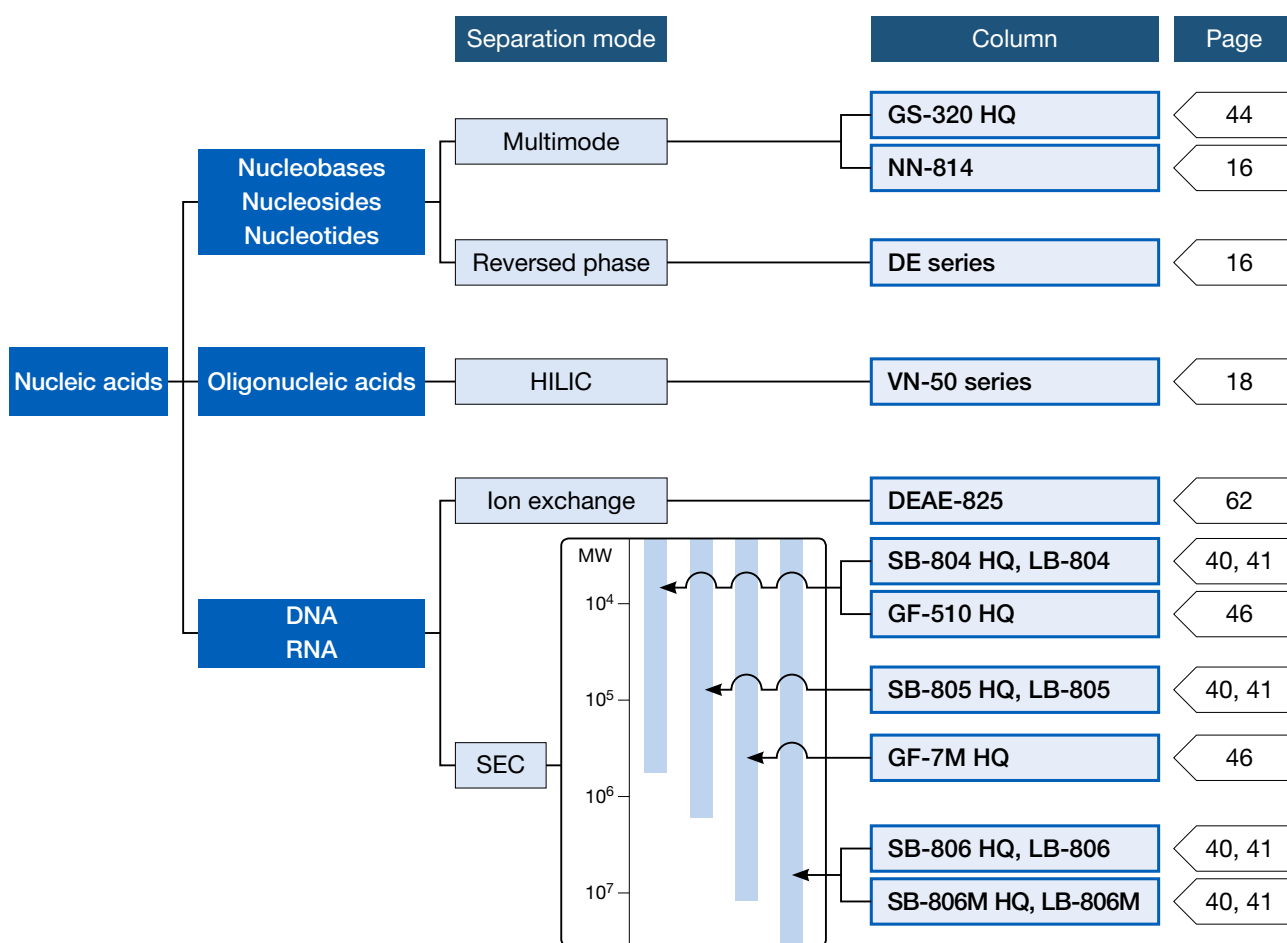
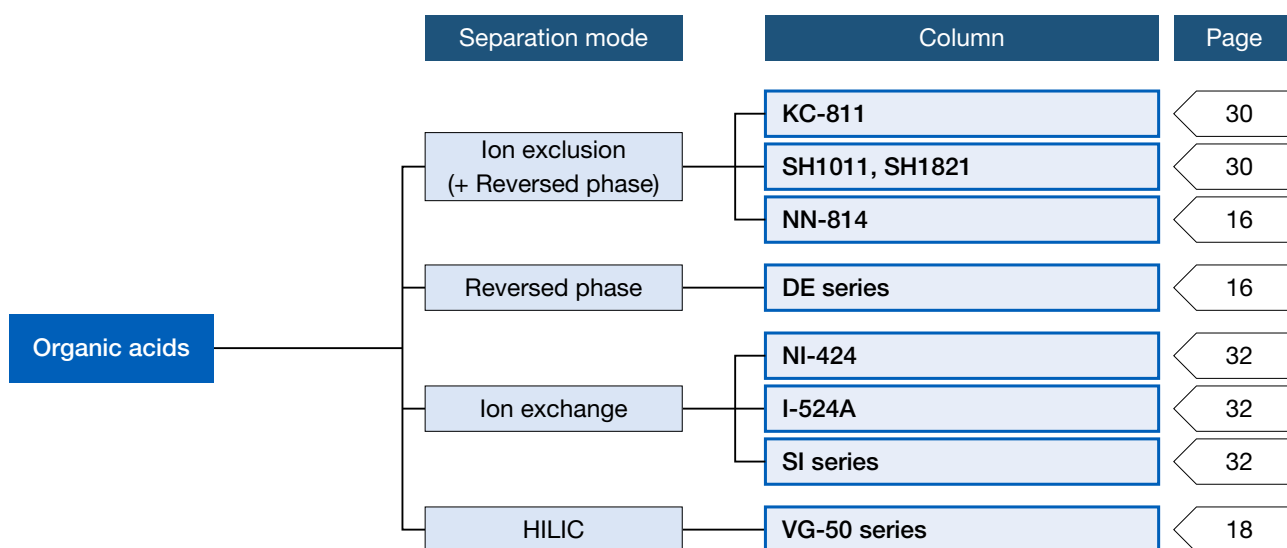


