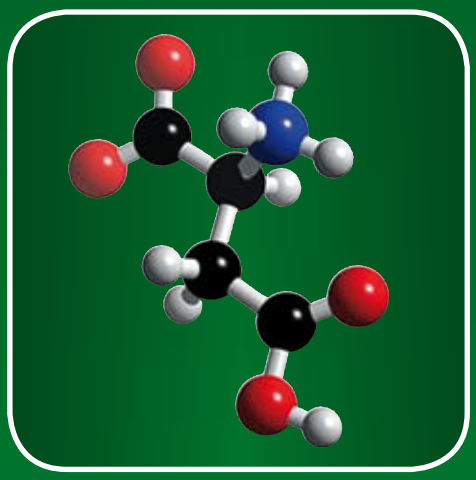
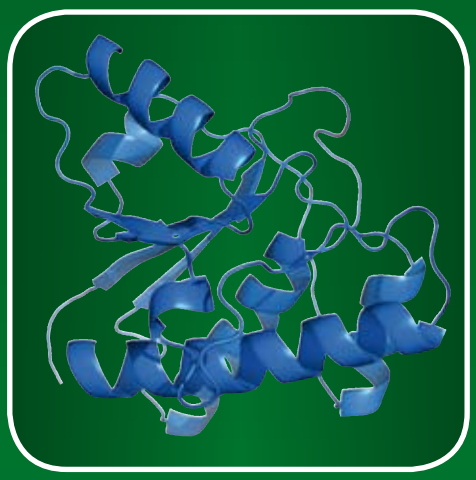
YMC
EUROPE GMBH

# YMC Phases for Biochromatography





IEX
SEC
RP
NP/HILIC

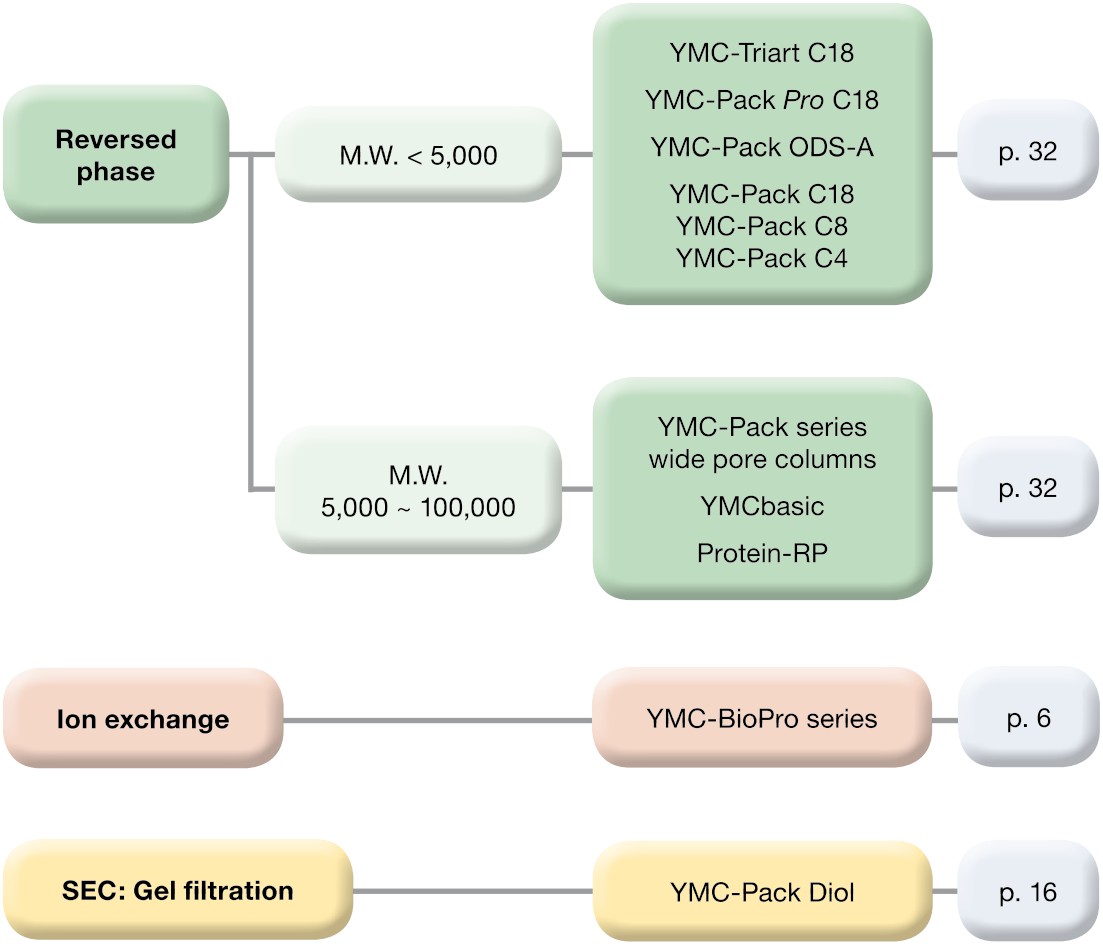
# YMC Phases for Biochromatography

Historically, small molecules have played the major role in diagnosis and therapy. However with the recent developments in the fields of genomics, proteomics and metabolomics, biological molecules have become an important tool for the treatment of diseases or help understanding biological processes.

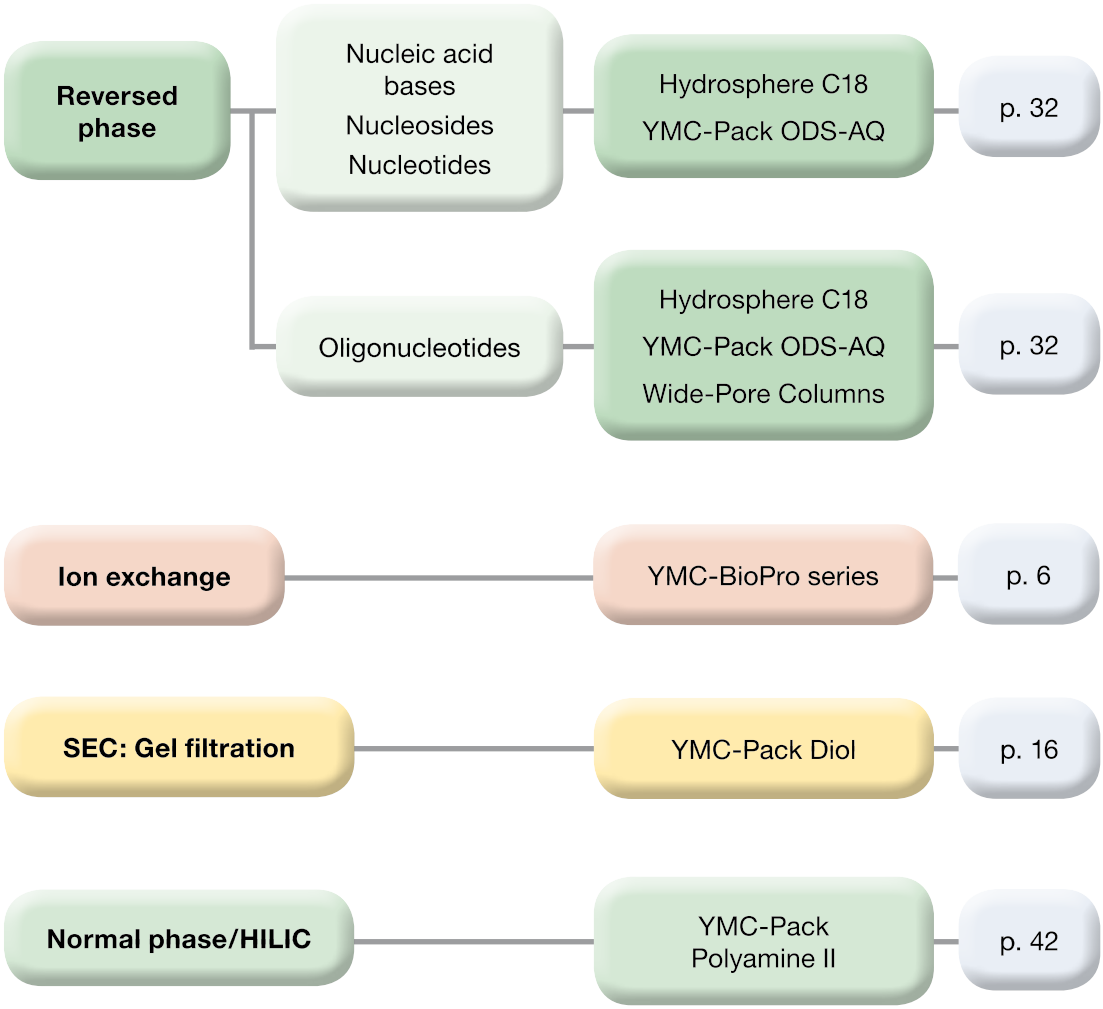
YMC has always played an important role in the provision of materials for bioseparations. With the constant driving force of innovation, the focus has always been on column design and stationary phase manufacturing. As a consequence, YMC offers state of the art reversed phase, ion-exchange, size exclusion and normal phase/HILIC columns and bulk materials.

# Contents

# Proteins, Peptides

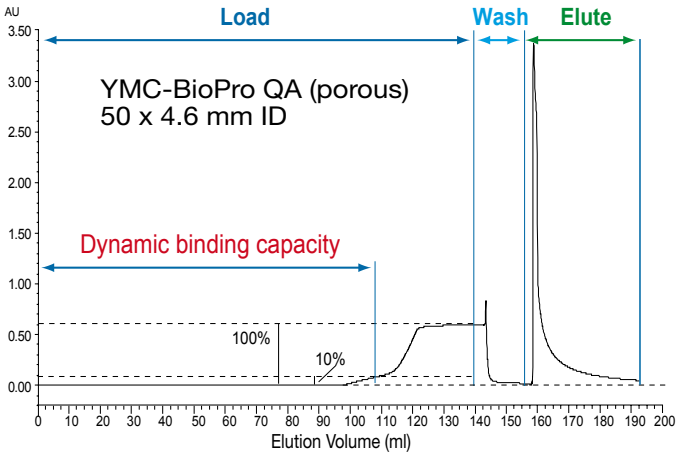


# Nucleic acids




# Polysaccharides

## Determination of DBC*




* Application data by courtesy YMC Co., Ltd.

Before determination, equilibrate the column with equilibration buffer.

### Step 1: Load

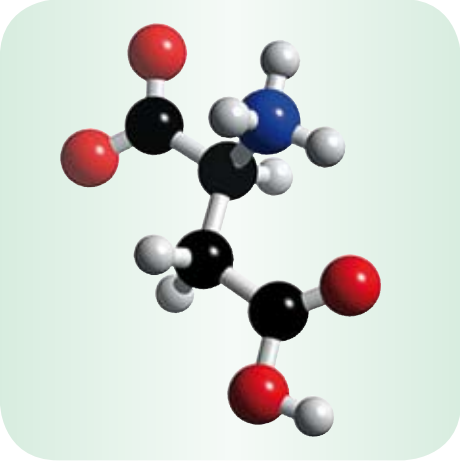
A protein solution of known concentration is continuously loaded at the desired flow rate and the absorbance of the eluate is monitored until full saturation is achieved (100% UV absorbance of the pure sample solutions).

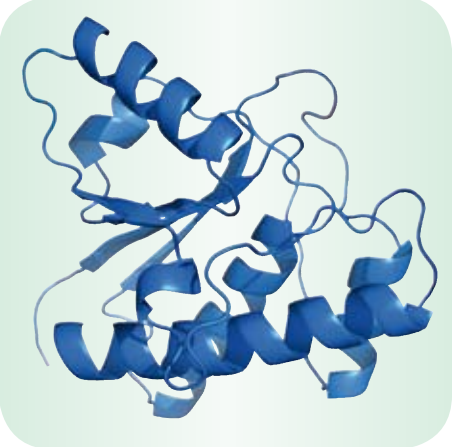
### Step 2: Wash

Wash the column with equilibration buffer until no more protein elutes (0% UV ab-sorbance).

### Step 3: Elute

The DBC of the medium is a measure of the volume of protein solution that has been applied up to a specific breakthrough point (usually 5 or 10%).





IEX

# YMC-BioPro: IEX-Columns

Ion exchange chromatography (IEX) is widely used for analysis and purification of biomolecules. YMC-BioPro ion exchange columns are specifically designed for separation of proteins, peptides and nucleic acids.

YMC-BioPro IEX columns are based on 5 µm porous and non-porous hydrophilic polymer beads with low nonspecific adsorption.

They also show higher binding capacity and higher recovery of biomolecules compared to conventional IEX-columns.

The completely spherical, monodisperse beads, together with optimal packing technology, provide high theoretical plate numbers and symmetrical peak shapes!

## High binding capacity and high recovery for porous type

The porous version of YMC-BioPro show high dynamic binding capacity and excellent recovery, making them useful for semi-preparative separations of proteins and antibodies.

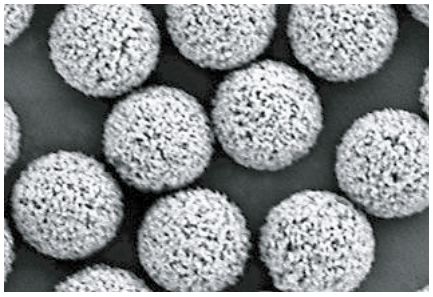
### Comparison of dynamic binding capacity (DBC) for BSA

| | Dynamic binding capacity (mg/ml-gel, 10% breakthrough) | Eluted amount (mg/ml-gel) | Recovery* (%) |
|---|---|---|---|
| **YMC-BioPro QA** | 126 | 120 | 95 |
| **Mono Q (GE Healthcare)** | 100 | 35 | 35 |
| **BioAssist Q (Tosoh Bioscience)** | 73 | 58 | 79 |

* Recovery: (Eluted amount/Dynamic binding capacity) x 100

High recovery rates for YMC-BioPro

Compared with conventional porous polymer anion exchange columns, YMC-BioPro QA gives higher DBC and recovery rates. This indicates that YMC-BioPro has a much lower nonspecific adsorption compared to conventional columns.



