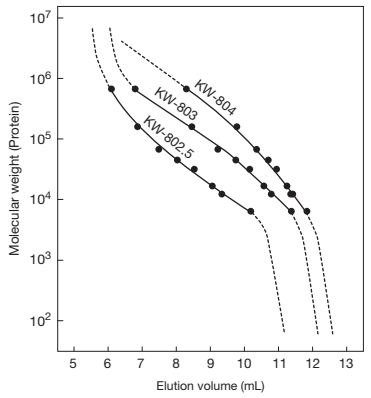
* ( ) Estimated value

● Measured with pullulan (eluent: ultrapure water)

Product Name	Target Molecular Weight Range	Exclusion Limit
KW-802.5	2,000 - 50,000	60,000
KW-803	5,000 - 100,000	170,000
KW-804	20,000 - 300,000	500,000
KW402.5-4F	2,000 - 40,000	60,000
KW403-4F	3,000 - 50,000	80,000
KW404-4F	20,000 - 300,000	400,000
KW405-4F	100,000 - 700,000	1,300,000

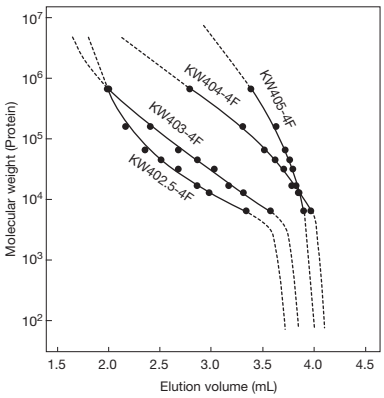
Please use the above table for reference purposes only when selecting columns.

Calibration curves for KW-800 series using protein



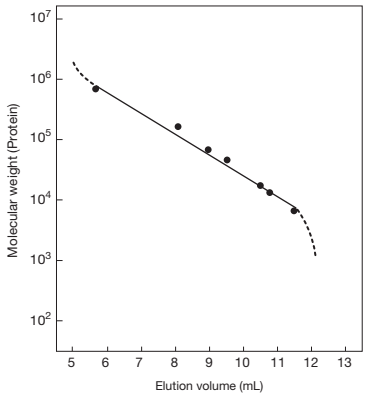
Column : **Shodex PROTEIN KW-800 series**
Eluent : 50 mM Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : 30 ℃

Calibration curves for KW400 series using protein



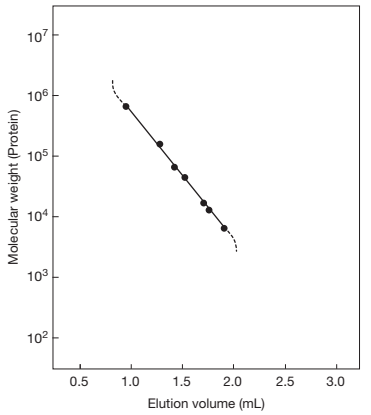
Column : **Shodex KW400-4F series**
Eluent : 50 mM Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : 0.33 mL/min
Detector : UV (280 nm) (small cell volume)
Column temp. : 30 ℃

Calibration curve for LW-803 using protein



Column : **Shodex PROTEIN LW-803**
Eluent : 50 mM Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : Room temp.

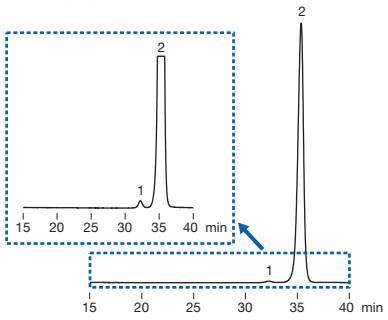
Calibration curve for LW-403 4D using protein



Column : **Shodex PROTEIN LW-403 4D**
Eluent : 50 mM Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : 0.35 mL/min
Detector : UV (280 nm) (small cell volume)
Column temp. : 30 ℃

Analysis of impurities (high molecular weight proteins) in insulin glargine according to USP-NF method

Sample : 100 µL
System suitability solution
(prepared following USP-NF method)
1. High molecular weight proteins
2. Insulin glargine

