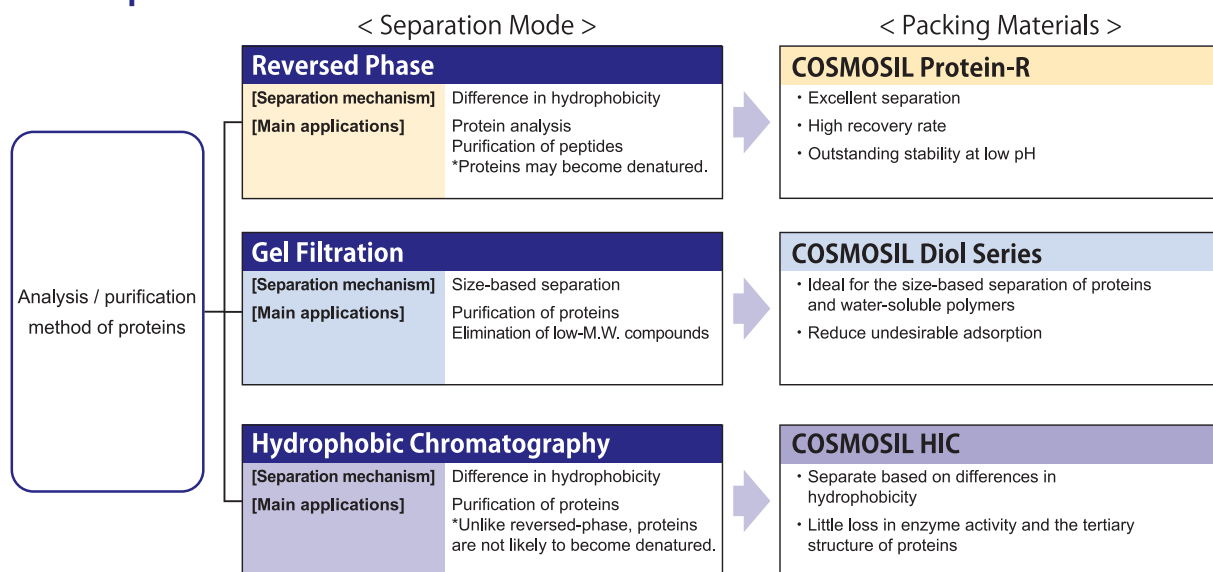


9. Protein Separation Columns

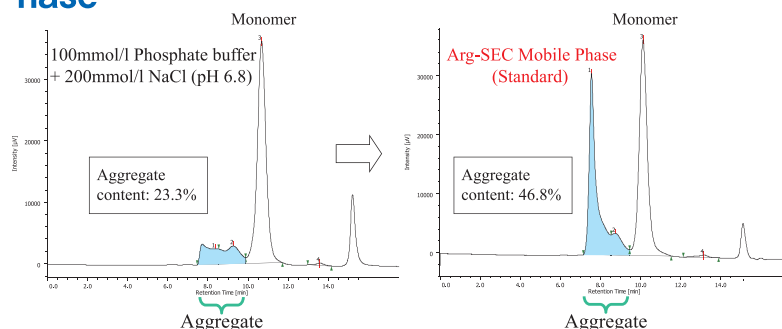
Protein Separation with HPLC



Arginine Mobile Phase / Arginine Buffer for Protein Purification

Mobile Phase for Size-Exclusion Chromatography Arg-SEC Mobile Phase

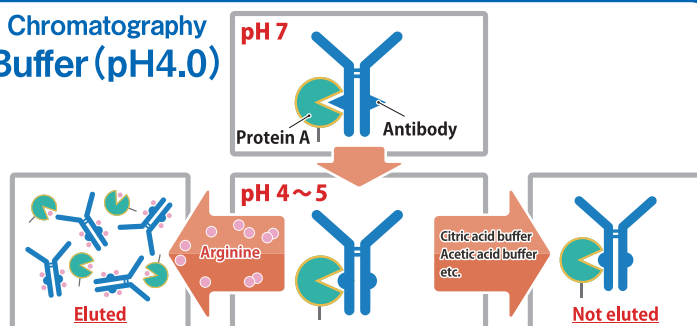
- Increased recovery of proteins and peptides that are more hydrophobic and have stronger tendency to stick to the columns.
- Effective separation of protein conjugates, e.g., ADC and sticky cytokines.