Porous polymer beads

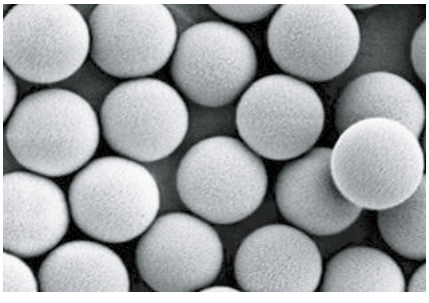
### YMC-BioPro QA / YMC-BioPro SP

**Pore size / nm:** 100
**Particle size / µm:** 5
**Charged group:** $-CH_2N^+(CH_3)_3$ / $-CH_2CH_2CH_2SO_3$
**Counter ion:** $Cl^-$ / $Na^+$
**pH range:** 2.0 - 12.0

Also available in 30 µm and 75 µm for preparative scale

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

**Pore size / nm:** non-porous
**Particle size / µm:** 5
**Charged group:** $-CH_2N^+(CH_3)_3$ / $-CH_2CH_2CH_2SO_3$
**Counter ion:** $Cl^-$ / $Na^+$
**pH range:** 2.0 - 12.0

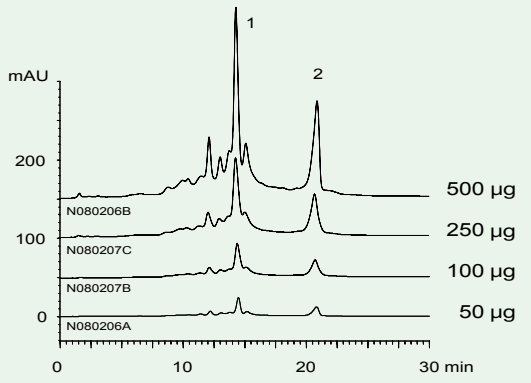


Nonporous polymer beads

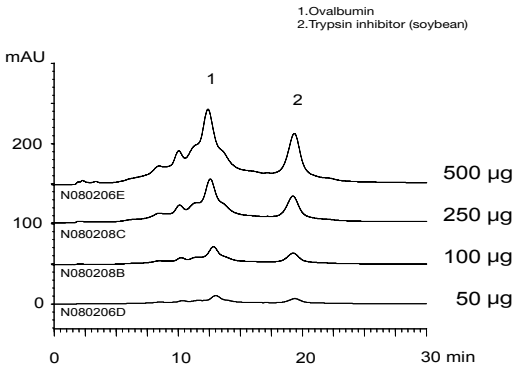
# Applications for porous YMC-BioPro

## Loading study for YMC-BioPro QA (porous) – Proteins*

YMC-BioPro QA
5 µm, 100 nm (50 x 4.6 mm ID)




GE Healthcare (Mono Q)
10 µm (50 x 5.0 mm ID)

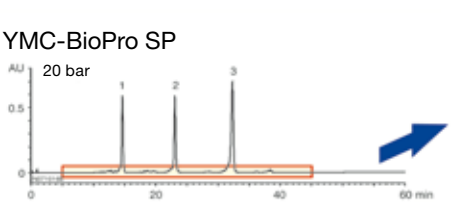



| | | | |
|---|---|---|---|
| Eluent: | A) 20 mM Tris-HCl (pH 8.1)<br>B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl | Flow rate: | 0.5 ml/min |
| Gradient: | 10-80%B (0-30 min) | Temperature: | 25°C |
| | | Detection: | UV at 280 nm |
| | | Injection: | 100 µl |

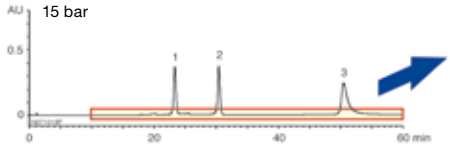
# Superior resolution

## Comparison of standard protein separation on YMC-BioPro SP and commercial SP or S type products*

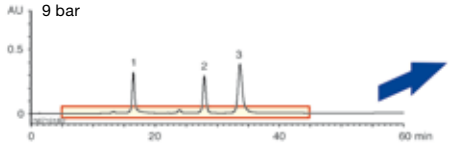
YMC-BioPro SP

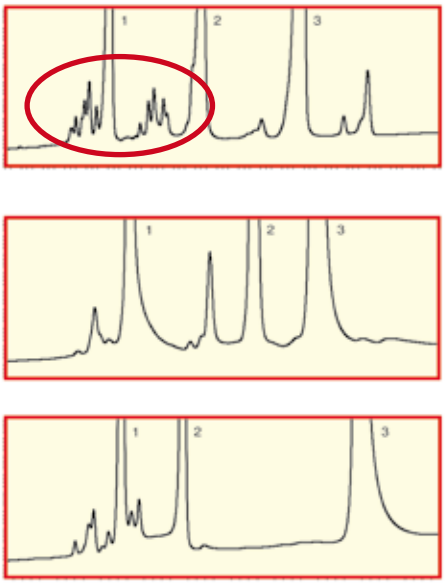



TSKgel BioAssist S, 10 µm




GE Healthcare Mono S




Superior resolution

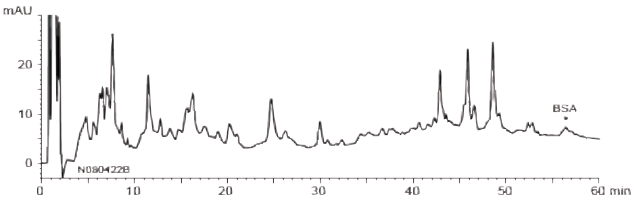
| | | | |
|---|---|---|---|
| Eluent: | A) 20 mM $KH_2PO_4$-$K_2HPO_4$ (pH 6.8)<br>B) 20 mM $KH_2PO_4$-$K_2HPO_4$ (pH 6.8) containing 0.5 M NaCl | Sample: | 1. Ribonuclease A (0.5 mg/ml)<br>2. Cytochrome c (0.5 mg/ml)<br>3. Lysozyme (0.5 mg/ml) |
| Gradient: | 0-100%B (0-60 min) | | |
| Flow rate: | YMC-BioPro SP, TSKgel BioAssist S 0.5 ml/min<br>GE Healthcare Mono S 0.59 ml/min | | |
| Temperature: | 25°C | | |
| Detection: | UV at 220 nm | | |
| Injection: | YMC-BioPro SP, TSKgel BioAssist S 20 µl<br>GE Healthcare Mono S 23.6 µl | | |

* Application data by courtesy YMC Co., Ltd.

# Applications for porous YMC-BioPro

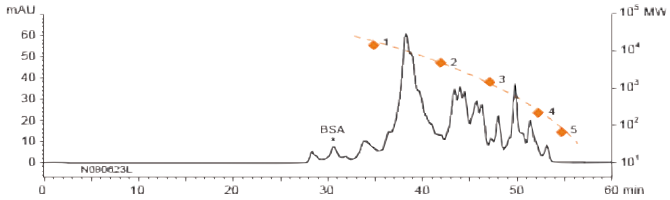
## Peptide mapping*

IEX: YMC-BioPro QA 5 µm, 50 x 4.6 mm ID




Eluent: A) 20 mM Tris-HCl (pH 8.6)
B) 20 mM Tris-HCl (pH 8.6)
+ 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

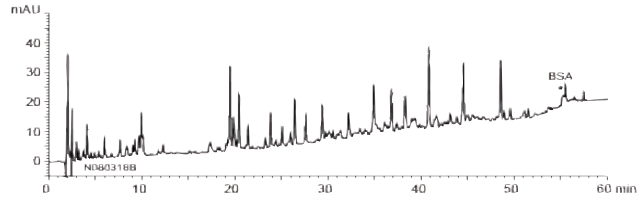
SEC: YMC-Pack Diol-120 x Diol-60 5 µm, 500 x 8.0 mm ID x 2




Calibration curve of proteins and peptides
1. Myoglobin (MW 17,000)
2. Insulin (Bovine) (MW 5,700)
3. Neurotensine (MW 1,672)
4. Tetraglyzine (MW 246)
5. Glyzine (MW 75)

Eluent: 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0)
+ 0.2 M NaCl/Acetonitrile (70/30)
Flow rate: 0.7 ml/min
Temperature: 25 °C
Detection: UV at 220 nm
Injection: 5 µl

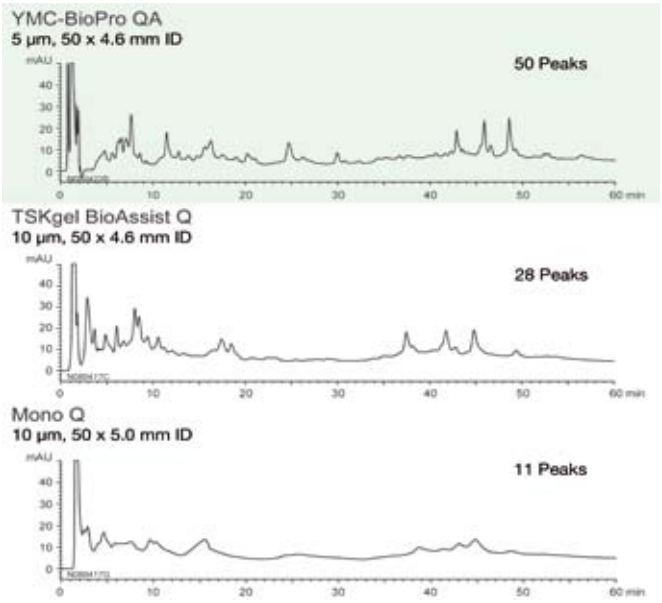
RP: YMCbasic 5 µm, 150 x 2.0 mm ID




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

Tryptic digest of BSA (MW: 66,000)

## Peptide mapping of tryptic digest of BSA*



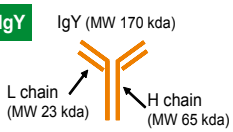

Eluent: A) 20 mM Tris-HCl (pH 8.6)
B) 20 mM Tris-HCl (pH 8.6)
containing 0.5 M NaCl
Gradient: 0-15%B (0-30 min), 15-60%B (30-60 min)
Flow rate: 0.5 ml/min
Temperature: 25°C
Detection: UV at 220 nm
Injection: 20 µl
Sample: Tryptic digest of BSA

# Capture purification by ion-exchange chromatography (IEX)

## Two step purification of IgY to produce reference standard material from crude egg yolk extract*

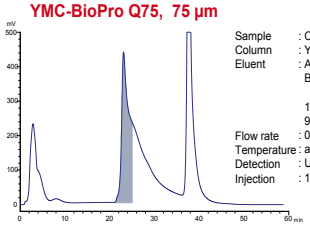
**Crude extract from egg yolk containing IgY**

Crude extract is the supernatant after precipitation of the lipids and lipoproteins from egg yolk.




***Step 1: Capture purification by ion-exchange chromatography (IEX)***

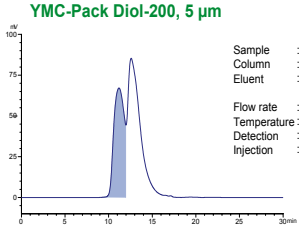
**YMC-BioPro Q75, 75 µm**



Sample : Crude extract from egg yolk
Column : YMC-BioPro Q75, 75 µm, 50 X 4.6 mm ID
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 : 0.5 ml/min (180 cm/hr)
Temperature : ambient
Detection : UV at 280 nm
Injection : 1 ml (approx. 20 mg protein)

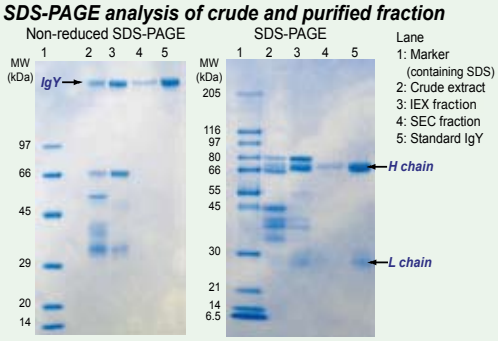
***Step 2: Final purification by size-exclusion chromatography (SEC)***

**YMC-Pack Diol-200, 5 µm**

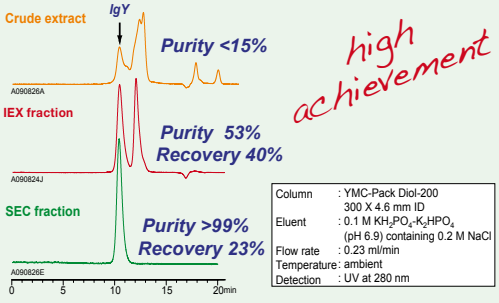


Sample : Fraction from capture purification by IEC
Column : YMC-Pack Diol-200, 5 µm, 300 X 8.0 mm ID
Eluent : 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 6.9) containing 0.2 M NaCl
Flow rate : 0.7 ml/min (180 cm/hr)
Temperature : ambient
Detection : UV at 280 nm
Injection : 1 ml (approx. 0.45 mg IgY)

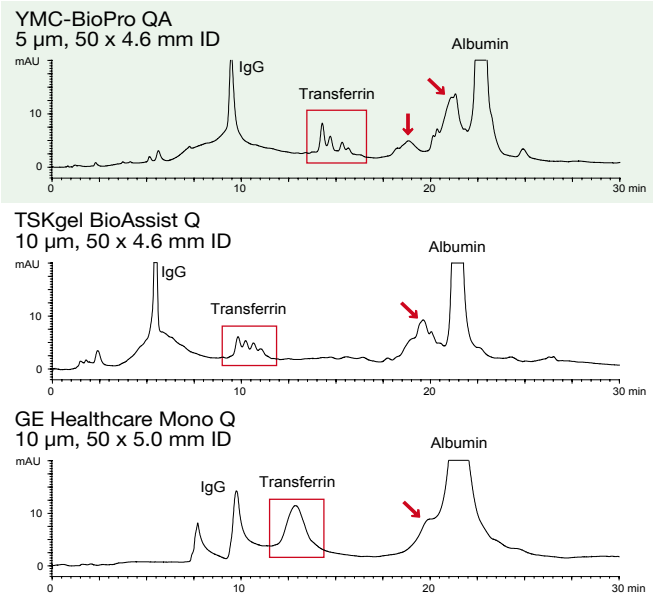
***SDS-PAGE analysis of crude and purified fraction***




***SEC analysis of crude and purified fraction***




## Separation of proteins in human serum on YMC-BioPro QA and commercial Q type products*




For high resolution YMC-BioPro QA/SP, porous IEX material, is recommended!

Eluent : A) 20 mM Tris-HCl (pH 8.6)
B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl
Gradient : 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
Sample : Human serum (100 ml/ml)

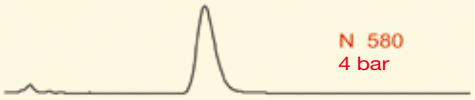
* Application data by courtesy YMC Co., Ltd.

## Applications for non-porous YMC-BioPro: High Throughput IEX

### High efficiency with a lower column pressure with non-porous type*

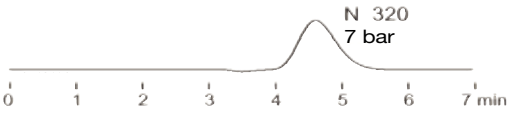
Anion exchange column

YMC-BioPro QA-F
5 µm, 30 x 4.6 mm i.d.




TSKgel DEAE-NPR
2.5 µm, 35 x 4.6 mm i.d.

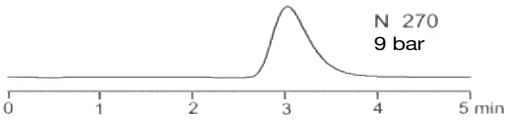

Mini Q 4.6/50 PE
3 µm, 50 x 4.6 mm i.d.




Cation exchange column

YMC-BioPro SP-F
5 µm, 30 x 4.6 mm i.d.


TSKgel SP-NPR
2.5 µm, 35 x 4.6 mm i.d.