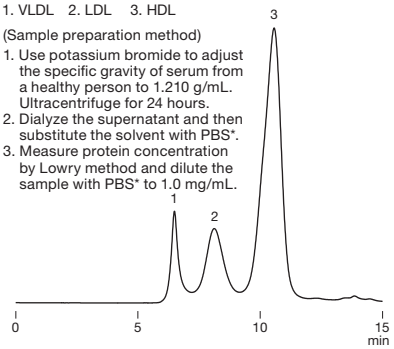


Column : **Shodex PROTEIN KW-802.5 x 2**
Eluent : $CH_3COOH/CH_3CN/H_2O$=20/30/50 (pH to 3.0 adjusted with 25 % NH_3 aq.)
Flow rate : 0.5 mL/min
Detector : UV (276 nm)
Column temp. : Ambient

Lipoproteins in serum

Sample : 40 µL
Whole lipoproteins from serum of a healthy person 1.0 mg/mL
1. VLDL 2. LDL 3. HDL

(Sample preparation method)
1. Use potassium bromide to adjust the specific gravity of serum from a healthy person to 1.210 g/mL. Ultracentrifuge for 24 hours.
2. Dialyze the supernatant and then substitute the solvent with PBS*.
3. Measure protein concentration by Lowry method and dilute the sample with PBS* to 1.0 mg/mL.

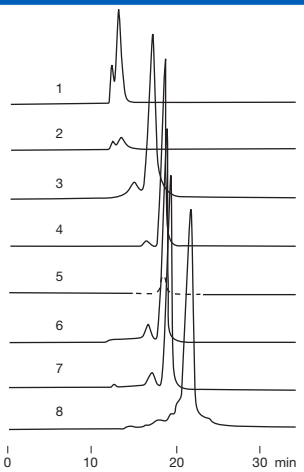


Column : **Shodex PROTEIN KW-G + KW-804**
Eluent : 10-fold diluted x 10 PBS* with H_2O
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : 30 ℃

x10 PBS* : 80 g NaCl + 29 g $Na_2HPO_4 \cdot 12H_2O$ + 2 g KCl + 2 g KH_2PO_4 in 1000 mL of H_2O

Data provided by Ohkawa Ryunosuke, Graduate School of Health Care Sciences, Analytical Laboratory Chemistry, Tokyo Medical and Dental University

Proteins in human blood serum



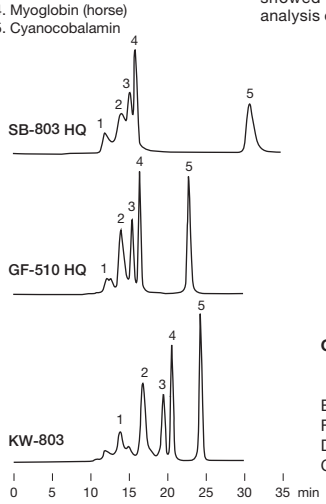
Sample : 0.1 % each

1. Fibrinogen	50 µL
2. α_2 -Macroglobulin	50 µL
3. IgG	50 µL
4. Transferrin	50 µL
5. Plasminogen	50 µL
6. Albumin	100 µL
7. Antitrypsin	100 µL
8. Hemoglobin	100 µL

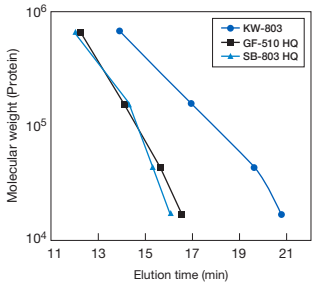
Column : **Shodex PROTEIN KW-803**
Eluent : 50 mM Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : Room temp.

Comparing three GFC columns for the separation of common proteins

Sample :
1. Thyroglobulin (bovine)
2. γ-Globulin (bovine)
3. Ovalbumin (chicken)
4. Myoglobin (horse)
5. Cyanocobalamin



Separation performances of three aqueous SEC columns (SB-803 HQ, GF-510 HQ, and KW-803) were compared. KW-803, silica-based column, showed the best separation performance for the analysis of protein standards.