This product is manufactured with permission from Ajinomoto Co., Inc. based on the patent JP 4941882.²
¹ Arginine is effective in suppressing non-specific molecular interactions. ² JP: 4941882, US: 7501495, EP: 1698637

Elution Buffer for Protein Affinity Chromatography Arg-Antibody Elution Buffer (pH4.0)

- Enables effective elution of antibodies from protein A column, reducing potential risk of acid denaturation and resultant aggregation.



This product is manufactured with permission from Ajinomoto Co., Inc. based on the patent JP 4826995.
^{*}JP: 4826995, US: 8084032, 8435527, 2012-0264918, EP: 1568710, CN: 1680426

For ordering information, please refer to page 77.

Reversed Phase Columns

COSMOSIL Protein-R



- Excellent separation
- High recovery rate
- Outstanding stability at low pH

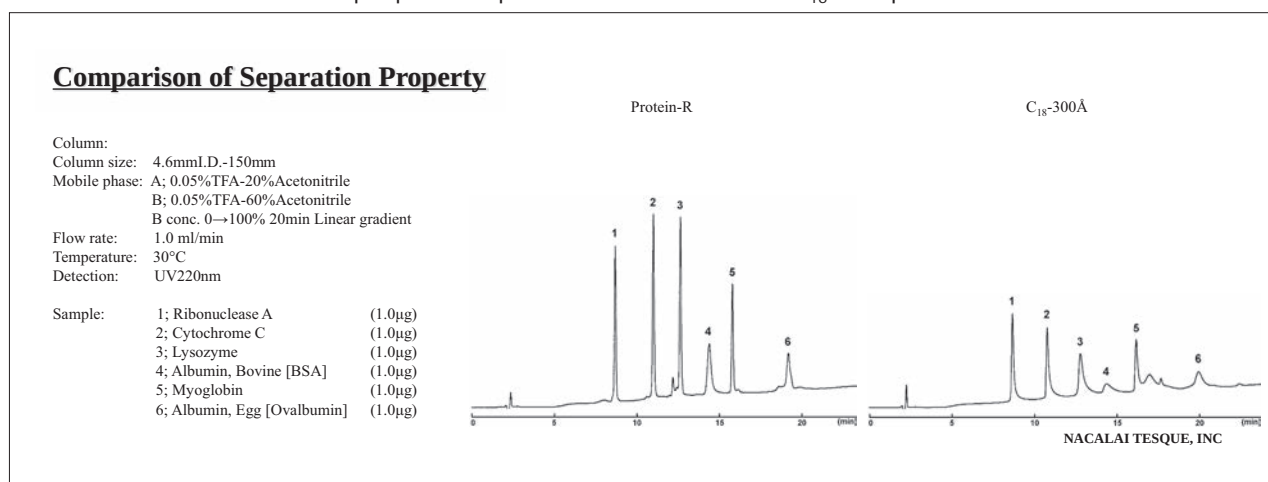
Specifications

Packing Material	Protein-R
Silica Gel	High purity porous spherical silica
Average Particle Size	5 μm
Average Pore Size	300 Å
Specific Surface Area	150 m ² /g
Bonded Phase	Octadecyl group
Bonding Type	Polymeric
Main Interaction	Hydrophobic interaction
End-Capping Treatment	Near-perfect treatment
Usable pH Range	1.5 ~ 7.5*
Features	• High recovery rate • Acid-resistance