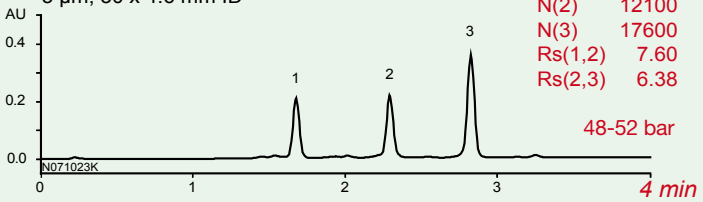



| | |
|---|---|
| Eluent: | 20 mM Tris-HCl (pH 8.1)<br>(for anion exchange columns)<br>20 mM $KH_2PO_4$-$K_2HPO_4$ (pH 6.8)<br>(for cation exchange columns) |
| Flow rate: | 0.12 ml/min |
| Temperature: | 25 °C |
| Detection: | UV at 220 nm |
| Injection: | 20 µl |
| Sample: | Formamide (2 µl/ml) |

**Compared to the competitors' columns, YMC-BioPro QA-F and SP-F show higher theoretical plate counts, excellent peak shapes, and lower backpressure. This makes YMC-BioPro QA-F and SP-F most suitable for high-throughput analysis.**

### Comparison of standard protein separation on YMC-BioPro SP-F and a commercial SP-type product*

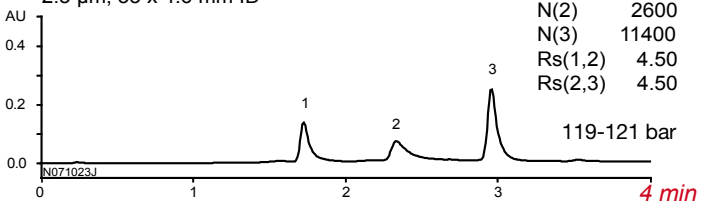
YMC-BioPro SP-F
5 µm, 30 x 4.6 mm ID




| | |
|---|---|
| N(1) | 7100 |
| N(2) | 12100 |
| N(3) | 17600 |
| Rs(1,2) | 7.60 |
| Rs(2,3) | 6.38 |

48-52 bar

TSKgel SP-NPR
2.5 µm, 35 x 4.6 mm ID




| | |
|---|---|
| N(1) | 4400 |
| N(2) | 2600 |
| N(3) | 11400 |
| Rs(1,2) | 4.50 |
| Rs(2,3) | 4.50 |

119-121 bar

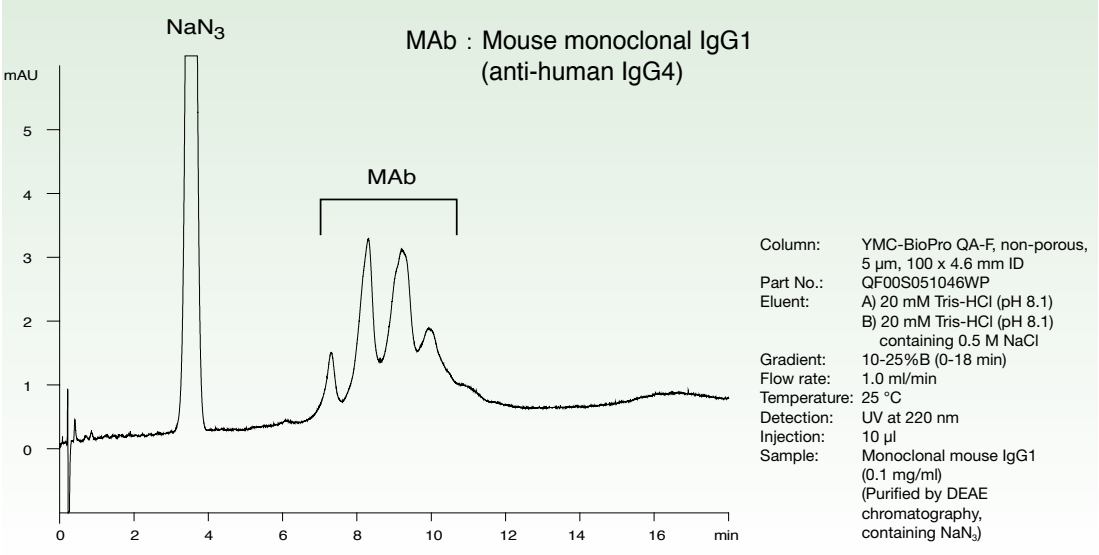
| | |
|---|---|
| Eluent: | A) 20 mM $KH_2PO_4$-$K_2HPO_4$ (pH 6.8)<br>B) 20 mM $KH_2PO_4$-$K_2HPO_4$ (pH 6.8)<br>containing 0.5 M NaCl |
| Gradient: | YMC-BioPro SP-F 0-100%B (0-4 min)<br>TSKgel SP-NPR 0-100%B (0-4.67 min) |
| Flow rate: | 1.5 ml/min |
| Temperature: | 25°C |
| Detection: | UV at 220 nm |
| Injection: | 20 µl |
| Sample: | 1. Ribonuclease A (0.1 mg/ml)<br>2. Cytochrome c (0.1 mg/ml)<br>3. Lysozyme (0.1 mg/ml) |

**YMC-BioPro SP-F elutes the proteins as sharper peaks without peak-tailing than TSKgel SP-NPR.**

**Despite the larger particle size, the theoretical plate count for YMC-BioPro SP-F is higher than that for TSKgel SP-NPR.**

## Applications for non-porous YMC-BioPro

### Analysis of monoclonal antibody (MAb) against human IgG4*




| | |
|---|---|
| Column: | YMC-BioPro QA-F, non-porous, 5 µm, 100 x 4.6 mm ID |
| Part No.: | QF00S051046WP |
| Eluent: | A) 20 mM Tris-HCl (pH 8.1)<br>B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl |
| Gradient: | 10-25%B (0-18 min) |
| Flow rate: | 1.0 ml/min |
| Temperature: | 25 °C |
| Detection: | UV at 220 nm |
| Injection: | 10 µl |
| Sample: | Monoclonal mouse IgG1 (0.1 mg/ml) (Purified by DEAE chromatography, containing $NaN_3$) |

## The optimum package!

### YMC-BioPro IEX + ECO/ECO$^{PLUS}$ Glass Columns

**YMC's ECO/ECO$^{PLUS}$ Glass Columns**

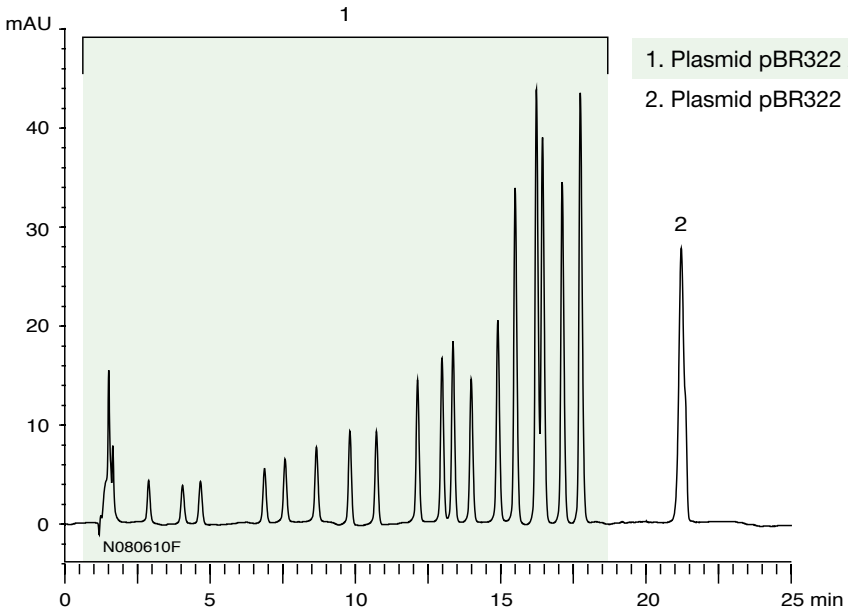
- **biocompatible**
- **easy to use**
- **compatible with any LC systems**
- **solvent resistant version (optional)**

**—> Find more at page 47!**

* Application data by courtesy YMC Co., Ltd.

# Applications for non-porous YMC-BioPro: High Throughput IEX

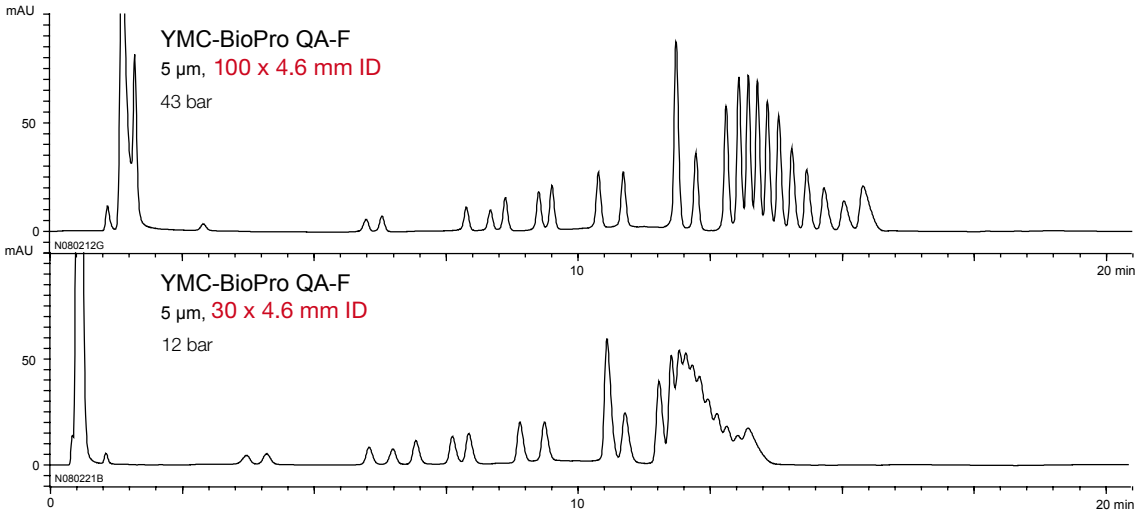
## Fast analysis on non-porous YMC-BioPro QA-F*




1. Plasmid pBR322 *Hae* III digest (8-587 bp)
2. Plasmid pBR322 (4,361 bp)

| | | | |
|---|---|---|---|
| Column: | YMC-BioPro QA-F, 5 µm, 100 x 4.6 mm ID | Temperature: | 35°C |
| Part No.: | QF00S051046WP | Detection: | UV at 260 nm |
| Eluent: | A) 20 mM Tris-HCl (pH 8.1) | Injection: | 10 µl |
| | B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl | Sample: | Plasmid pBR322 Hae III digest (0.13 mg/ml) |
| Gradient: | 70-85%B (0-20 min), 85%B (20-25 min) | | Plasmid pBR322 (0.03 mg/ml) |
| Flow rate: | 0.5 ml/min | | |

## Excellent resolution in analysis of complex mixtures*

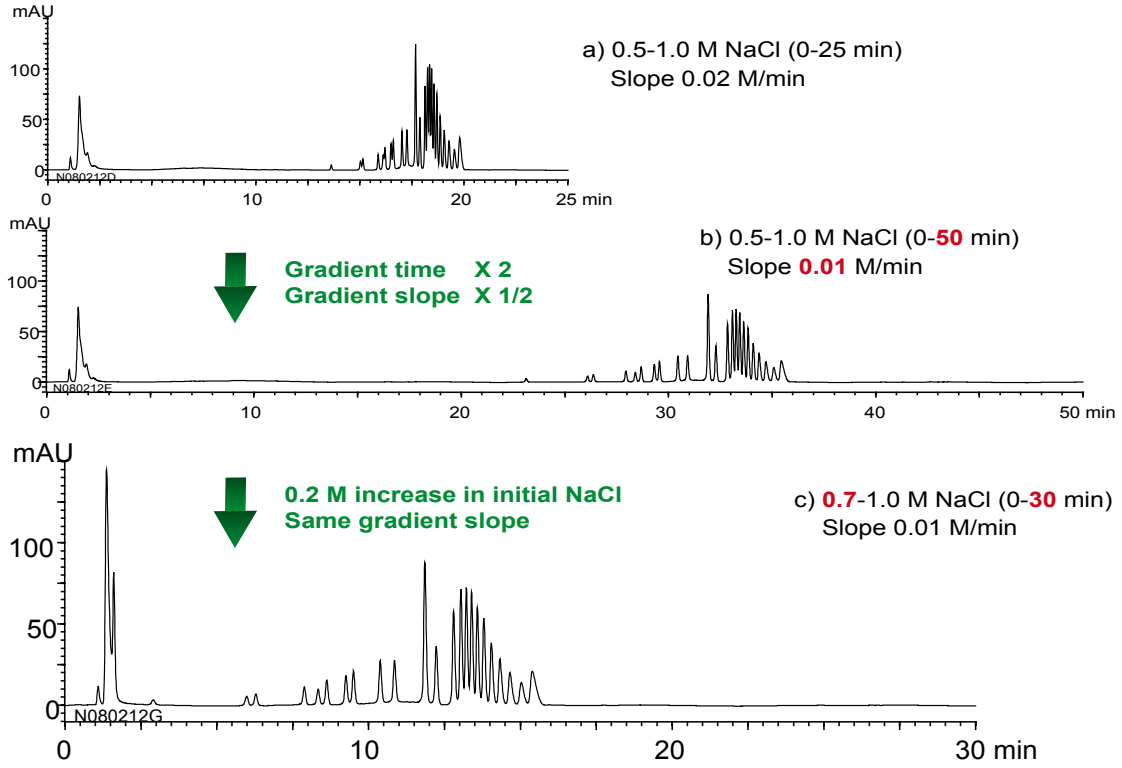



| | | | |
|---|---|---|---|
| Eluent | : A) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl | Temperature | : 25°C |
| | B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl | Detection | : UV at 260 nm |
| Gradient | : 40-100%B (0-30 min) | Injection | : 20 µl |
| Flow rate | : 0.5 ml/min | Sample | : 1 kb DNA Ladder (0.25 mg/ml) |

**Comparison of DNA fragment separation on 100 mm and 30 mm length YMC-BioPro QA-F columns.**

## Applications for non-porous YMC-BioPro: High Throughput IEX

### Method development for DNA-fragments*




| | | | |
|---|---|---|---|
| Column: | YMC-BioPro QA-F, 5 µm, 100 x 4.6 mm ID | Temperature: | 25 °C |
| Part No.: | QF00S051046WP | Detection: | UV at 260 nm |
| Eluent: | A) 20 mM Tris HCl (pH 8.1) containing 0.5 M NaCl<br>B) 20 mM Tris HCl (pH 8.1) containing 1.0 M NaCl | Injection: | 20 µl |
| | | Sample: | 1 Kb DNA Ladder (0.25 mg/ml) |
| Flow rate: | 0.5 ml/min | | |

**Elution of DNA fragments is optimised on 100 mm columns. The sensitivity is improved by reducing the gradient development time by half. In addition, the analysis time is reduced by increasing the buffer concentration, while maintaining excellent resolution.**

# *Ordering information*

## 5 µm analytical columns

| | Column dimension | | |
|---|---|---|---|
| **Phase** | **30 x 4.6 mm ID** | **50 x 4.6 mm ID** | **100 x 4.6 mm ID** |
| YMC-BioPro QA | — | QAA0S050546WP | — |
| YMC-BioPro SP | — | SPA0S050546WP | — |
| YMC-BioPro QA-F | QF00S0510346WP | — | QF00S052046WP |
| YMC-BioPro SP-F | SF00S050346WP | — | SF00S051046WP |

Olther dimensions on demand

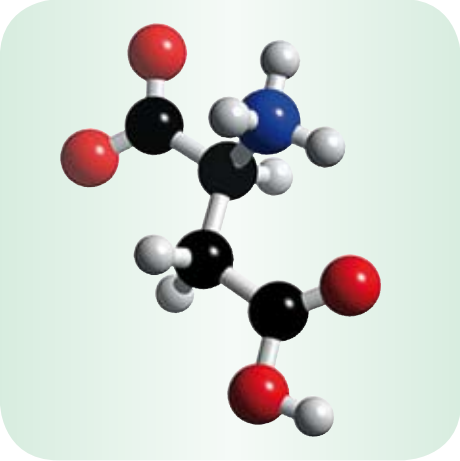
**Preparative grade YMC-BioPro also available as bulk media! (See next page!)**

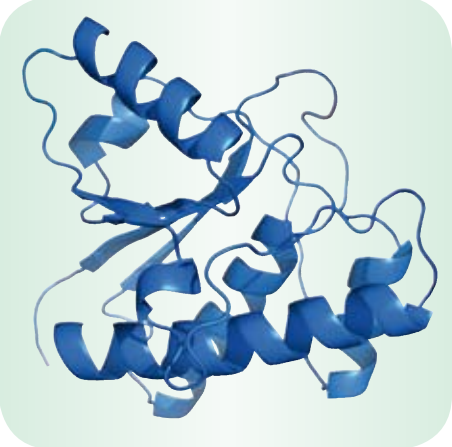
* Application data by courtesy YMC Co., Ltd.