## Column Selection (Nucleic Acids)

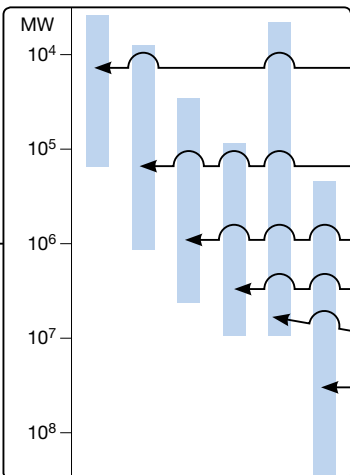
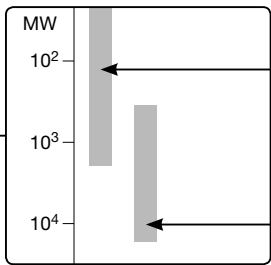


## Column Selection (Organic Acids)



# Column Selection (Saccharides)

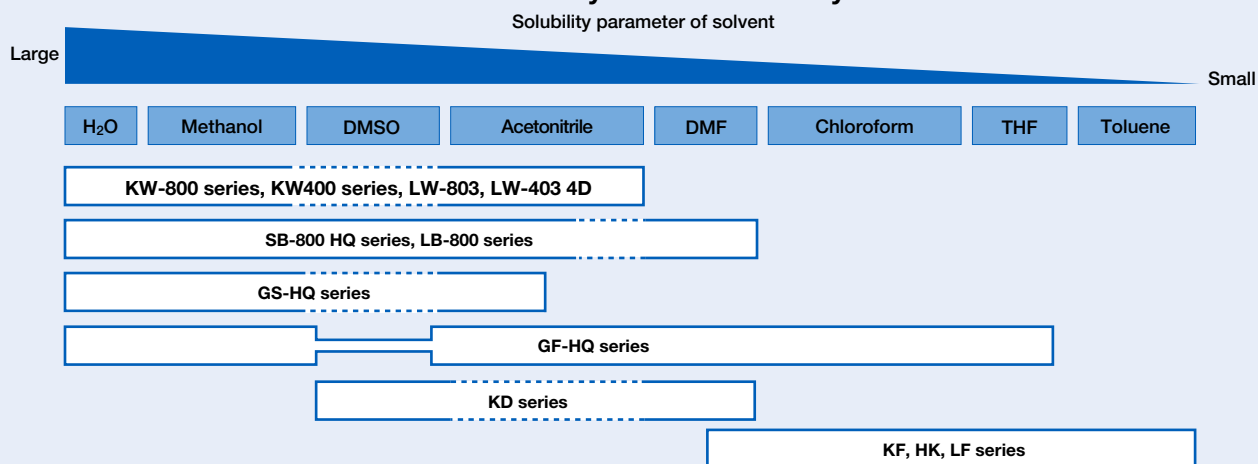
	Separation mode	Column	Page	
Mono-, di-saccharides, and sugar alcohols Saccharides and sugar alcohols	Ligand exchange + SEC	SP0810 (Pb <sup>2+</sup> )	26	
		SC1011 (Ca <sup>2+</sup> )	26	
		KS-801 (Na <sup>+</sup> )	26	
	Ligand exchange + HILIC	SZ5532 (Zn <sup>2+</sup> )	26	
		DC-613 (Na <sup>+</sup> )	26	
	HILIC	VG-50 series	18	
		NH2P series	22	
Sugar alcohols	Ligand exchange + HILIC	SC1211 (Ca <sup>2+</sup> )	26	
Oligosaccharides and sugar alcohols	Ligand exchange + SEC	KS-801 (Na <sup>+</sup> ) + KS-802 (Na <sup>+</sup> )	26	
Amino sugars	HILIC	VG-50 series	18	
		NH2P series	22	
	Ion exchange	SC1011 (Ca <sup>2+</sup> )	26	
Acidic sugars	Ion exclusion	SH1011 (H <sup>+</sup> )	30	
		KC-811	30	
	Ion exchange	VT-50 2D	18	
		NH2P series	22	
Saccharides and organic acids	Ion exclusion + SEC	SH1011 (H <sup>+</sup> ), SH1821 (H <sup>+</sup> )	30	
Oligosaccharides	SEC	KS-801 (Na <sup>+</sup> )	26	
		SB-802 HQ	40	
		GS-220 HQ	44	
	HILIC	KS-802 (Na <sup>+</sup> )	26	
		SB-802.5 HQ, LB-802.5	40, 41	