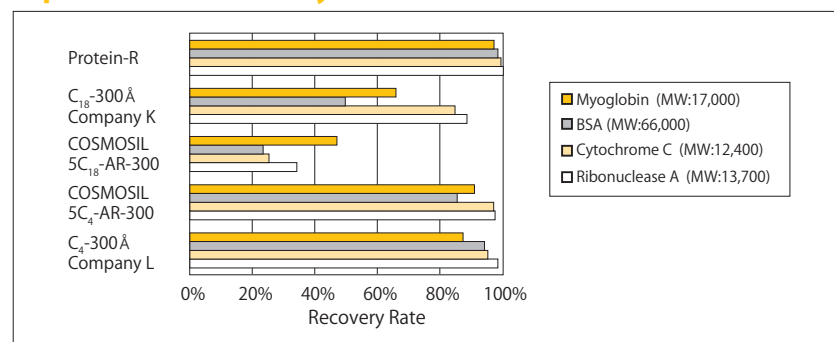
*Optimal pH range of silica-based columns is between 2 and 7.5. Extreme pH may significantly decrease column lifetime.

Comparison of Separation

COSMOSIL Protein-R shows sharper peaks for proteins than conventional C₁₈ wide pore columns.



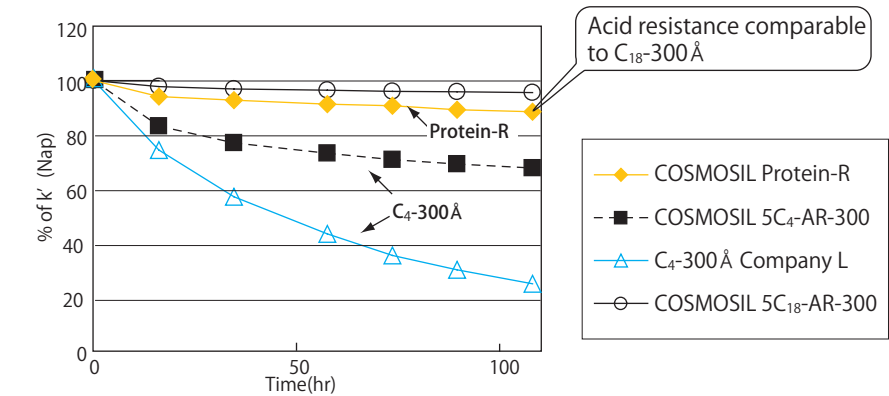
Comparison of Recovery Rate



The figure below shows recovery rates for proteins using different columns. Protein-R shows a higher recovery rate than C₄-300 and a much higher recovery rate than C₁₈-300.

Comparison of durability against acidic mobile phase

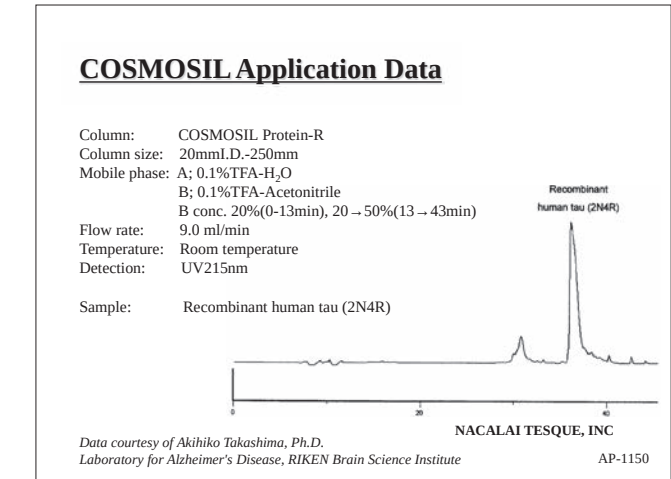
The figure below shows durability against acidic mobile phase of various columns. Protein-R shows a higher acid durability than C₄-300.