# Ordering information

## Bulk media

| Phase | Particle Size | Part-No. |
|---|---|---|
| YMC-BioPro Q30 | 30 µm | QAA0S30 |
| YMC-BioPro S30 | 30 µm | SPA0S30 |
| YMC-BioPro Q75 | 75 µm | QAA0S75 |
| YMC-BioPro S75 | 75 µm | SPA0S75 |





SEC

# YMC Column for SEC: YMC-Pack Diol

Size Exclusion Chromatography (SEC) also referred to as Gel Permeation Chromatography (GPC) is a mode of liquid chromatography in which the components of a mixture are separated according to differences in their size. It is an important tool for the analysis and separation of natural and synthetic biopolymers. The column packing for SEC is a rigid, totally porous silica gel that is derivatised with 1,2-dihydroxypropylsilane and has pores of known size. The variety of available pore sizes allows the analysis of compounds over a broad range of molecular weights, starting from about 1000 g/mol for oligomers and going up to 1,000,000 g/mol.

## What is special about YMC SEC columns?

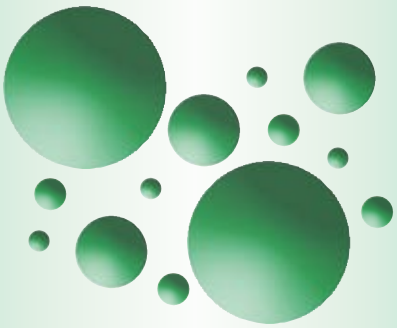
- Method development
- Scaleability
- Reproducibility
- Cost-efficiency

YMC-Pack Diol is available in four porosities and is, therefore, suitable for separation and molecular weight determination of a wide range of peptides, proteins, oligonucleotides, carbohydrates and other biopolymers with molecular weights of 10,000 to several hundred thousand.

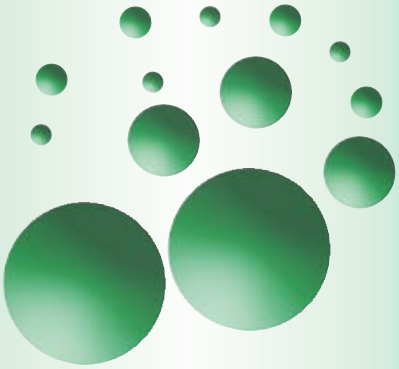
Furthermore, YMC offers a wide range of column dimensions suitable for analytical determinations to preparative separations.

## YMC-Pack Diol Columns

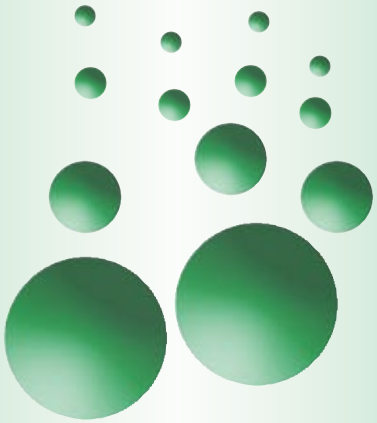
| YMC-Pack Diol | for peptides and small proteins | for intermediate proteins | for large proteins | for very large proteins |
|---|---|---|---|---|
| pore size / nm | 6 | 12 | 20 | 30 |
| particle size / µm | 5 | 5 | 5 | 5 |
| surface area / $m^2g^{-1}$ | 650 | 330 | 175 | 100 |
| recommended pH range | 5.0 - 7.5 | 5.0 - 7.5 | 5.0 - 7.5 | 5.0 - 7.5 |



1. Injection



2. Separation

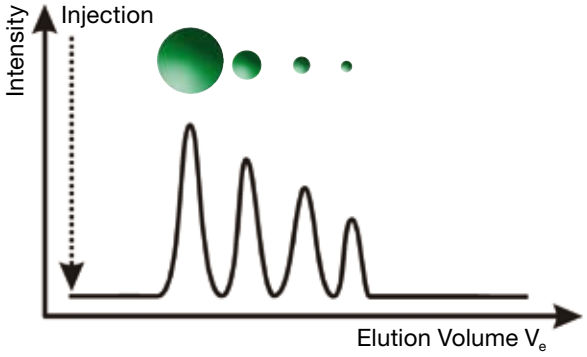


3. Elution of Fraction

## Principles of separation

Molecules with shapes such as rigid rods, random chains and spheres but with the same molecular weight behave differently in SEC. The principle of separation is based on differences in the hydrodynamic radius of the molecules in solution. Molecules with a larger radius elute earlier and those with the smallest radius are retained longer.

The separation limit is such that only those compounds which differ by more than 10% in MW can be separated by SEC.



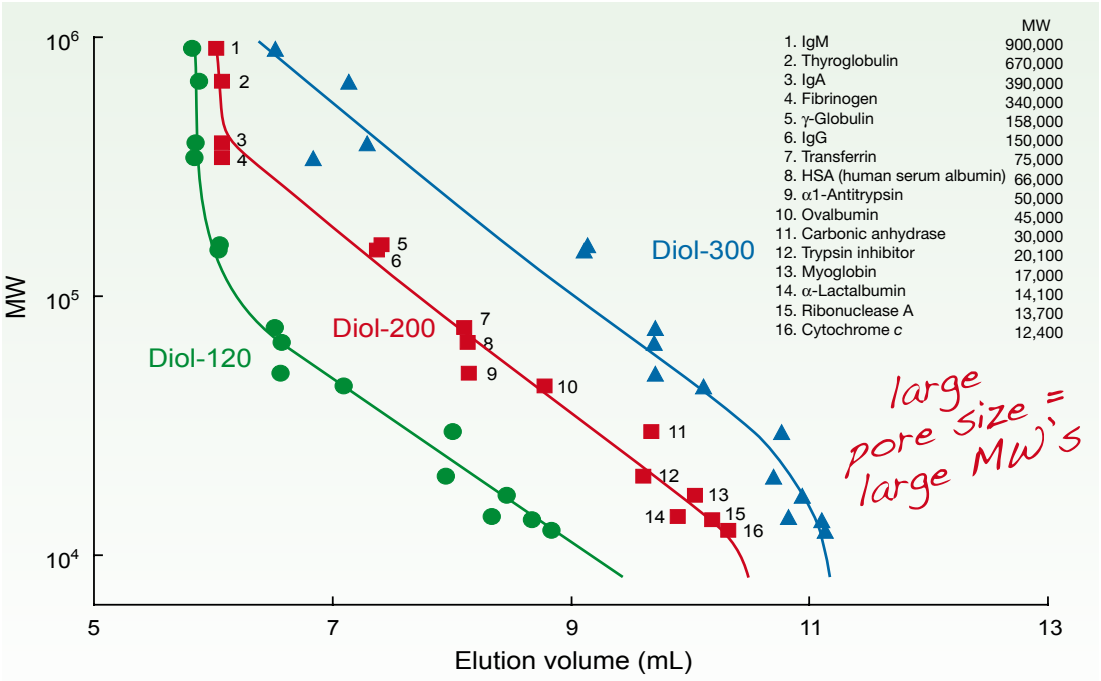

**small molecules = long retention time**
**bigger molecules = short retention time**

Large molecules exit the column more rapidly as they cannot permeate the porous structure of stationary phase. Smaller ones with the lowest hydrodynamic volume elute with longer retention times because they are able to penetrate some or all of the pores of the stationary phase. Molecules of intermediate size elute in an intermediate position.

* Application data by courtesy YMC Co., Ltd.

## SEC Applications for YMC-Pack Diol

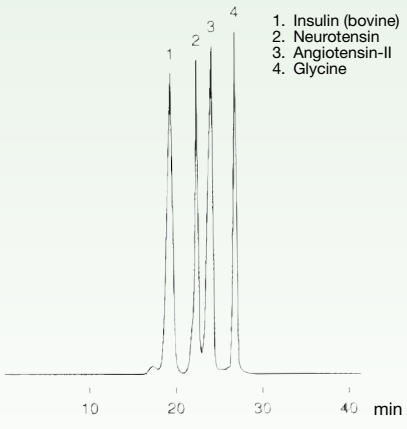
### For separation of proteins with molecular weights from 10.000 to several 100.000

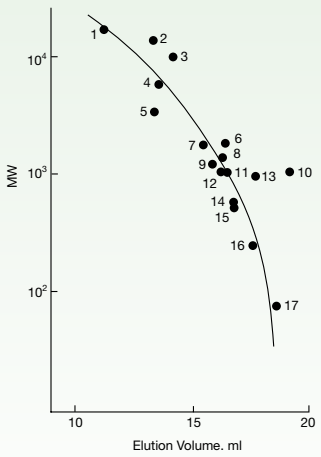



| | MW |
|---|---|
| 1. IgM | 900,000 |
| 2. Thyroglobulin | 670,000 |
| 3. IgA | 390,000 |
| 4. Fibrinogen | 340,000 |
| 5. γ-Globulin | 158,000 |
| 6. IgG | 150,000 |
| 7. Transferrin | 75,000 |
| 8. HSA (human serum albumin) | 66,000 |
| 9. α1-Antitrypsin | 50,000 |
| 10. Ovalbumin | 45,000 |
| 11. 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 mm ID | Flow rate: | 0.5 ml/min |
| Part No.: | DL20S053008WT | Temperature: | 25 °C |
| Eluent: | 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) containing 0.2 M NaCl | Detection: | UV at 280 nm |

### For separation of proteins with molecular weights below 10.000



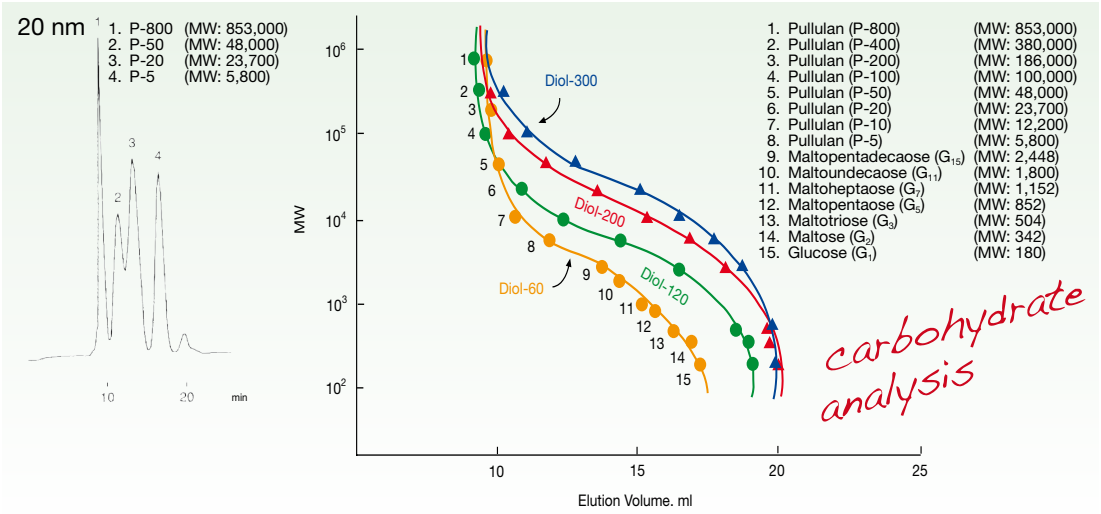

| | | |
|---|---|---|
| 1. Myoglobin | (MW: 17,000) |
| 2. Ribonuclease A | (MW: 13,700) |
| 3. Cytochrome c | (MW: 12,400) |
| 4. Insulin (bovine) | (MW: 5,700) |
| 5. Insulin B chain | (MW: 3,496) |
| 6. α-Mating factor | (MW: 1,684) |
| 7. Neurotensin | (MW: 1,672) |
| 8. Angiotensin-I | (MW: 1,296) |
| 9. CCK-octapeptide | (MW: 1,143) |
| 10. Bradykinin | (MW: 1,060) |
| 11. Angiotensin-II | (MW: 1,040) |
| 12. Oxytocin | (MW: 1,007) |
| 13. Angiotensin-III | (MW: 931) |
| 14. Met-enkephalin | (MW: 573) |
| 15. Leu-enkephalin | (MW: 555) |
| 16. Tetraglycine | (MW: 246) |
| 17. Glycine | (MW: 75) |

small pore size = small MW`s

| | |
|---|---|
| Column: | YMC-Pack Diol (6 nm) 500 x 8.0 mm ID |
| Part No.: | DL06S055008WT |
| Eluent: | 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 7) containing 0.2 M NaCl / acetonitrile (70/30) |
| Flow rate: | 0.7 ml/min |
| Temperature: | ambient |
| Detection: | UV at 215 nm |

# SEC Applications for YMC-Pack Diol

## For molecular weight determination of oligosaccharides and polysaccharides




Column: YMC-Pack Diol (20 nm) 500 x 8.0 mm ID
Part No.: DL20S055008WT
Eluent: water
Flow rate: 1.0 ml/min
Temperature: ambient
Detection: RI

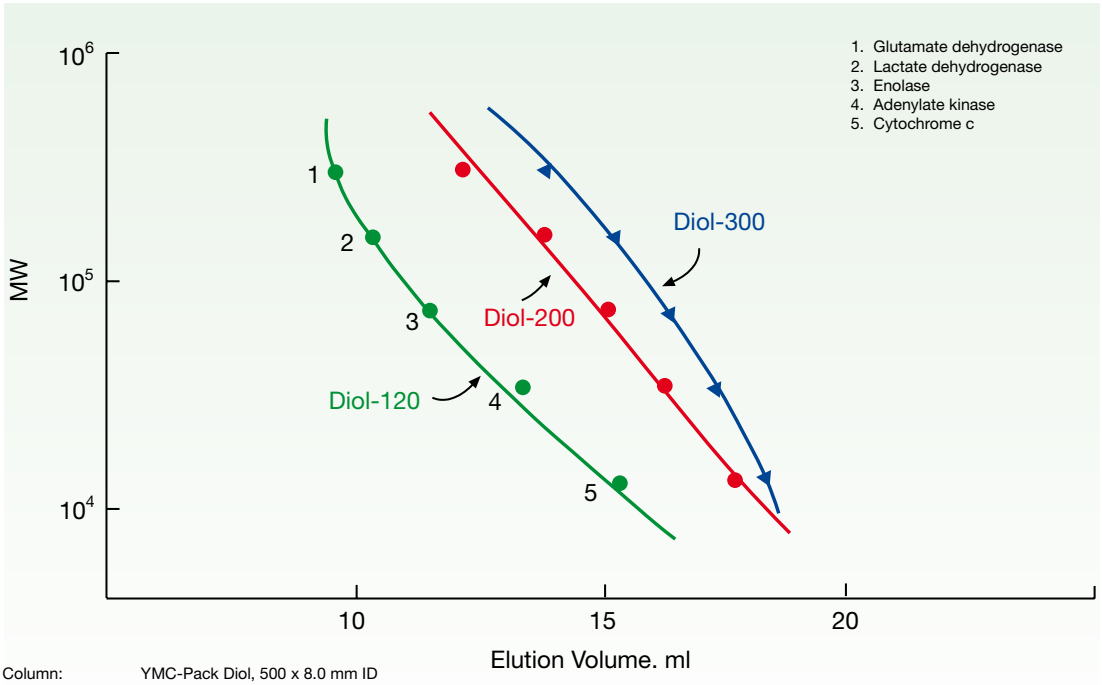
### Column Selection Tool

| | |
|---|---|
| **YMC-Pack Diol-60** | **for MW < 10.000** |
| **YMC-Pack Diol-120** | **for MW 5.000 to 100.000** |
| **YMC-Pack Diol-200** | **for MW 10.000 to several 100.000** |
| **YMC-Pack Diol-300** | **for MW several 10.000 to 1.000.000** |

* Application data by courtesy YMC Co., Ltd.