		GS-320 HQ	44	
		VN-50 series	18	
		NH2P series	22	
		SEC	KS-803 (Na <sup>+</sup> )	26
			SB-803 HQ, LB-803	40, 41
KS-804 (Na <sup>+</sup> )	26			
SB-804 HQ, LB-804	40, 41			
SB-805 HQ, LB-805	40, 41			
Polysaccharides	SEC	SB-806 HQ, LB-806	40, 41	
		SB-806M HQ, LB-806M	40, 41	
		SB-807 HQ	40	



# 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
		Chloroform	LF series	56
			KF-800 series	48
			HK-400 series	54
	Polar polymers (Polyvinylpyrrolidone etc.)	DMF	LF series	56
			SB-800 HQ series	40
			LB-800 series	41
			KD-800 series	50
			HK-400 series	54
			LF series	56
	Engineering plastics (Polyamides etc.)	HFIP	SB-800 HQ series	40
			LB-800 series	41
			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

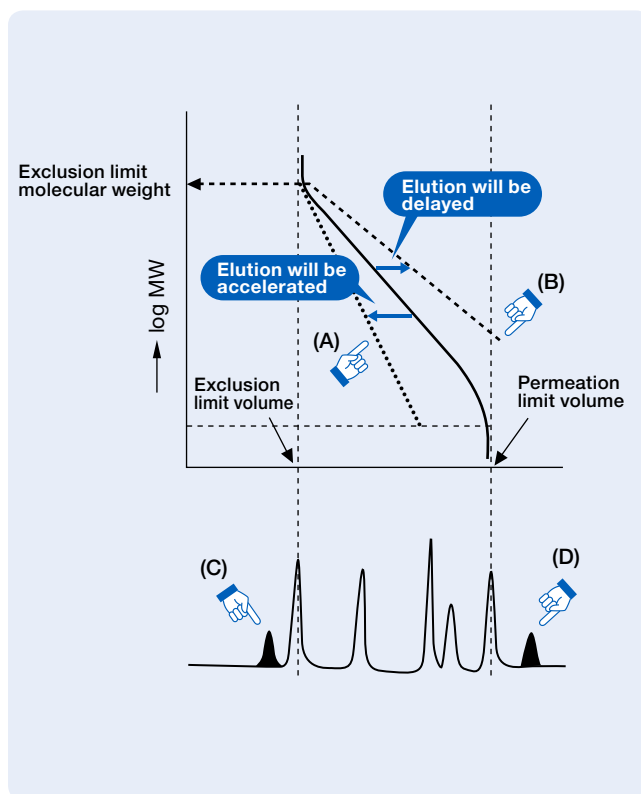
- ◆ Hydrophobic interaction
  - The analyte is adsorbed on the packing material.
  - This delays the analyte elution and results in under estimating the analyte's molecular weight. See (B) and (D).
- ◆ Ionic interaction
  - (1) Ion Exclusion
    - The analyte is repelled from the packing material.
    - 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  $\text{CF}_3\text{COONa}$  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: Polymer-based