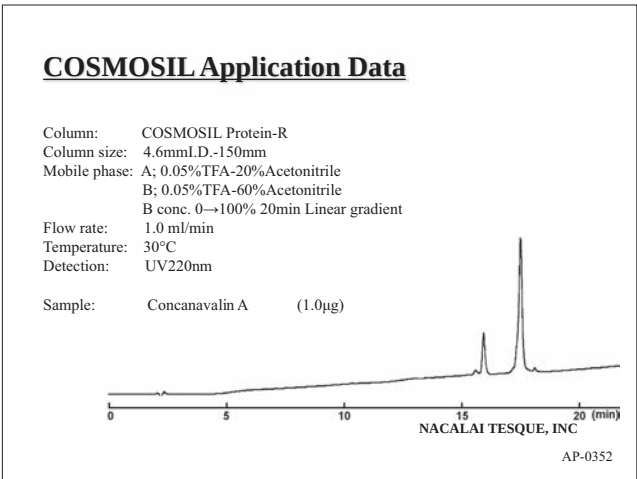
Degradation test with 0.1% Trifluoroacetic Acid at 60°C
(k) : Naphthalene in the mobile phase (methanol : water = 50 : 50)

Applications

● Tau Protein



● Glycoproteins



Ordering Information

● COSMOSIL Protein-R Analytical / Preparative Columns (Particle Size: 5 µm)

Packed Column

I.D. x Length (mm)	Product Number	I.D. x Length (mm)	Product Number
2.0×150	06514-71	10×150	06529-91
4.6× 50	06525-31	10×250	06530-51
4.6×150	06526-21	20×150	06531-41
4.6×250	06527-11	20×250	06532-31

Guard Column / Guard Cartridge

I.D. x Length (mm)	Product Number
4.6×10	06518-31
4.6×10 Cartridge*	21249-04
10×20	06528-01
20×20	08692-81

* 2 cartridges included. Guard cartridge holder required; refer to page 78.

COSMOSIL C₁₈-AR-300, C₈-AR-300, C₄-AR-300, Ph-AR-300



- Wide-pore reversed-phase columns
- 4 types of phases (octadecyl, octyl, butyl and phenyl)