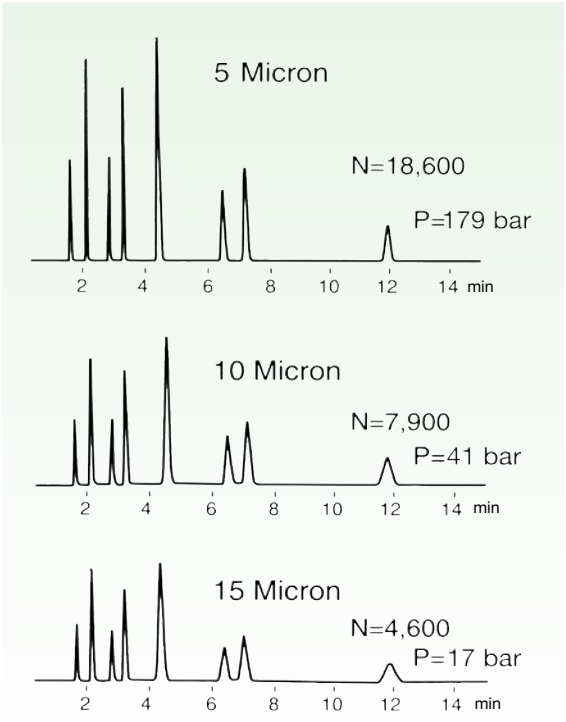
## High flexibility

### Proteins and Peptides*




| | |
|---|---|
| Column: | YMC-Pack Diol, 500 x 8.0 mm ID |
| Part No.: | DL20S055008WT |
| Eluent: | 0.1M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) containing 0.2M NaCl |
| Flow rate: | 0.7 ml/min |
| Temperature: | ambient |
| Detection: | UV at 280 nm |

## Scalability

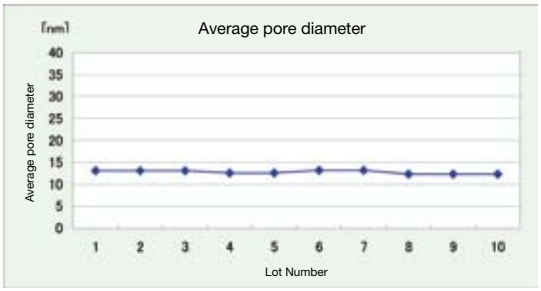


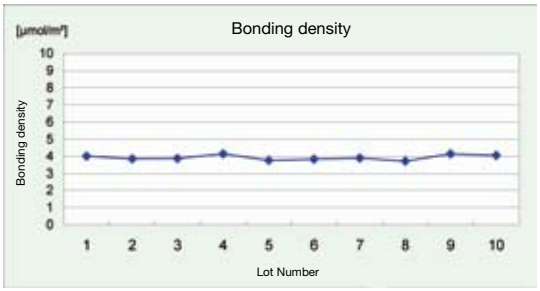

YMC guarantees a seamless, reproducible scale up through all particle sizes for all stationary phases

## Reproducibility

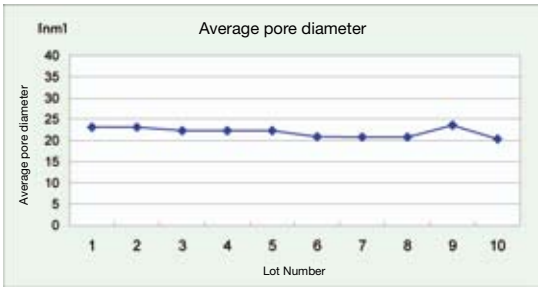
YMC-Pack Diol columns have the reputation, not only for their high versatility and excellent cost/performance ratio, but also for their high degree of lot-to-lot reproducibility.

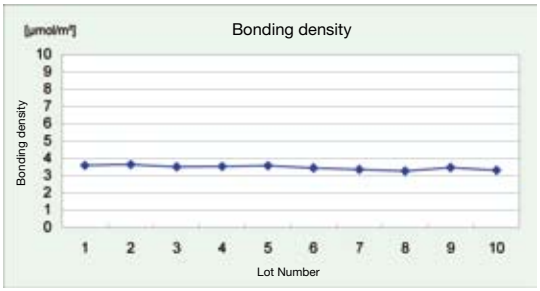
### YMC-Pack Diol-120



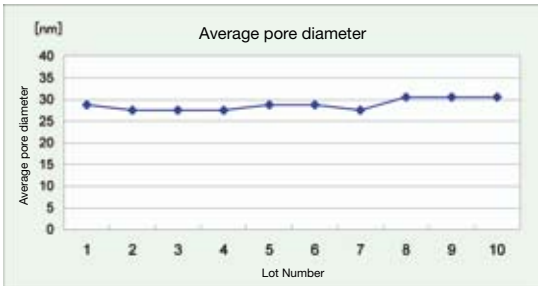



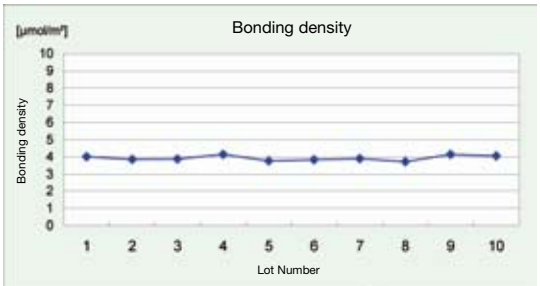

### YMC-Pack Diol-200




### YMC-Pack Diol-300

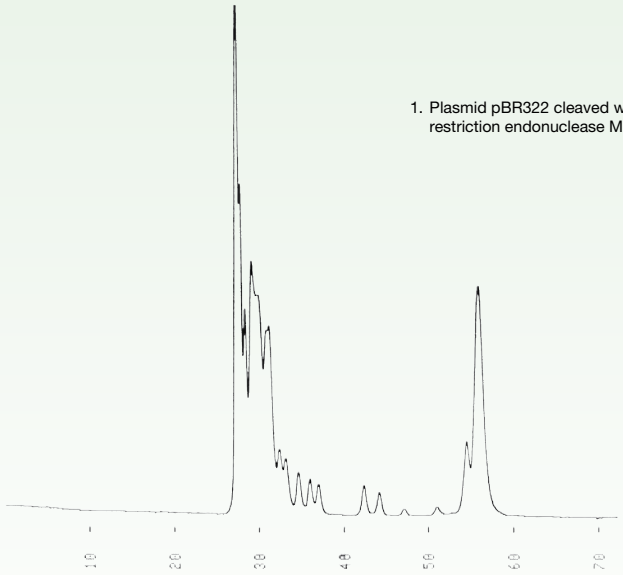



* Application data by courtesy YMC Co., Ltd.

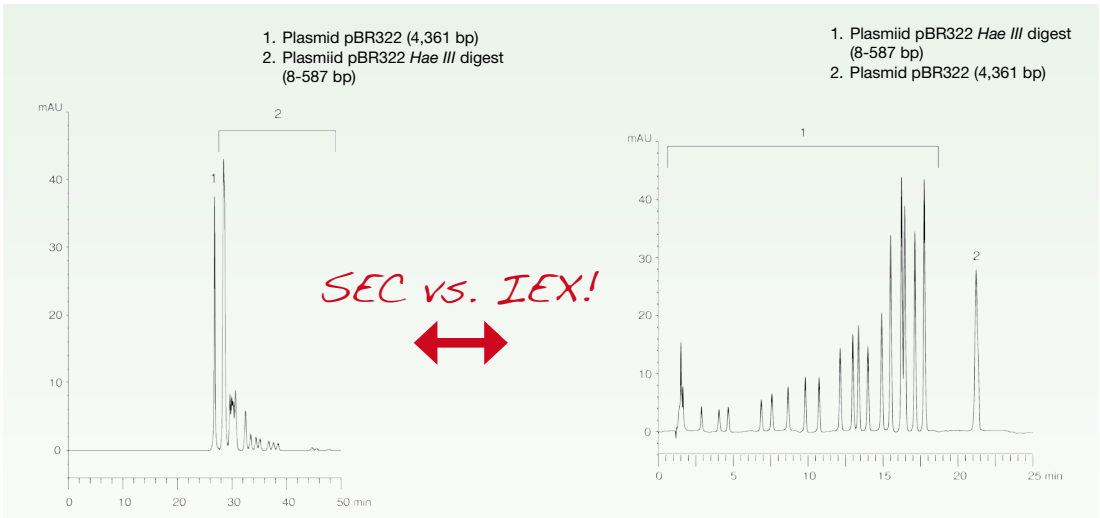
# SEC Applications for YMC-Pack Diol

## Plasmid pBR322 restriction fragment




| | | | |
|---|---|---|---|
| Column: | YMC-Pack Diol-300 + Diol-200, 500 x 8.0 mm ID x 2 | Detection: | UV at 260 nm, 0.01 AUFS |
| Part No.: | DL30S055008WT + DL20S055008WT | Injection: | 3 µl (0.49 mg/ml) |
| Eluent: | 0.1M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) containing 0.2M NaCl | Sample: | Plasmid pBR322 cleaved with restriction endonuclease Msp I |
| Flow rate: | 0.7 ml/min | | |
| Temperature: | ambient (26 °C) | | |

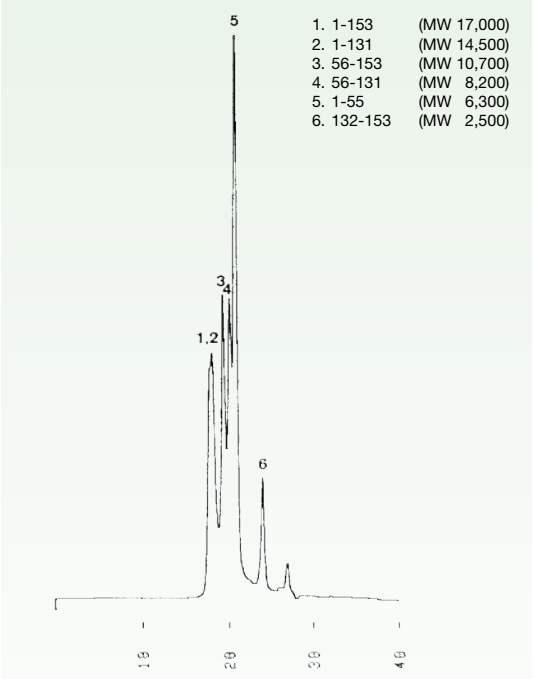
## Plasmid pBR322 restriction and pBR322 *Hae III* restriction fragment




| | | | |
|---|---|---|---|
| Column: | YMC-Pack Diol-300 + Diol-200, 500 x 8.0 mm ID x 2 | Column: | YMC-BioPro QA-F (5 µm), 100 x 4.6 mm ID |
| Part No.: | DL30S055008WT + DL20S055008WT | Part No.: | QF00S051046WT |
| Eluent: | 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) containing 0.2 M NaCl | Eluent: | A) 20 mM Tris-HCl (pH 8.1)<br>B) 20 mM Tris-HCl (pH 8.1) containing 0.1 M NaCl |
| Flow rate: | 0.7 ml/min | Gradient: | 70-85% B (0-20 min), 85% B (20-25 min) |
| Temperature: | ambient (25 °C) | Flow rate: | 0.5 ml/min |
| Detection: | UV at 260 nm | Temperature: | 35 °C |
| Injection: | 10 µl | Detection: | UV at 260 nm |
| | | Injection: | 10 µl |

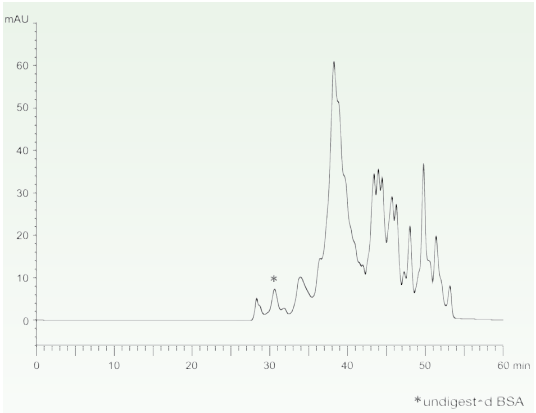
# SEC Applications for YMC-Pack Diol

## Peptide fragments from myoglobin



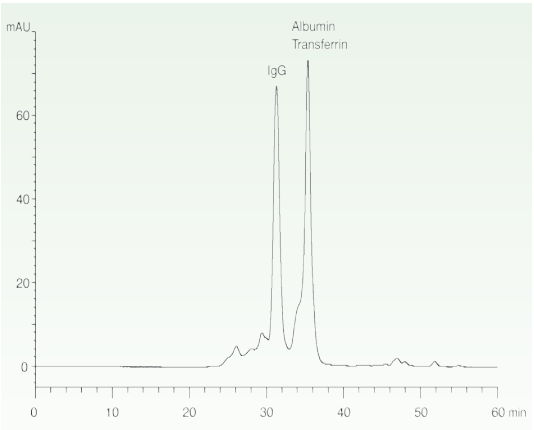

Column: YMC-Pack Diol-120, 500 x 8.0 mm ID
Part No.: DL12S055008WT
Eluent: 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) containing 0.2 M NaCl/ acetonitrile (70/30)
Flow rate: 0.7 ml/min
Temperature: ambient (25 °C)
Detection: UV at 215 nm, 0.32 AUFS
Injection: 20 µl (2.0 mg/ml)
Sample: Cyanogen bromide cleavages of horse heart myoglobim. Molecular Weight Marker for proteins, manufactured by Fluka Chemie AG.