<https://www.shodex.de/ohpak-sb-lb-columns>

## Features

### SB-800 HQ

- Polymer-based packed columns for aqueous SEC (GFC) analysis
- Supports a wide range of molecular weight sample analysis
- The eluent can be replaced with DMF (except SB-802 HQ and SB-807 HQ), enabling the analysis of polar polymers
- Method using SB-804 HQ or SB-805 HQ for gelatin's mean molecular weight determination is comparable with PAGI method (Ver. 10, Japan)
- Fulfills USP-NF L38 and L39 requirements
- SB-802 HQ fulfills USP-NF L25 requirements
- SB-802.5 HQ fulfills USP-NF L25 and L89 requirements
- SB-803 HQ fulfills USP-NF L37 requirements

### SB-807 HQ

- Column for the analysis of water-soluble ultra high molecular weight polymers
- Large particle-size gel prevents shear degradation of polymers
- Fulfills USP-NF L38 and L39 requirements

### LB-800

- Polymer-based packed columns for aqueous SEC (GFC) analysis
- Low column bleeding allows its use with light scattering detectors
- The eluent can be replaced with DMF enabling the analysis of polar polymers
- Fulfills USP-NF L38 and L39 requirements
- LB-802.5 fulfills USP-NF L25 and L89 requirements
- LB-803 fulfills USP-NF L37 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
F6429100	<b>OHpak SB-802 HQ</b>	≥ 12,000	8	100	<b>8.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.
F6429101	<b>OHpak SB-802.5 HQ</b>	≥ 16,000	6	200	<b>8.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.
F6429102	<b>OHpak SB-803 HQ</b>	≥ 16,000	6	800	<b>8.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.
F6429103	<b>OHpak SB-804 HQ</b>	≥ 16,000	10	2,000	<b>8.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.
F6429104	<b>OHpak SB-805 HQ</b>	≥ 12,000	13	7,000	<b>8.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.
F6429105	<b>OHpak SB-806 HQ</b>	≥ 12,000	13	15,000	<b>8.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.
F6429106	<b>OHpak SB-806M HQ</b>	≥ 12,000	13	15,000	<b>8.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.
F6709430	<b>OHpak SB-G 6B</b>	(guard column)	10	—	<b>6.0 x 50</b>	0.02 % NaN <sub>3</sub> aq.

SB-806M HQ is a mixed-gel column capable of analyzing samples over a wide range of molecular weight distribution.

Base Material: Polyhydroxymethacrylate  
Usable pH Range: pH 3 - 10

## Aqueous high molecular weight analysis column

## • 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
F6429108	<b>OHpak SB-807 HQ</b>	≥ 1,500	35	30,000	<b>8.0 x 300</b>	H <sub>2</sub> O
F6709431	<b>OHpak SB-807G</b>	(guard column)	35	—	<b>8.0 x 50</b>	H <sub>2</sub> O

Base Material: Polyhydroxymethacrylate  
Usable pH Range: pH 3 - 10

## • Preparative columns [ Preparative columns are made to order. ]

Product Code	Product Name	Plate Number (TP/column)	Particle Size (µm)	Column Size (mm) I.D. x Length	Shipping Solvent	Standard Column
F6516011	<b>OHpak SB-2002</b>	≥ 9,000	15	<b>20.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.	SB-802 HQ
F6516012	<b>OHpak SB-2002.5</b>	≥ 12,000	10	<b>20.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.	SB-802.5 HQ
F6516013	<b>OHpak SB-2003</b>	≥ 12,000	10	<b>20.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.	SB-803 HQ
F6516014	<b>OHpak SB-2004</b>	≥ 12,000	18	<b>20.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.	SB-804 HQ
F6516015	<b>OHpak SB-2005</b>	≥ 12,000	20	<b>20.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.	SB-805 HQ
F6516016	<b>OHpak SB-2006</b>	≥ 12,000	20	<b>20.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.	SB-806 HQ
F6516017	<b>OHpak SB-2006M</b>	≥ 12,000	20	<b>20.0 x 300</b>	0.02 % NaN <sub>3</sub> aq.	SB-806M HQ
F6709555	<b>OHpak SB-G 8B</b>	(guard column)	18	<b>8.0 x 50</b>	0.02 % NaN <sub>3</sub> aq.	(guard column)