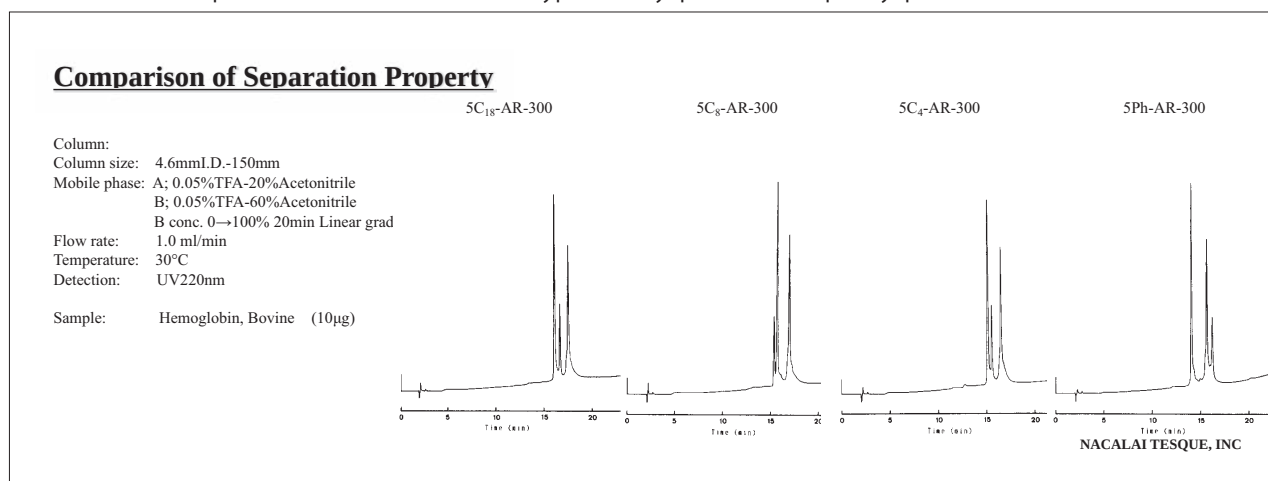
Specifications

Packing Material	C ₁₈ -AR-300	C ₈ -AR-300	C ₄ -AR-300	Ph-AR-300
Silica Gel	High purity porous spherical silica			
Average Particle Size	5 μm			
Average Pore Size	300 Å			
Specific Surface Area	150 m ² /g			
Bonded Phase Structure				
Bonded Phase	Octadecyl group	Octyl group	Butyl group	Phenyl group
Bonding Type	Polymeric			
Main Interaction	Hydrophobic interaction			Hydrophobic interaction π-π interaction
End-Capping	Near-perfect treatment			
Usable pH Range	1.5 ~ 7.5*			
Carbon Content	12%	7%	6%	7%

*Optimal pH range of silica-based columns is between 2 and 7.5. Extreme pH may significantly decrease column lifetime.

Comparison of Separation

COSMOSIL AR-300 packed column series offers 3 types of alkyl phases and a phenyl phase.



Ordering Information

● COSMOSIL 5C₁₈-AR-300 Analytical / Preparative Columns (Particle Size: 5 µm)

Packed Column

I.D. x Length (mm)	Product Number
4.6 × 50	37911-01
4.6 × 150	37913-81
4.6 × 250	37914-71

I.D. x Length (mm)	Product Number
10 × 150	37917-41
10 × 250	37918-31
20 × 150	37919-21
20 × 250	37920-81

Guard Column

I.D. x Length (mm)	Product Number
4.6 × 10	37910-11
10 × 20	37965-11

● COSMOSIL 5C₈-AR-300 Analytical / Preparative Columns (Particle Size: 5 µm)

Packed Column

I.D. x Length (mm)	Product Number
4.6 × 50	37951-81
4.6 × 150	37953-61
4.6 × 250	37954-51

I.D. x Length (mm)	Product Number
10 × 150	34345-21
10 × 250	34247-11
20 × 150	05861-51
20 × 250	34364-71

Guard Column

I.D. x Length (mm)	Product Number
4.6 × 10	37950-91
10 × 20	34464-61

● COSMOSIL 5C₄-AR-300 Analytical / Preparative Columns (Particle Size: 5 µm)

Packed Column

I.D. x Length (mm)	Product Number
4.6 × 50	37956-31
4.6 × 150	37958-11
4.6 × 250	37959-01

I.D. x Length (mm)	Product Number
10 × 150	34249-91
10 × 250	38047-11
20 × 150	34477-01
20 × 250	38048-01

Guard Column

I.D. x Length (mm)	Product Number
4.6 × 10	37955-41
10 × 20	05862-41

● COSMOSIL 5Ph-AR-300 Analytical / Preparative Columns (Particle Size: 5 µm)

Packed Column

I.D. x Length (mm)	Product Number
4.6 × 50	37961-51
4.6 × 150	37963-31
4.6 × 250	37964-21

I.D. x Length (mm)	Product Number
10 × 150	05865-11
10 × 250	34267-51
20 × 150	05866-01
20 × 250	34468-21

Guard Column

I.D. x Length (mm)	Product Number
4.6 × 10	37960-61
10 × 20	34268-41

Gel Filtration Columns (Aqueous)

COSMOSIL Diol-120-II, Diol-300-II, Diol-1000-II



- Ideal for the size-based separation of proteins and water-soluble polymers
- Reduce undesirable adsorption