## Peptide mapping



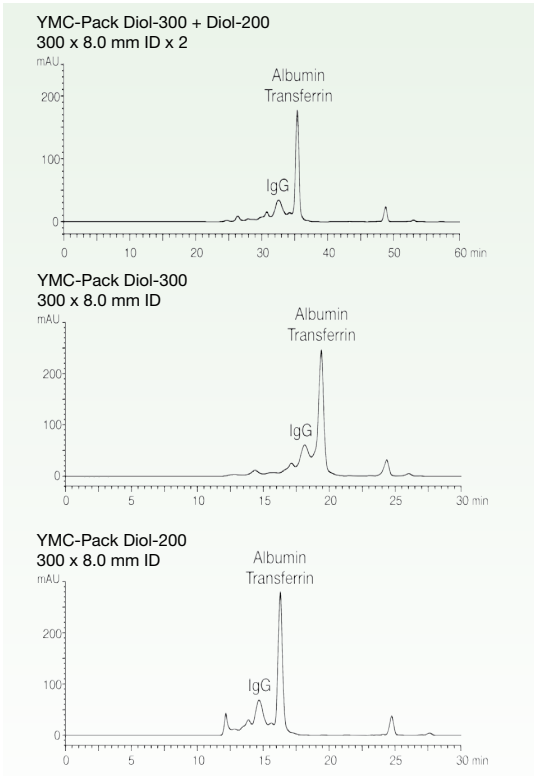

Column: YMC-Pack Diol-120 + Diol-60, 500 x 8.0 mm ID x 2
Part No.: DL12S055008WT + DL06S055008WT
Eluent: 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) containing 0.2 M NaCl/ acetonitrile (70/30)
Flow rate: 0.7 ml/min
Temperature: ambient (25 °C)
Detection: UV at 220 nm
Injection: 5 µl
Sample: Tryptic digest of BSA

## Proteins in mouse ascites fluid




Column: YMC-Pack Diol-300 + Diol-200, 300 x 4.6 mm ID x 2
Part No.: DL30S053046WT + DL20S053046WT
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 (60 times dilution with water)

## Proteins in human serum

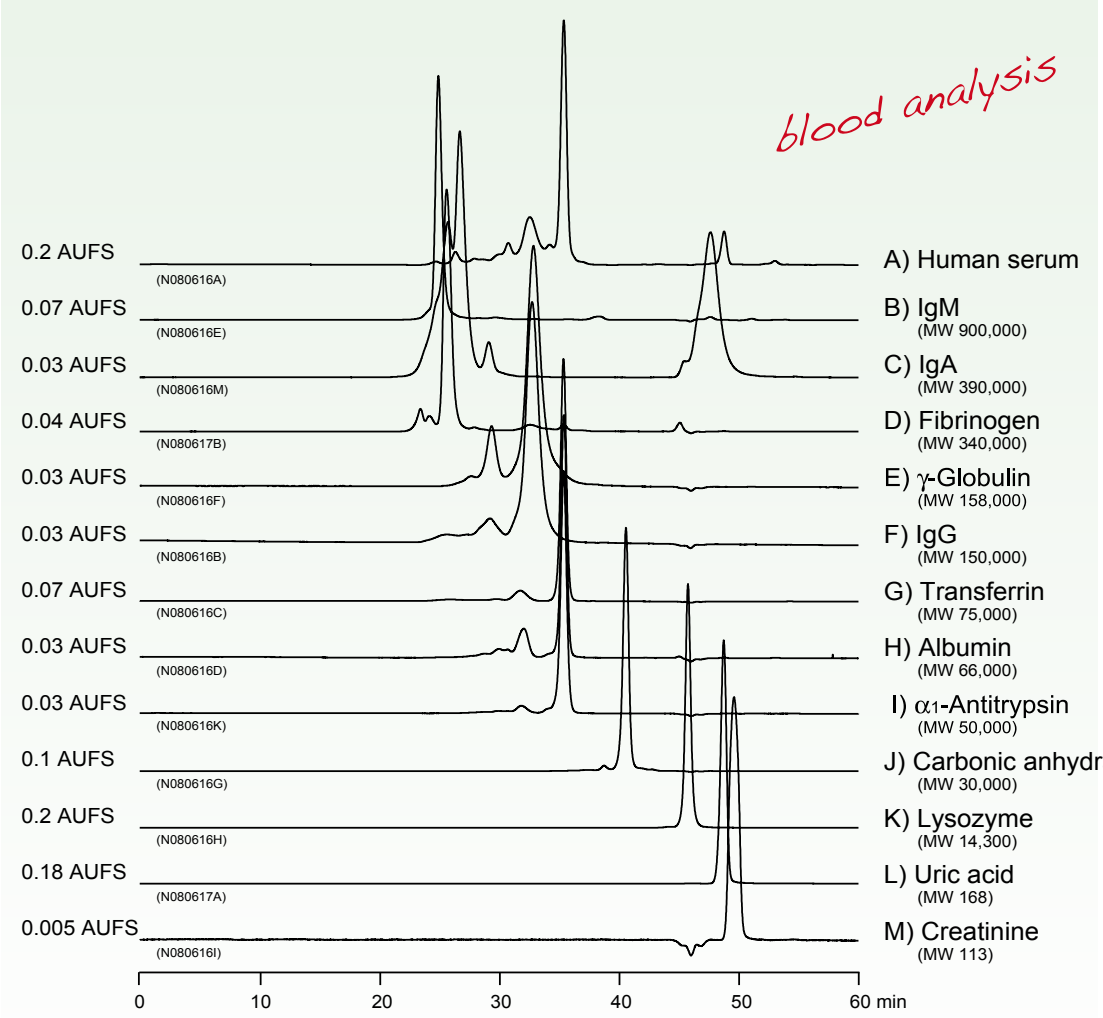



Eluent: 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) containing 0.2 M NaCl
Flow rate: 0.5 ml/min
Temperature: ambient (25 °C)
Detection: UV at 280 nm
Injection: 20 µl
Sample: Human serum (100 µl/ml)

* Application data by courtesy YMC Co., Ltd.

## SEC Applications for YMC-Pack Diol

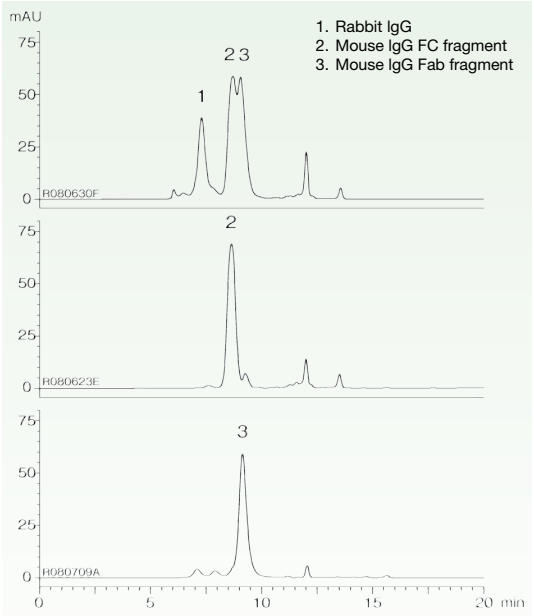
### Plasma constituents




| | | | |
|---|---|---|---|
| Column: | YMC-Pack Diol-300 + Diol-200, 300 x 8.0 mm ID x 2 | Temperature: | ambient (25 °C) |
| Part No.: | DL30S053008WT + DL20S053008WT | Detection: | UV at 280 nm |
| Eluent: | 0.1 M $KH_2PO_4K_2HPO_4$ (pH 7.0) containing 0.2 M NaCl | Injection: | 20 µl (L: 1 µl) |
| Flow rate: | 0.5 ml/min | Sample: | A) 100 µl/ml; B-M) 1.0 mg/ml |

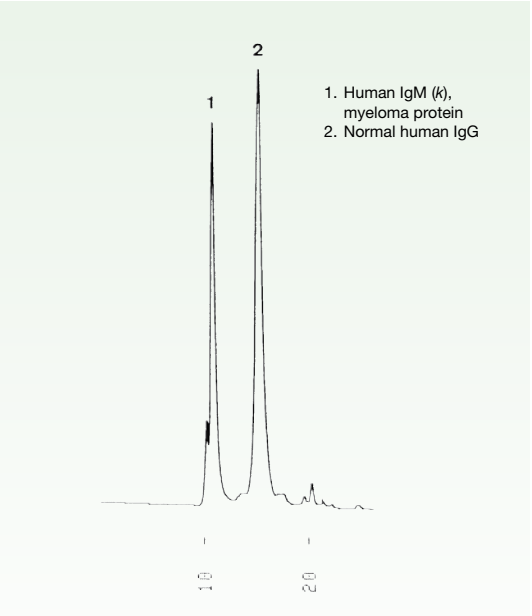
# SEC Applications for YMC-Pack Diol

## IgG, Fab and Fc fragments



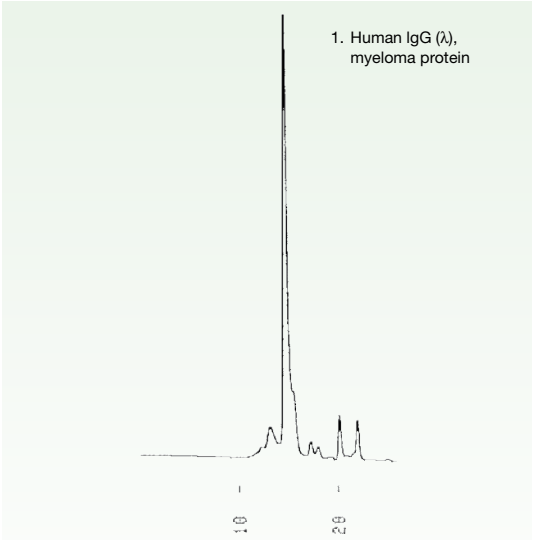

Column: YMC-Pack Diol-200, 300 x 8.0 mm ID
Part No.: DL20S053008WT
Eluent: 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 6.9) containing 0.2 M NaCl
Flow rate: 1.0 ml/min
Temperature: ambient (27 °C)
Detection: UV at 220 nm
Injection: 5 µl (0.4, 0.5 mg/ml)

## Human Immunglobulin



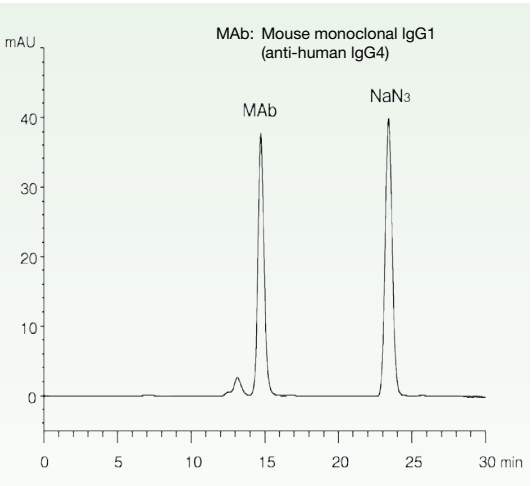

Column: YMC-Pack Diol-300, 500 x 8.0 mm ID
Part No.: DL30S055008WT
Eluent: 0.1M $NaH_2PO_4$-$Na_2HPO_4$ (pH 6.8) containing 0.1M $Na_2SO_4$
Flow rate: 1.0 ml/min
Temperature: ambient (24 °C)
Detection: UV at 280 nm, 0.04 AUFS
Injection: 40 µl (0.5 mg/ml)

## Human IgG (λ), myeloma protein




Column: YMC-Pack Diol-300 + Diol-200, 500 x 8.0 mm ID x 2
Part No.: DL30S055008WT + DL20S055008WT
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

## Monoclonal antibody (MAb)

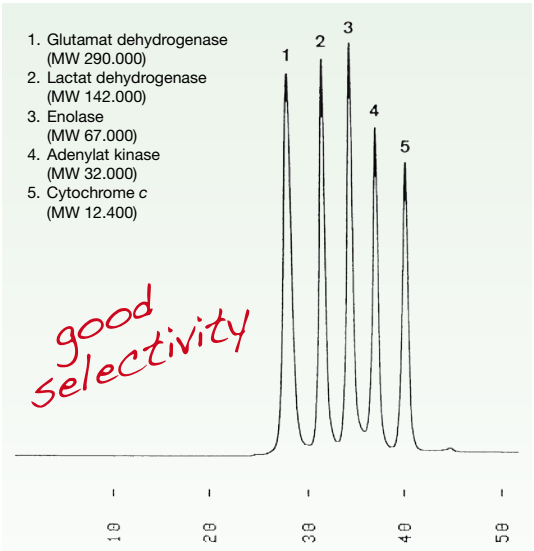



Column: YMC-Pack Diol-200, 300 x 4.6 mm ID
Part No.: DL20S053046WT
Eluent: 0.1 M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0)
Flow rate: 0.17 ml/min
Temperature: ambient (25 °C)
Detection: UV at 220 nm
Injection: 10 µl
Sample: a commercially available mouse monoclonal IgG1 (0.05 mg/ml) (purified by DEAE chromatography, containing $NaN_3$)

* Application data by courtesy YMC Co., Ltd.

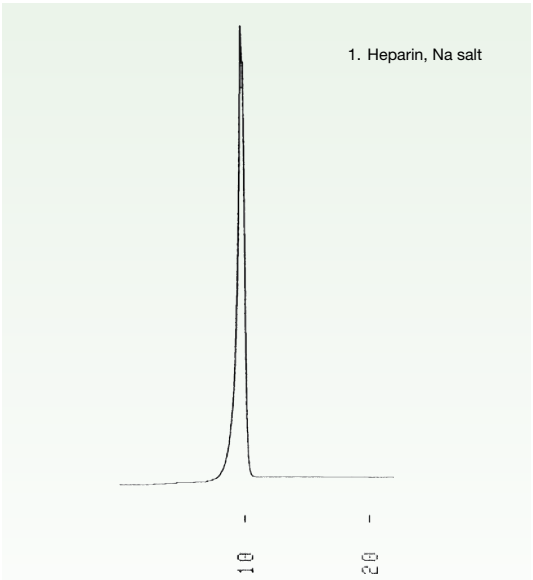
# SEC Applications for YMC-Pack Diol

## Proteins for molecular weight markers



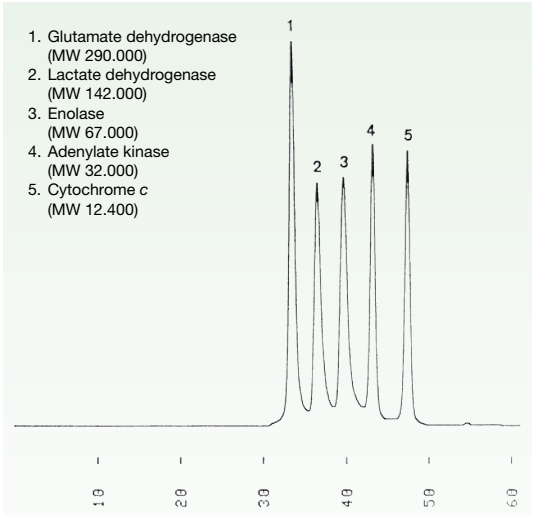

| | |
|---|---|
| Column: | YMC-Pack Diol-200, 500 x 8.0 mm ID + 300 x 8.0 mm ID |
| Part No.: | DL20S055008WT + DL20S053008WT |
| Eluent: | 0.1M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) containing 0.2M NaCl |
| Flow rate: | 0.7 ml/min |
| Temperature: | ambient (26 °C) |
| Detection: | UV at 280 nm, 0.08 AUFS |
| Injection: | 15 µl (100 µl/1 vial) |
| Sample: | MW-Marker (HPLC) manufactured by ORIENTAL YEAST CO., LTD. |

## Heparin



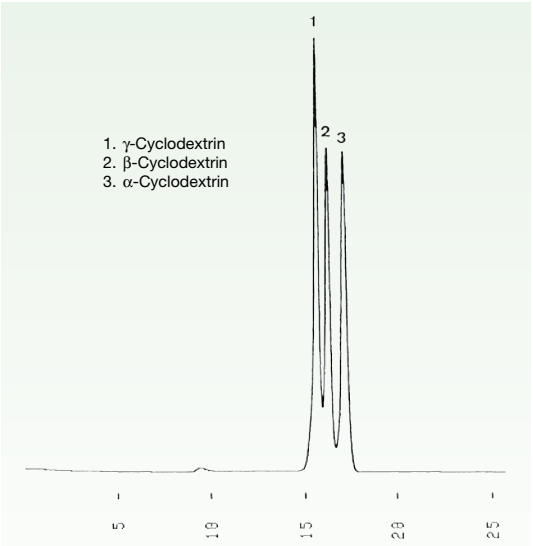

| | |
|---|---|
| Column: | YMC-Pack Diol-300, 500 x 8.0 mm ID |
| Ordering-No.: | DL30S055008WT |
| Eluent: | water |
| Flow rate: | 1.0 ml/min |
| Temperature: | ambient (24 °C) |
| Detection: | RI, 32 x $10^{-6}$ RIU/FS |
| Injection: | 20 µl (5.0 mg/ml) |

## Proteins for molecular weight markers




| | |
|---|---|
| Column: | YMC-Pack Diol-120 + Diol-300, 500 x 8.0 mm ID x 2 |
| Part No.: | DL12S055008WT + DL30S055008WT |
| Eluent: | 0.1M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) containing 0.2M NaCl |
| Flow rate: | 0.7 ml/min |
| Temperature: | ambient (26 °C) |
| Detection: | UV at 280 nm, 0.08 AUFS |
| Injection: | 15 µl (100 µl/1 vial) |
| Sample: | MW-Marker (HPLC) manufactured by ORIENTAL YEAST CO., LTD. |