Base Material: Polyhydroxymethacrylate

## GFC columns to be used with light scattering detector

## ● 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
F6429206	<b>OHpak LB-802.5</b>	≥ 16,000	6	200	<b>8.0 x 300</b>	H <sub>2</sub> O
F6429201	<b>OHpak LB-803</b>	≥ 16,000	6	800	<b>8.0 x 300</b>	H <sub>2</sub> O
F6429204	<b>OHpak LB-804</b>	≥ 16,000	10	2,000	<b>8.0 x 300</b>	H <sub>2</sub> O
F6429203	<b>OHpak LB-805</b>	≥ 12,000	13	7,000	<b>8.0 x 300</b>	H <sub>2</sub> O
F6429205	<b>OHpak LB-806</b>	≥ 12,000	13	15,000	<b>8.0 x 300</b>	H <sub>2</sub> O
F6429202	<b>OHpak LB-806M</b>	≥ 12,000	13	15,000	<b>8.0 x 300</b>	H <sub>2</sub> O
F6709434	<b>OHpak LB-G 6B</b>	(guard column)	13	—	<b>6.0 x 50</b>	H <sub>2</sub> O

LB-806M is a mixed-gel column capable of analyzing samples over a wide range of molecular weight distribution.

Base Material: Polyhydroxymethacrylate  
Usable pH Range: pH 3 - 10

## Usable solvents

Product Name	Maximum Usable Concentration (%)		
	Methanol	Acetonitrile	N,N-Dimethylformamide (DMF)
<b>SB-802 HQ</b>	0	0	0
<b>SB-802.5 HQ, SB-803 HQ</b>	100	75	100
<b>SB-804 HQ - SB-806M HQ</b>	75	75	100
<b>SB-G 6B</b>	75	75	100
<b>SB-807 HQ, SB-807G</b>	30	30	0
<b>LB-802.5 - LB-806M, LB-G 6B</b>	100	100	100

(Note)

The maximum solvent tolerance of SB-2000 series, preparative columns of SB-800 HQ series, is 50 % methanol, acetonitrile, or DMF. (SB-2002 is not tolerant to organic solvents)

## Target molecular weight range and exclusion limit

## ● Measured with pullulan (eluent: ultrapure water)

Product Name	Target Molecular Weight Range	Exclusion Limit
<b>SB-802 HQ</b>	200 - 1,000	1,000
<b>SB-802.5 HQ</b>	500 - 10,000	10,000
<b>SB-803 HQ</b>	1,000 - 100,000	100,000
<b>SB-804 HQ</b>	5,000 - 400,000	1,000,000
<b>SB-805 HQ</b>	100,000 - 1,000,000	* (4,000,000)
<b>SB-806 HQ</b>	100,000 - * (20,000,000)	* (20,000,000)
<b>SB-806M HQ</b>	500 - * (20,000,000)	* (20,000,000)
<b>SB-807 HQ</b>	500,000 - * (500,000,000)	* (500,000,000)
<b>LB-802.5</b>	500 - 10,000	10,000
<b>LB-803</b>	1,000 - 100,000	100,000
<b>LB-804</b>	5,000 - 400,000	1,000,000
<b>LB-805</b>	100,000 - 1,000,000	* (4,000,000)
<b>LB-806</b>	100,000 - * (20,000,000)	* (20,000,000)
<b>LB-806M</b>	500 - * (20,000,000)	* (20,000,000)

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

\* ( ) Estimated value

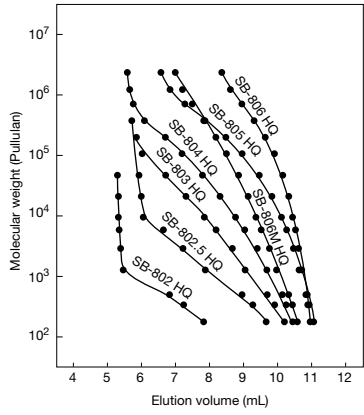
## ● Measured with \*PEG/PEO (eluent: DMF)