Specifications

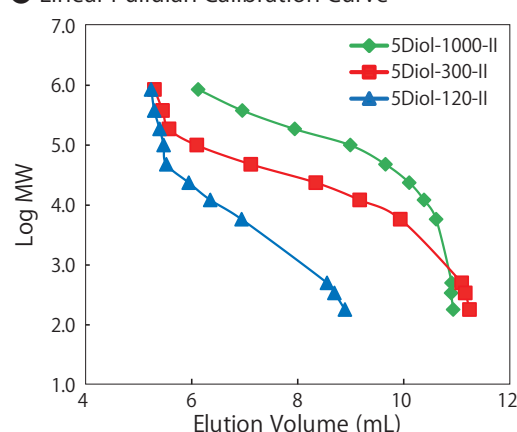
Packing Material	Diol-120-II	Diol-300-II	Diol-1000-II
Silica Gel	High purity porous spherical silica ⁽¹⁾		
Average Particle Size	5 μm		
Average Pore Size	120 $\text{\AA}$	300 $\text{\AA}$	1,000 $\text{\AA}$ ⁽²⁾
Bonded Phase	Diol group		
Flow Rate (mL/min)	0.5 ~ 1.0		
Pressure	20 MPa or less		15 MPa or less
Sample MW (Protein)	5,000 ~ 100,000	10,000 ~ 700,000	—
Sample MW (Water-Soluble Polymers)	1,000 ~ 20,000	5,000 ~ 100,000	50,000 ~ 500,000

(1) With the silica-based gel, organic solvents, including methanol and acetonitrile, can be used.

(2) If you require pore sizes greater than 1000 $\text{\AA}$, please contact us.

Calibration Curve

● Linear Pullulan Calibration Curve



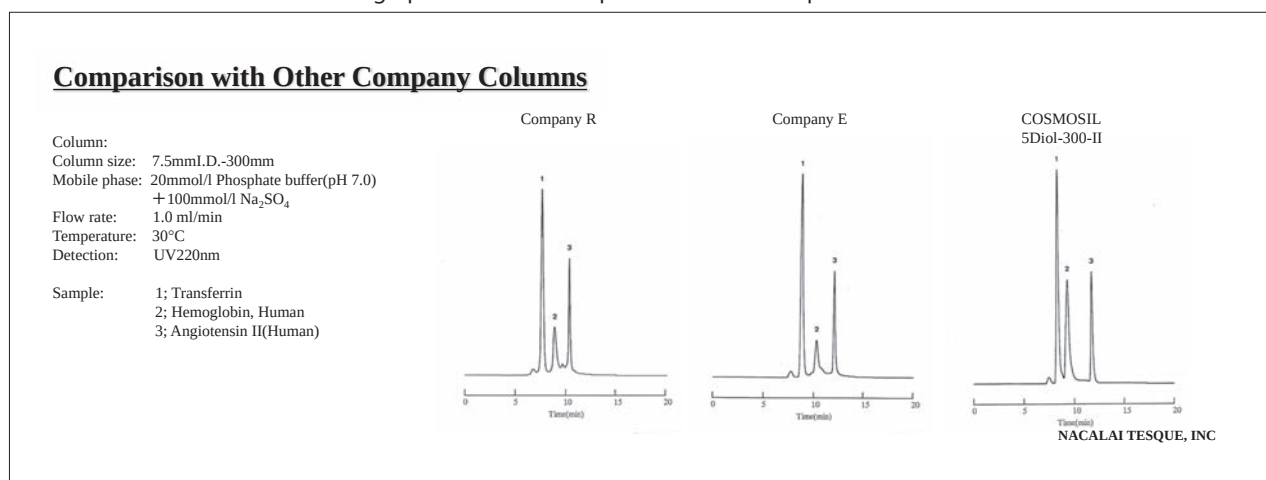
Column
Mobile Phase
Flow Rate
Temperature
Detection
Sample

COSMOSIL 5Diol-II (7.5 mmI.D. $\times$ 300 mm)
Water
1.0 mL/min
30°C
RI
Linear Pullulan

Sample	M.W.
1; P-800	853,000
2; P-400	380,000
3; P-200	186,000
4; P-100	100,000
5; P-50	48,000
6; P-20	23,700
7; P-10	12,200
8; P-5	5,800
9; Maltotriose	504
10; Maltose	342
11; Glucose	180

Comparison with Other Companies' Columns

COSMOSIL Diol-II demonstrates high performance compared to other companies' columns.