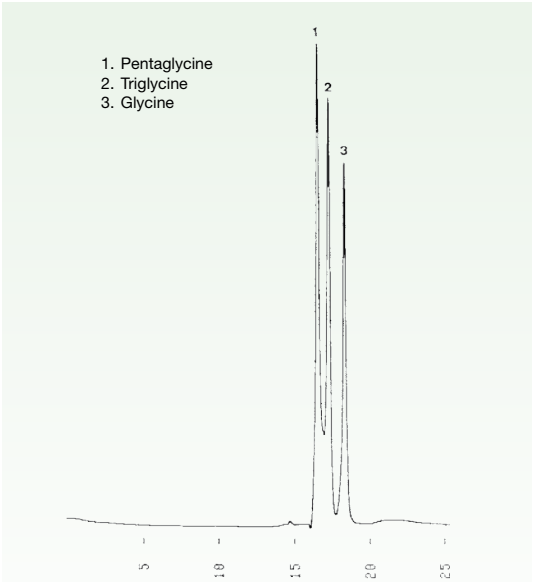
## Cyclodextrins




| | |
|---|---|
| Column: | YMC-Pack Diol 60, 500 x 8.0 mm ID |
| Ordering-No.: | DL06S055008WT |
| Eluent: | water |
| Flow rate: | 1.0 ml/min |
| Temperature: | ambient (24 °C) |
| Detection: | RI 32 x 10-6 RIU/FS |
| Injection: | 30 µl (1.67 mg/ml) |

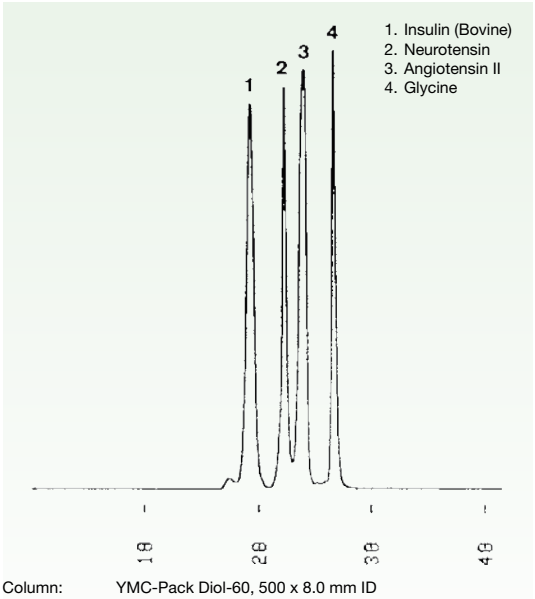
# SEC Applications for YMC-Pack Diol

## Glycine oligomers



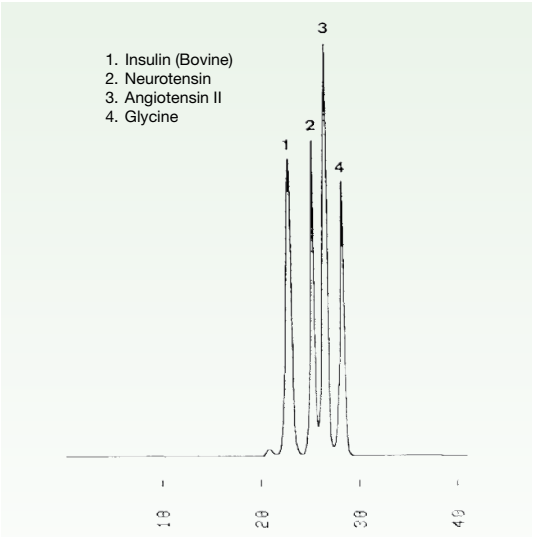

Column: YMC-Pack Diol-60, 500 x 8.0 mm ID
Ordering-No.: DL06S055008WT
Eluent: 0.1M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) / acetonitrile (70/30)
Flow rate: 1.0 ml/min
Temperature: ambient (24 °C)
Detection: UV at 215 nm, 0.08 AUFS
Injection: 20 µl (0.25 ~ 2.5 mg/ml)

## Peptides



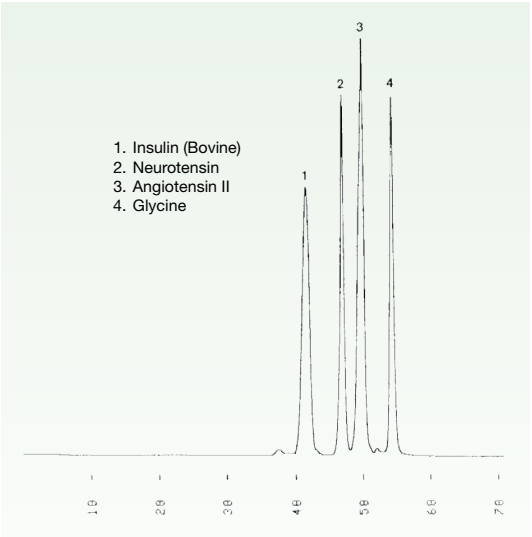

Column: YMC-Pack Diol-60, 500 x 8.0 mm ID
Ordering-No.: DL06S055008WT
Eluent: 0.1M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) / containing 0.2 M NaCl / acetonitrile (70/30)
Flow rate: 0.7 ml/min
Temperature: ambient (25 °C)
Detection: UV at 215 nm, 0.16 AUFS
Injection: 25 µl (0.07 ~ 5.3 mg/ml)

## Peptides




Column: YMC-Pack Diol-120, 500 x 8.0 mm ID
Ordering-No.: DL12S055008WT
Eluent: 0.1M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) / containing 0.2 M NaCl / acetonitrile (70/30)
Flow rate: 0.7 ml/min
Temperature: ambient (25 °C)
Detection: UV at 215 nm, 0.16 AUFS
Injection: 25 µl (0.07 ~ 5.3 mg/ml)

## Peptides



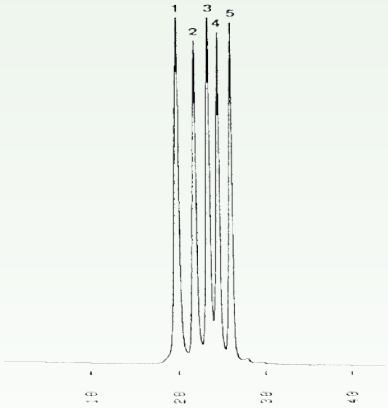

Column: YMC-Pack Diol-120 + 60, 500 x 8.0 mm ID x 2
Ordering-No.: DL12S055008WT + DL06S055008WT
Eluent: 0.1M $KH_2PO_4$-$K_2HPO_4$ (pH 7.0) / containing 0.2 M NaCl / acetonitrile (70/30)
Flow rate: 0.7 ml/min
Temperature: ambient (25 °C)
Detection: UV at 215 nm, 0.16 AUFS
Injection: 25 µl (0.07 ~ 5.3 mg/ml)

* Application data by courtesy YMC Co., Ltd.

# SEC Applications for YMC-Pack Diol

## Proteins for molecular weight markers

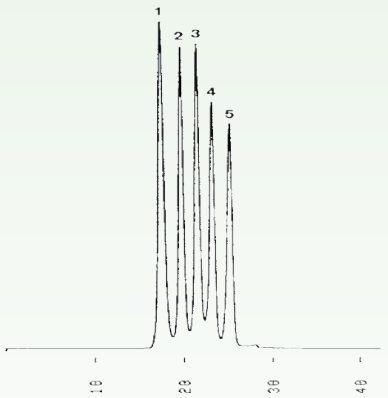
1. Glutamate dehydrogenase (MW 290,000)
2. Lactate dehydrogenase (MW 142,000)
3. Enolase (MW 67,000)
4. Adenylate kinase (MW 32,000)
5. Cytochrome c (MW 12,400)




| | |
|---|---|
| Column: | YMC-Pack Diol-300, 500 x 8.0 mm ID |
| Part No.: | DL30S055008WT |
| 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 (26 °C) |
| Detection: | UV at 280 nm, 0.08 AUFS |
| Injection: | 15 µl (100 µl / 1 vial) |
| Sample: | MW-Marker (HPLC), manufactured by ORIENTAL YEAST CO., LTD. |

## Proteins for molecular weight markers

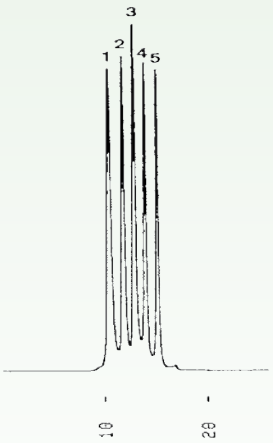
1. Glutamate dehydrogenase (MW 290,000)
2. Lactate dehydrogenase (MW 142,000)
3. Enolase (MW 67,000)
4. Adenylate kinase (MW 32,000)
5. Cytochrome c (MW 12,400)




| | |
|---|---|
| Column: | YMC-Pack Diol-200, 500 x 8.0 mm ID |
| Part No.: | DL20S055008WT |
| 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 (26 °C) |
| Detection: | UV at 280 nm, 0.08 AUFS |
| Injection: | 15 µl (100 µl / 1 vial) |
| Sample: | MW-Marker (HPLC), manufactured by ORIENTAL YEAST CO., LTD. |

## Proteins for molecular weight markers

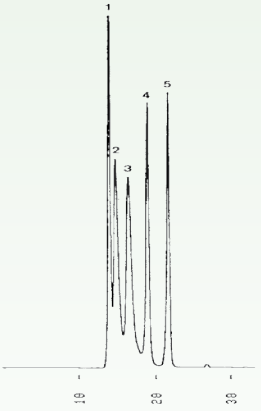
1. Glutamate dehydrogenase (MW 290,000)
2. Lactate dehydrogenase (MW 142,000)
3. Enolase (MW 67,000)
4. Adenylate kinase (MW 32,000)
5. Cytochrome c (MW 12,400)




| | |
|---|---|
| Column: | YMC-Pack Diol-200, 300 x 8.0 mm ID |
| Part No.: | DL20S053008WT |
| 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 (26 °C) |
| Detection: | UV at 280 nm, 0.08 AUFS |
| Injection: | 15 µl (100 µl / 1 vial) |
| Sample: | MW-Marker (HPLC), manufactured by ORIENTAL YEAST CO., LTD. |

## Proteins for molecular weight markers

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




| | |
|---|---|
| Column: | YMC-Pack Diol-120, 500 x 8.0 mm ID |
| Part No.: | DL12S055008WT |
| 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 (26 °C) |
| Detection: | UV at 280 nm, 0.08 AUFS |
| Injection: | 15 µl (100 µl / 1 vial) |
| Sample: | MW-Marker (HPLC), manufactured by ORIENTAL YEAST CO., LTD. |

# Ordering Information

## YMC-Pack Diol, 6 nm, 5 µm

| | Column ID (mm) | | | |
|---|---|---|---|---|
| **Column length** | **4.6** | **6.0** | **8.0** | **10.0** |
| 250 mm | DL06S052546WT | DL06S052506WT | n.a. | DL06S052510WT |
| 300 mm | DL06S053046WT | DL06S053006WT | DL06S053008WT | DL06S053010WT |
| 500 mm | n.a. | DL06S055006WT | DL06S055008WT | DL06S055010WT |

## YMC-Pack Diol, 12 nm, 5 µm

| | Column ID (mm) | | | |
|---|---|---|---|---|
| **Column length** | **4.6** | **6.0** | **8.0** | **10.0** |
| 250 mm | DL12S052546WT | DL12S052506WT | n.a. | DL12S052510WT |
| 300 mm | DL12S053046WT | DL12S053006WT | DL12S053008WT | DL12S053010WT |
| 500 mm | n.a. | DL12S055006WT | DL12S055008WT | DL12S055010WT |

## YMC-Pack Diol, 20 nm, 5 µm

| | Column ID (mm) | | | |
|---|---|---|---|---|
| **Column length** | **4.6** | **6.0** | **8.0** | **10.0** |
| 250 mm | DL20S052546WT | DL20S052506WT | n.a. | DL20S052510WT |
| 300 mm | DL20S053046WT | DL20S053006WT | DL20S053008WT | DL20S053010WT |
| 500 mm | n.a. | DL20S055006WT | DL20S055008WT | DL20S055010WT |

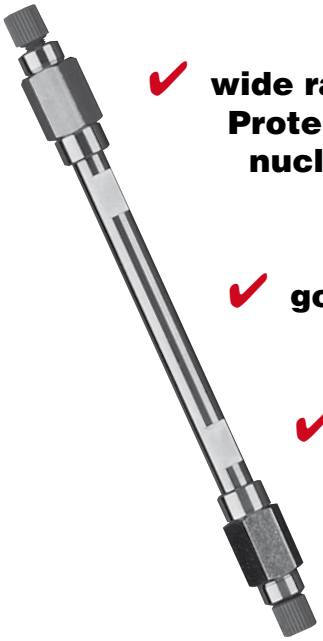
## YMC-Pack Diol, 30 nm, 5 µm

| | Column ID (mm) | | | |
|---|---|---|---|---|
| **Column length** | **4.6** | **6.0** | **8.0** | **10.0** |
| 250 mm | DL30S052546WT | DL30S052506WT | n.a. | DL30S052510WT |
| 300 mm | DL30S053046WT | DL30S053006WT | DL30S053008WT | DL30S053010WT |
| 500 mm | n.a. | DL30S055006WT | DL30S055008WT | DL30S055010WT |

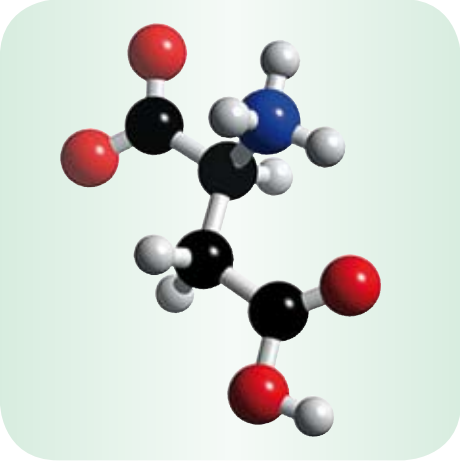
Guard Columns are available for the different column dimensions.
For more details please contact us: Phone 02064-427-0 or email info@ymc.de.

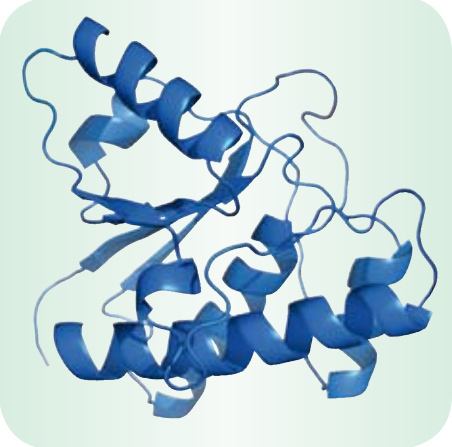
* Application data by courtesy YMC Co., Ltd.