Product Name	Target Molecular Weight Range
<b>SB-802.5 HQ</b>	100 - 2,000
<b>SB-803 HQ</b>	200 - 40,000
<b>SB-804 HQ</b>	500 - 300,000
<b>SB-805 HQ</b>	50,000 - 700,000
<b>SB-806 HQ</b>	70,000 - ** (20,000,000)
<b>SB-806M HQ</b>	200 - ** (20,000,000)
<b>LB-802.5</b>	100 - 5,000
<b>LB-803</b>	500 - 50,000
<b>LB-804</b>	500 - 300,000
<b>LB-805</b>	50,000 - 700,000
<b>LB-806</b>	70,000 - ** (20,000,000)
<b>LB-806M</b>	200 - ** (20,000,000)

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

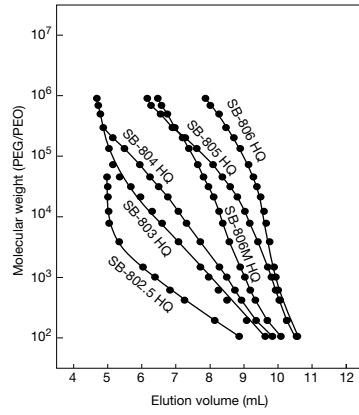
\*PEG : polyethylene glycol  
\*PEO : polyethylene oxide  
\*\* ( ) Estimated value

**Calibration curves for SB-800 HQ series using pullulan (eluent: H<sub>2</sub>O)**



**Column** : Shodex OHpak SB-800 HQ series  
**Eluent** : H<sub>2</sub>O  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
**Column temp.** : 30 °C

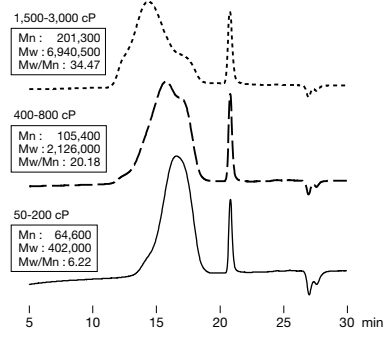
**Calibration curves for SB-800 HQ series using PEG/PEO (eluent: DMF)**



**Column** : Shodex OHpak SB-800 HQ series  
**Eluent** : DMF  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
**Column temp.** : 40 °C

**Carboxymethylcellulose**

Sample : Carboxymethylcellulose 0.1 % each, 50 µL



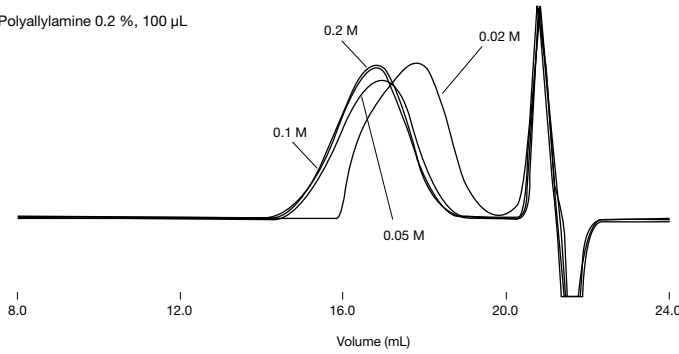
Molecular weight was determined from the calibration curve of pullulan.

**Column** : Shodex OHpak SB-806M HQ x 2  
**Eluent** : 0.1 M NaCl aq.  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
**Column temp.** : 40 °C

**Effects of sodium nitrate in eluent on the analysis of polyallylamine**

For the analysis of cationic polymers, such as polyallylamine, the polymer is observed to adsorb on the column or delayed in elution when low sodium nitrate eluent was used. These phenomena can be suppressed by increasing the concentration of sodium nitrate in the eluent. In the case of polyallylamine, a good shape chromatogram is obtained when sodium nitrate concentration is 0.1 M or higher.

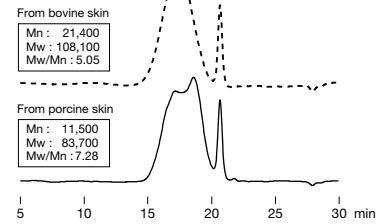
Sample : Polyallylamine 0.2 %, 100 µL



**Column** : Shodex OHpak SB-806M HQ x 2  
**Eluent** : 0.5 M CH<sub>3</sub>COOH + NaNO<sub>3</sub> aq.  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
**Column temp.** : 40 °C

**Gelatin**

Sample : 0.1 % each, 100 µL  
 Gelatin from bovine skin (Acid treatment, Gel strength : 225 g)  
 Gelatin from porcine skin (Alkali treatment, Gel strength : 90-100 g)