Protein Separation Columns
1
2
3
4
5
6
7
8
9
10
11
12
13
14

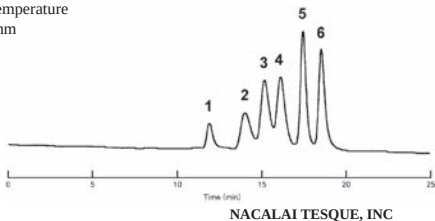
Applications

● Proteins

COSMOSIL Application Data

Column: COSMOSIL 5Diol-300-II
Column size: 7.5mmI.D.-600mm
Mobile phase: 20mmol/l Phosphate buffer(pH 7.0)
+ 100mmol/l Na₂SO₄
Flow rate: 1.0 ml/min
Temperature: Room temperature
Detection: UV220nm

Sample:
1; Thyroglobulin
2; Glucose Oxidase
3; Conalbumin
4; Peroxidase
5; Myoglobin
6; Aprotinin



NACALAI TESQUE, INC

AP-0391

● Anti-IgG(H+L), Mouse, Goat-Poly

COSMOSIL Application Data

Column: COSMOSIL 5Diol-300-II
Column size: 7.5mmI.D.-300mm
Mobile phase: 20mmol/l Phosphate buffer(pH7.0),
100mmol/l Na₂SO₄
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV280nm

Sample: Anti-IgG(H+L), Mouse, Goat-Poly,
Unlabeled, Serum (10mg/ml)
Inj. Vol.: 2μl



NACALAI TESQUE, INC

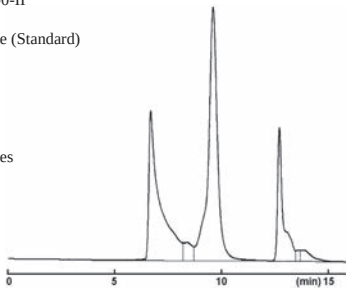
AP-1477

● Mouse IgG1, with aggregates

COSMOSIL Application Data

Column: COSMOSIL 5Diol-300-II
Column size: 7.5mmI.D.-300mm
Mobile phase: Arg-SEC mobile phase (Standard)
(#17000-51)
Flow rate: 0.8 ml/min
Temperature: 30°C
Detection: UV280nm

Sample: mIgG1, with aggregates
Injection Vol. 10μl



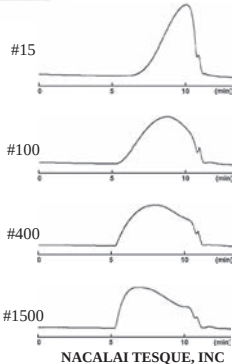
NACALAI TESQUE, INC

● Methyl Cellulose

COSMOSIL Application Data

Column: COSMOSIL 5Diol-1000-II
Column size: 7.5mmI.D.-300mm
Mobile phase: H₂O
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: RI

Sample: Methyl Cellulose (5mg/ml)
Inj. Vol.: 20μl



NACALAI TESQUE, INC

AP-1449

Ordering Information

● COSMOSIL 5Diol-120-II Analytical Columns (Particle Size: 5 μm)

Packed Column

I.D. x Length (mm)	Product Number
7.5×300	38050-51
7.5×600	38051-41

Guard Column

I.D. x Length (mm)	Product Number
7.5×50	38049-91

● COSMOSIL 5Diol-300-II Analytical Columns (Particle Size: 5 μm)

Packed Column

I.D. x Length (mm)	Product Number
7.5×300	38053-21
7.5×600	38054-11

Guard Column

I.D. x Length (mm)	Product Number
7.5×50	38052-31

Guard Cartridge

I.D. x Length (mm)	Product Number
4.6×10 Cartridge*	19180-54

* 2 cartridges included. Guard cartridge holder required; refer to page 78.