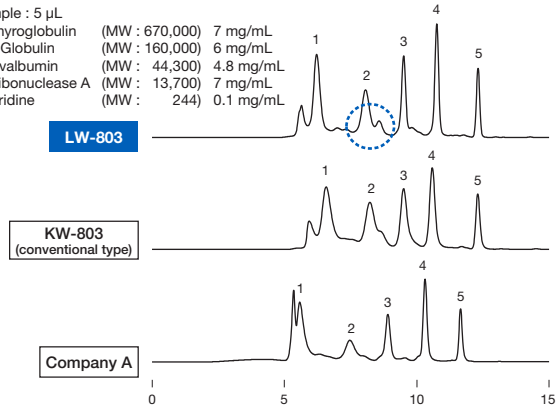
Column : **Shodex OHpak SB-803 HQ**
Shodex Asahipak GF-510 HQ
Shodex PROTEIN KW-803
Eluent : 0.2 M Phosphate buffer (pH6.9)
Flow rate : 0.5 mL/min
Detector : UV (280 nm)
Column temp. : 30 ℃

Comparison of LW-803, conventional column, and other manufacturer's column

PROTEIN LW-803 is suitable for analyzing a few-hundred-thousand molecular weight size proteins. When comparing LW-803 to our conventional columns and other manufacturer's columns, LW-803 provides a better separation around 160,000 molecular weight range that is about the size of Globulin. This improved separation efficiency is advantageous for the separation of monomer and dimer of IgG which is a mainstream of antibody drug.

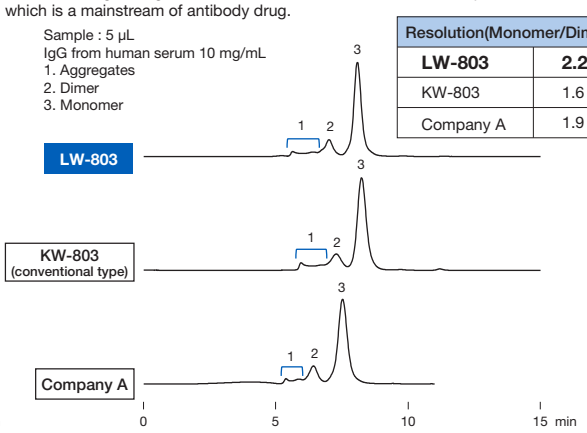
Sample : 5 µL

1. Thyroglobulin	(MW : 670,000)	7 mg/mL
2. γ-Globulin	(MW : 160,000)	6 mg/mL
3. Ovalbumin	(MW : 44,300)	4.8 mg/mL
4. Ribonuclease A	(MW : 13,700)	7 mg/mL
5. Uridine	(MW : 244)	0.1 mg/mL



Sample : 5 µL
IgG from human serum 10 mg/mL
1. Aggregates
2. Dimer
3. Monomer

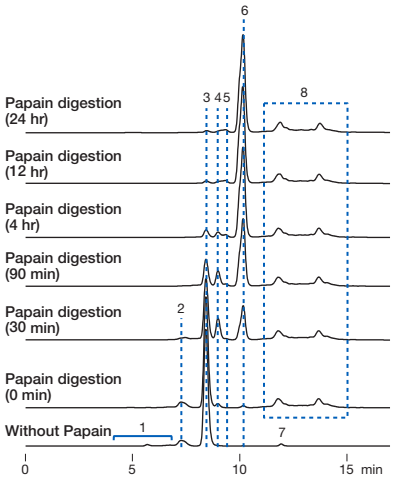
Resolution(Monomer/Dimer)	
LW-803	**2.2**
KW-803	1.6
Company A	1.9



Column : **Shodex PROTEIN LW-803, Shodex PROTEIN KW-803, Silica-based SEC column from other manufacturer**
Eluent : 50 mM Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : Room temp.

Monitoring papain digestion of humanized monoclonal IgG

Papain digestion of humanized monoclonal IgG was monitored using PROTEIN LW-803, an aqueous SEC (GFC) column. During the papain digestion of IgG, Fc and Fab fragments from the IgG and their decomposition intermediates are expected to be observed. LW-803 separates IgG and decomposed fragments and intermediates well from each other, thus it is suitable for the monitoring of papain digestion of IgG.