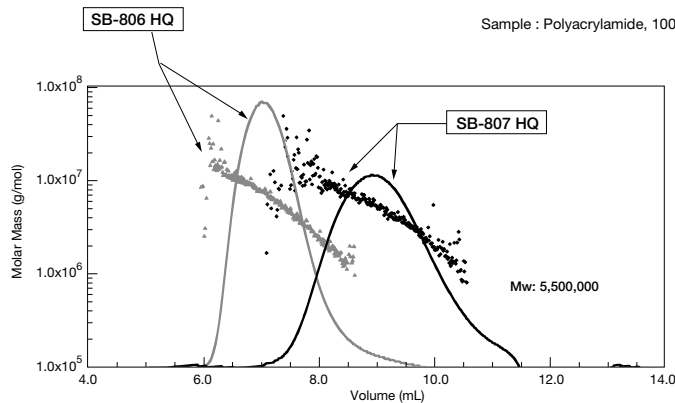


\*Molecular weight was determined from the calibration curve of pullulan.

**Column** : Shodex OHpak SB-806M HQ x 2  
**Eluent** : 0.1 M KH<sub>2</sub>PO<sub>4</sub> aq./ 0.1 M Na<sub>2</sub>HPO<sub>4</sub> aq. = 50/50  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
**Column temp.** : 40 °C

**Polyacrylamide**

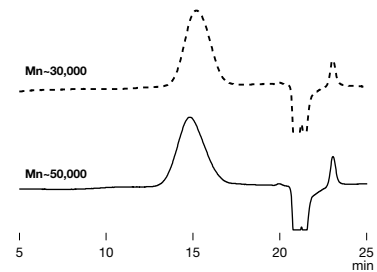
Sample : Polyacrylamide, 100 µL



**Column** : Shodex OHpak SB-807 HQ, SB-806 HQ  
**Eluent** : 0.2 M NaCl aq.  
**Flow rate** : 0.5 mL/min  
**Detector** : RI  
 MALS (Multi angle light scattering)  
**Column temp.** : 30 °C

**Cellulose acetate**

Sample : Cellulose acetate 0.1 % each, 100 µL



**Column** : Shodex OHpak SB-806M HQ x 2  
**Eluent** : 20 mM LiBr in DMF  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
**Column temp.** : 40 °C

### Copovidones

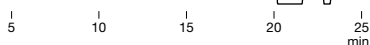
Sample : 100  $\mu$ L  
Poly(1-vinylpyrrolidone-co-vinyl acetate) 0.1 % each

Copolymer 7:3

Mn : 2,000  
Mw : 14,400  
Mw/Mn : 7.40

Copolymer 3:7

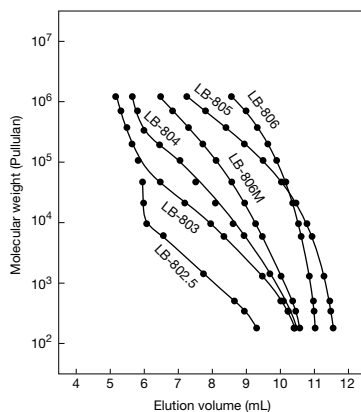
Mn : 6,400  
Mw : 28,900  
Mw/Mn : 4.53



Molecular weight was determined from the calibration curve of PEG/P EO.

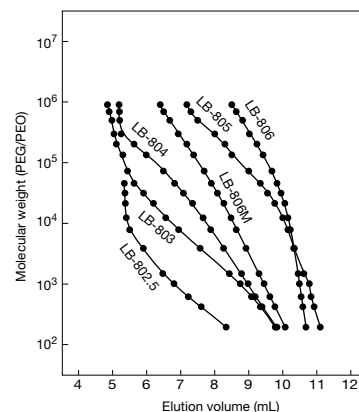
**Column** : Shodex OHpak SB-806M HQ x 2  
**Eluent** : 20 mM LiBr in DMF  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
**Column temp.** : 40 °C

### Calibration curves for LB-800 series using pullulan (eluent: H<sub>2</sub>O)



**Column** : Shodex OHpak LB-800 series  
**Eluent** : H<sub>2</sub>O  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
**Column temp.** : 30 °C