● COSMOSIL 5Diol-1000-II Analytical Columns (Particle Size: 5 μm)

Packed Column

I.D. x Length (mm)	Product Number
7.5×300	13338-71

Guard Column

I.D. x Length (mm)	Product Number
7.5×50	13337-81

● COSMOSIL 5Diol-2000-II Analytical Columns (Particle Size: 5 μm)

Packed Column

I.D. x Length (mm)	Product Number
7.5×300	19976-31

Hydrophobic Interaction Columns

COSMOSIL HIC



- Separate based on differences in hydrophobicity
- Little loss in enzyme activity and the tertiary structure of proteins

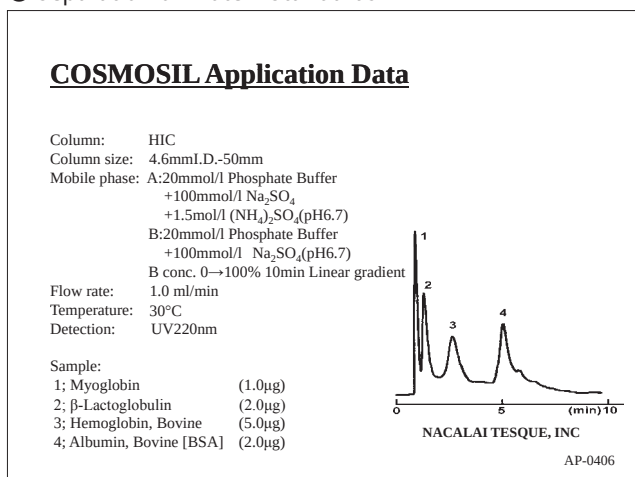
Specifications

Packing Material	HIC
Silica Gel	High purity porous spherical silica
Average Particle Size	5 μm
Average Pore Size	300 $\text{\AA}$
Specific Surface Area	150 m^2/g
Main Interaction	Hydrophobic interaction

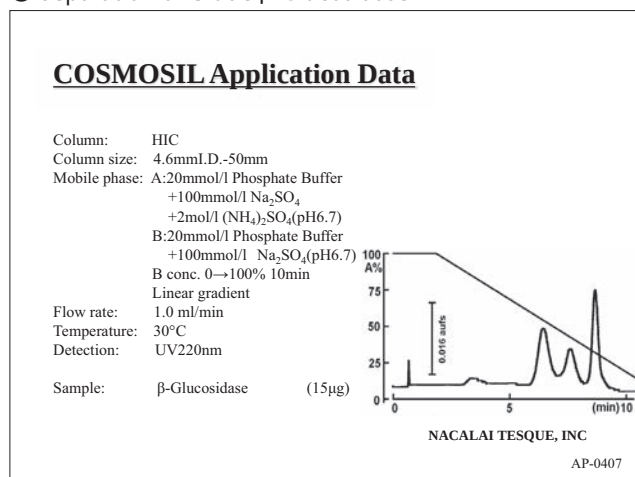
Applications

A buffer with high salt concentration, usually 1-2 mol/L of $(\text{NH}_4)_2\text{SO}_4$, is used as an initial mobile phase for adsorption of samples to a weakly hydrophobic stationary phase. The elution is done with a decreasing salt gradient. The application in lower left shows myoglobin elutes first than BSA under the buffer with high salt concentration, suggesting that myoglobin is less hydrophobic than BSA.

● Separation of Protein Standards



● Separation of Crude β -Glucosidase



Ordering Information

● COSMOSIL 5HIC Analytical Columns (Particle Size: 5 μm)

Packed Column

I.D. x Length (mm)	Product Number
4.6 $\times$ 50	04263-21