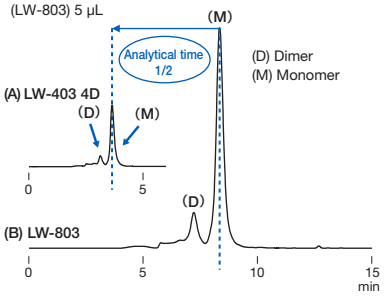
Sample : 10 µL
Humanized monoclonal IgG
1. Aggregates of IgG
2. Dimer of IgG
3. Monomer of IgG
4 - 6. Fragments of IgG from papain digestion
7. Citric acid
8. Papain

(Procedures for digestion monitoring)
(1) Dissolve 3 mg of humanized monoclonal IgG in 500 µL of the eluent. (6 mg/mL conc.)
(2) Dissolve 1 mg of papain in 500 µL of the eluent. (1 mg/mL conc.)
(3) Filer (1) and (2) using 0.2-µm membrane filters
(4) Mix two solutions in 1:1 ratio.
(5) Keep the mixture at 25 °C.
(6) Take samples at set timings and analyze them by HPLC.

Column : **Shodex PROTEIN LW-803**
Eluent : 0.1 M Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : 25 °C

Efficiencies of LW-403 4D over LW-803 for IgG separation

Sample : IgG from human serum 10 mg/mL
(LW-403 4D) 0.5 µL
(LW-803) 5 µL



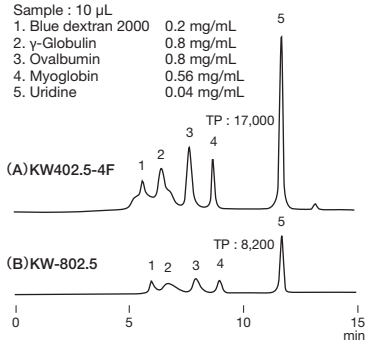
Column : **(A) Shodex PROTEIN LW-403 4D**
(B) Shodex PROTEIN LW-803
Eluent : 50mM Sodium phosphate buffer (pH7.0) + 0.3M NaCl
Flow rate : (A) 0.35 mL/min
(B) 1.0 mL/min
Detector : (A) UV (280 nm) (small cell volume)
(B) UV (280 nm) (conventional type)
Column temp. : Room temp.

Comparison of KW402.5-4F and KW-802.5

KW400 series is a high performance type semi-micro columns. It offers approximately 1.5 times larger theoretical plate number and 3 to 4 times higher detection sensitivity (peak height) than KW-800 series columns do.

Sample : 10 µL

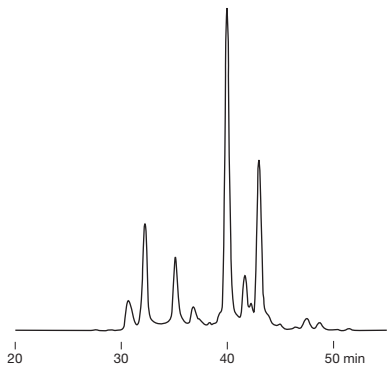
1. Blue dextran 2000	0.2 mg/mL
2. γ-Globulin	0.8 mg/mL
3. Ovalbumin	0.8 mg/mL
4. Myoglobin	0.56 mg/mL
5. Uridine	0.04 mg/mL



Column : **(A) Shodex KW402.5-4F**
(B) Shodex PROTEIN KW-802.5
Eluent : 50 mM Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : (A) 0.33 mL/min, (B) 1.0 mL/min
Detector : UV (280 nm) (small cell volume)
Column temp. : 25 °C

Whey in yogurt

Sample : Whey, 5 µL

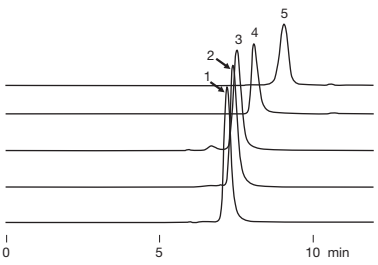


Column : **Shodex KW403-4F + KW402.5-4F**
Eluent : 50 mM Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : 0.20 mL/min
Detector : UV (280 nm) (small cell volume)
Column temp. : 30 °C

Lectins

Sample : 5 µL

1. Lectin from soybean	0.6 mg/mL
2. Lectin from arachis hypogaea	1.1 mg/mL
3. Lectin from canavalia ensiformis (Con A)	0.9 mg/mL
4. Lectin from lens culinaris (LCA)	0.7 mg/mL
5. Lectin from triticum vulgaris (WGA)	0.8 mg/mL



Column : **Shodex KW402.5-4F**
Eluent : 50 mM Sodium phosphate buffer (pH7.0) + 0.3 M NaCl
Flow rate : 0.33 mL/min
Detector : UV (220 nm) (small cell volume)
Column temp. : 30 °C