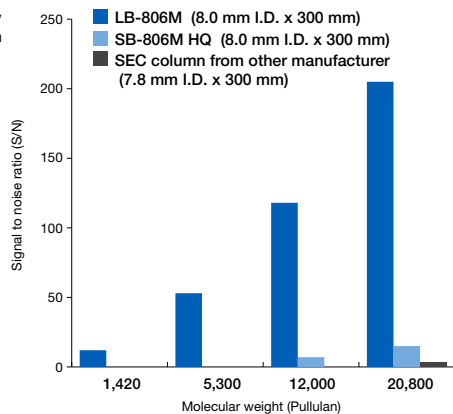
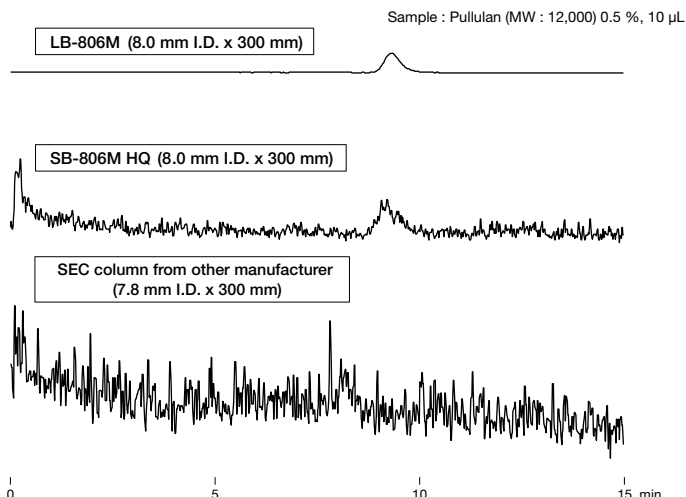
### Calibration curves for LB-800 series using PEG/P EO (eluent: DMF)



**Column** : Shodex OHpak LB-800 series  
**Eluent** : DMF  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
**Column temp.** : 40 °C

### Comparison of pullulan detection using multi angle light scattering detector

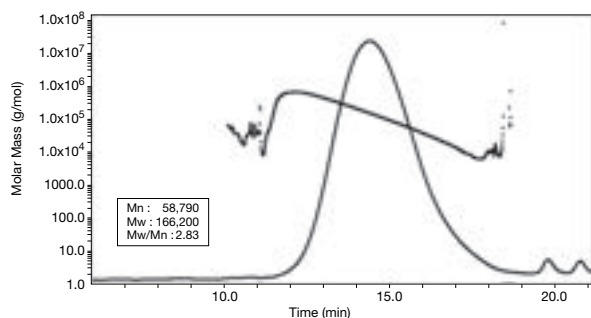
The OHpak LB-800 series is able to detect low molecular weight substances owing to its improved low baseline noise level while using it with a multiangle light scattering detector. This cannot be achieved with other manufacturer's SEC column.



**Column** : Shodex OHpak LB-806M  
Shodex OHpak SB-806M HQ  
SEC column from other manufacturer  
**Eluent** : 0.1 M NaNO<sub>3</sub> aq.  
**Flow rate** : 1.0 mL/min  
**Detector** : MALS (Multi angle light scattering) (90 °)  
**Column temp.** : 30 °C

### Sodium alginate

Sample : Sodium alginate 0.1 % , 100  $\mu$ L

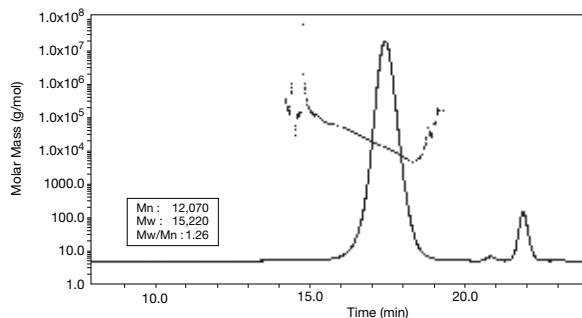


Mn : 58,790  
Mw : 166,200  
Mw/Mn : 2.83

**Column** : Shodex OHpak LB-806M x 2  
**Eluent** : 0.1 M NaNO<sub>3</sub> aq.  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
MALS (Multi angle light scattering)  
**Column temp.** : 30 °C

### Sodium heparin

Sample : Sodium heparin 0.1 % , 100  $\mu$ L



Mn : 12,070  
Mw : 15,220  
Mw/Mn : 1.26

**Column** : Shodex OHpak LB-806M x 2  
**Eluent** : 0.1 M NaNO<sub>3</sub> aq.  
**Flow rate** : 1.0 mL/min  
**Detector** : RI  
MALS (Multi angle light scattering)  
**Column temp.** : 30 °C