# YMC SEC columns provide:



- ✔ **wide range of applications: Proteins, peptides, carbohydrates and nucleic acid components**
- ✔ **good cost/performance ratio**
- ✔ **scalability: from 5 µm to 75 µm**
- ✔ **minimal secondary interactions**





RP

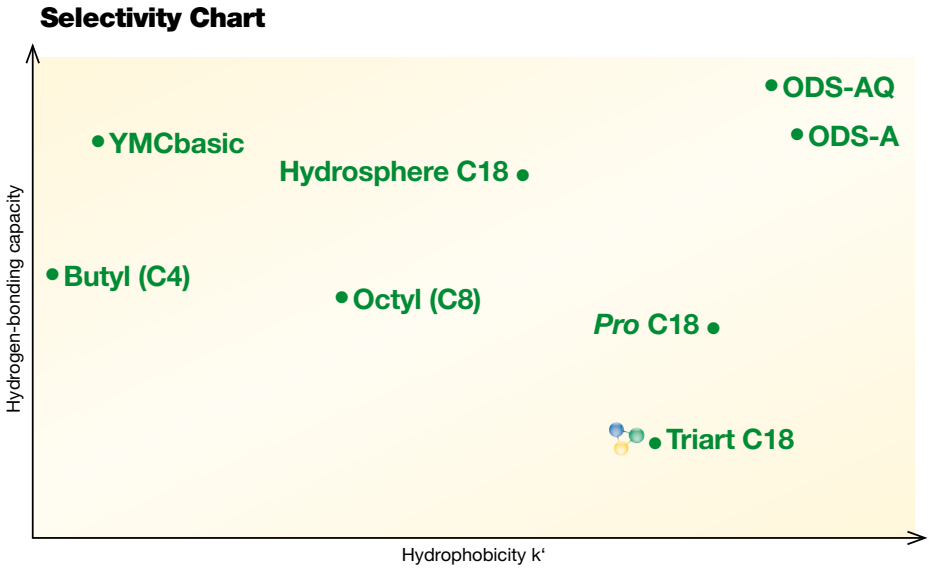
# Bioseparation columns

### C18-Selectivities for peptides

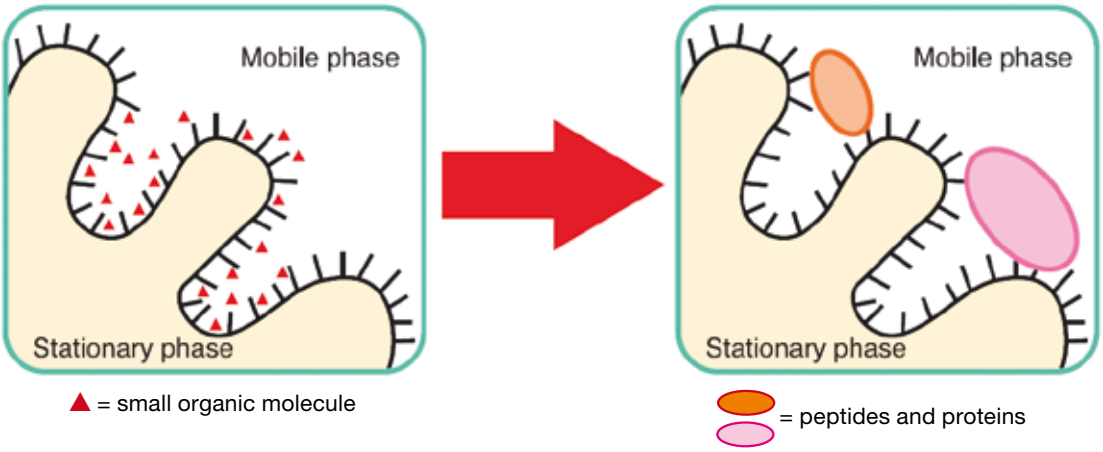
| | YMC-Triart C18 | YMC-Pack *Pro* C18 | YMC-Pack ODS-A | YMC-Pack ODS-AQ | Hydrosphere C18 |
|---|---|---|---|---|---|
| Particle size / µm | 2; 3; 5 | 2; 3; 5 | 3; 5 | 3; 5 | 2; 3; 5 |
| Pore size / nm | 12 | 12 | 12; 20; 30 | 12; 20 | 12 |
| Carbon content / % | 20 | 17 | 17; 12; 7 | 14; 10 | 12 |
| pH range | 1.0 - 12.0 | 2.0 - 8.0 | 2.0 - 7.5 | 2.0 - 7.5 | 2.0 - 8.0 |

### C8- and C4-Selectivities for peptides and proteins

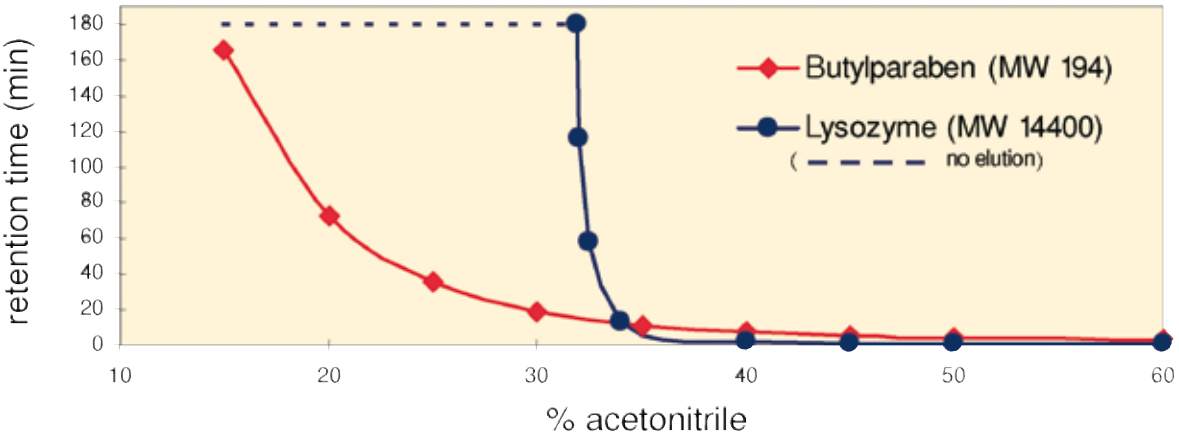
| | YMC-Pack C8 | YMCbasic | YMC-Pack C4 | YMC-Pack Protein RP |
|---|---|---|---|---|
| Particle size / µm | 3; 5 | 3; 5 | 3; 5 | 5 |
| Pore size / nm | 12; 20; 30 | proprietary | 12; 20; 30 | proprietary |
| Carbon content / % | 10; 7; 4 | 7 | 7; 5; 3 | 4 |
| pH range | 2.0 - 7.5 | 2.0 - 7.5 | 2.0 - 7.5 | 1.5 - 7.5 |



## Retention mechanism for peptides and proteins




## ON–OFF mechanism




By monitoring the dependency between the retention time of analytes and the percentage of organic solvent being used, an interesting difference in behaviour between small organic molecules and proteins is revealed.

Small organic molecules such as butylparaben are retained/eluted by a distribution mechanism as shown in the linear relationship between retention time and percentage of organic modifier. This is in sharp contrast to the retention behaviour of peptides and proteins, e.g. lysozyme. These are retained/eluted by an adsorption-desorption (on-off) mechanism and as a result the pore size has to be carefully considered.

* Application data by courtesy YMC Co., Ltd.

## Chromatographers know the problems during method development: “Which phase is suitable and allows a simple and robust separation?”

In the field of biochromatography, phase selection is a key to success!

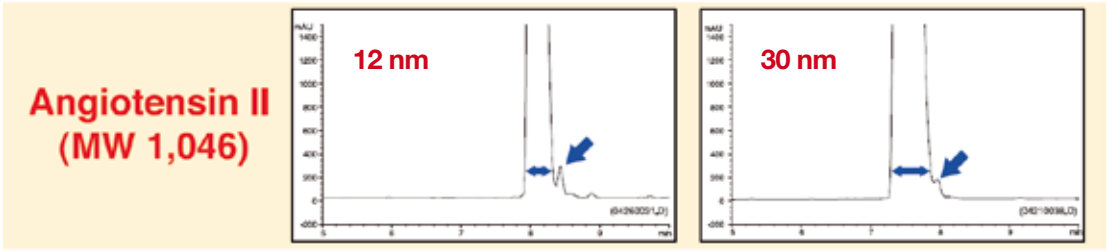
With the YMC’s ”Column Selection Tool“ for Bio-LC, stationary phase selection is almost too easy.

As shown in the table (below), the C18 column with 12 nm pore size is suitable for small peptides up to a MW 5000. The best efficiency for large peptides or small proteins can be obtained by employing a C8 phase characterised by a 20 nm pore size.

Furthermore, most proteins are eluted effectively by a C4 column with 30 nm.

However, the separation may also be influenced by the hydrophobicity of the peptide/protein and the nature of the column’s bonded phase. Therefore, for initial method development, it might be useful, in the first instance, to follow the arrow shown in the table for method optimisation.

### Comparision of peaks on C4 with 12 nm and 30 nm pore sizes




**For smaller peptides a small pore size is more successful. Larger molecules are separated much better with larger pore sizes!**

### Column Selection Tool*

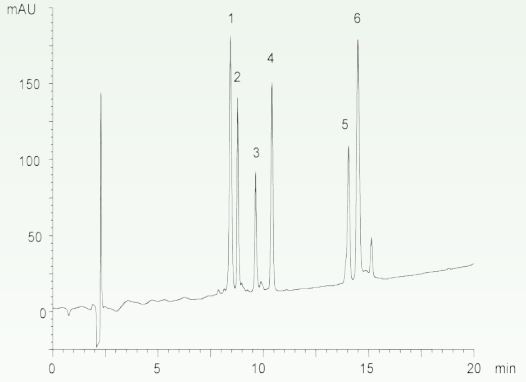
| MW | | C18 | C8 | C4 |
|---|---|---|---|---|
| 5000 | 12 nm | ◎ | ○ | △ |
| 20000 | 20 nm | ○ | ◎ | ○ |
| 100000 | 30 nm | △ | ○ | ◎ |

◎: excellent, ○: good, △: moderate

# Peptide and Protein Applications

## Peptides and Proteins

1. Cytochrome c (Horse heart) MW 12,400
2. Insulin (Bovine pancreas) MW 5,733
3. Amyloid β-protein (1-40) MW 4,330
4. Lysozyme (Chicken egg white) MW 14,300
5. α-Lactalbumin (Human milk) MW 14,100
6. Myoglobin (Horse skeletal muscle) MW 17,000

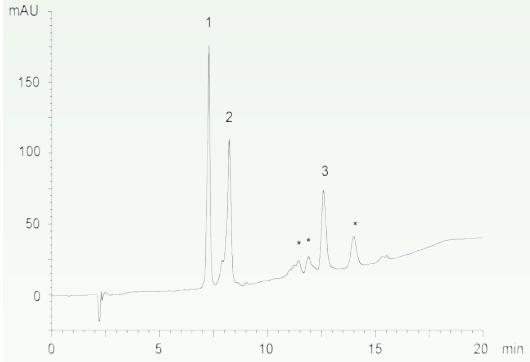



Column: YMC-Pack C8 (5 µm, 20 nm) 150 x 4.6 mm ID
Part No.: OC20S051546WT
Eluent: A) water / TFA (100/0.1)
B) acetonitrile / TFA (100/0.1)
Gradient: 25-60% B (0-20 min)
Flow rate: 1.0 ml/min
Temperature: 37 °C
Detection: UV at 220 nm
Injection: 10 µl (0.1 ~ 0.2 mg/ml)

## Proteins

1. BSA MW 66,000
2. Conalbumin (Chicken egg white) MW 77,000
3. Lipoxidase (Soybean) MW 96,000

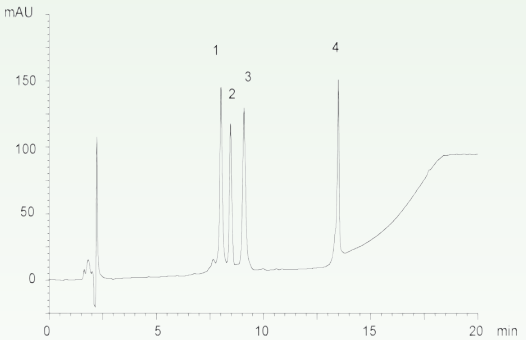
* Impurities in commercial lipoxidase




Column: YMC-Pack C4 (5 µm, 30 nm) 150 x 4.6 mm ID
Part No.: BU30S051546WT
Eluent: A) water / TFA (100/0.1)
B) acetonitrile / 2-propanol / TFA (50/50/0.1)
Gradient: 30-75% B (0-15 min), 75% B (15-20 min)
Flow rate: 1.0 ml/min
Temperature: 37 °C
Detection: UV at 220 nm
Injection: 10 µl (0.25 ~ 1.0 mg/ml)

## Proteins

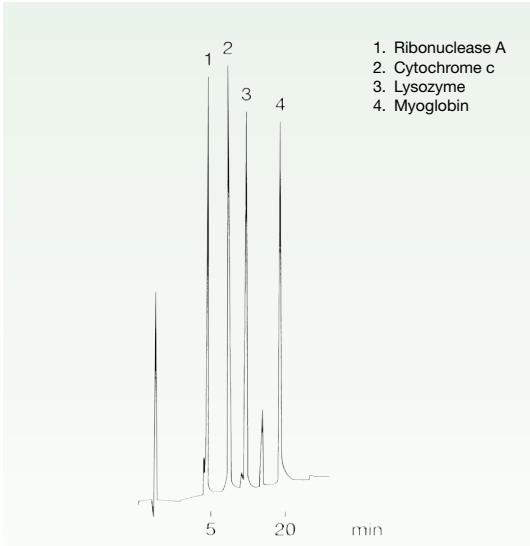
1. β-Lactoglobulin B (Bovine milk) MW 18,300
2. β-Lactoglobulin A (Bovine milk) MW 18,400
3. α-Chymotrypsinogen A (Bovine pancreas) MW 18,300
4. Ovalbumin MW 45,000




Column: YMC-Pack C4 (5 µm, 30 nm) 150 x 4.6 mm ID
Part No.: BU30S051546WT
Eluent: A) water / TFA (100/0.1)
B) acetonitrile / TFA (100/0.1)
Gradient: 40-50% B (0-10 min), 50-90% B (10-15 min), 90% B (15-20 min)
Flow rate: 1.0 ml/min
Temperature: 37 °C
Detection: UV at 220 nm
Injection: 10 µl (0.2 ~ 0.3 mg/ml)

## Proteins

1. Ribonuclease A
2. Cytochrome c
3. Lysozyme
4. Myoglobin

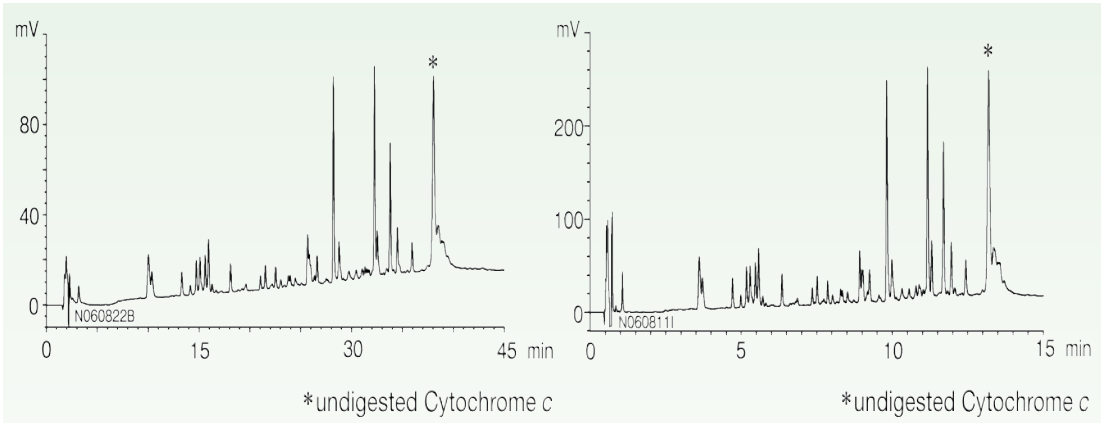



Column: YMC-Pack C4 (5 µm, 30 nm) 150 x 4.6 mm ID
Part No.: BU30S051546WT
Eluent: A) acetonitrile / water / TFA (5/95/0.1)
B) acetonitrile / water / TFA (60/40/0.1)
Gradient: 30%-90% B (0-20 min., linear), 90% B (20-50 min)
Flow rate: 1.0 ml/min
Temperature: 37 °C
Detection: UV at 220 nm

* Application data by courtesy YMC Co., Ltd.

## Peptide and Protein Applications

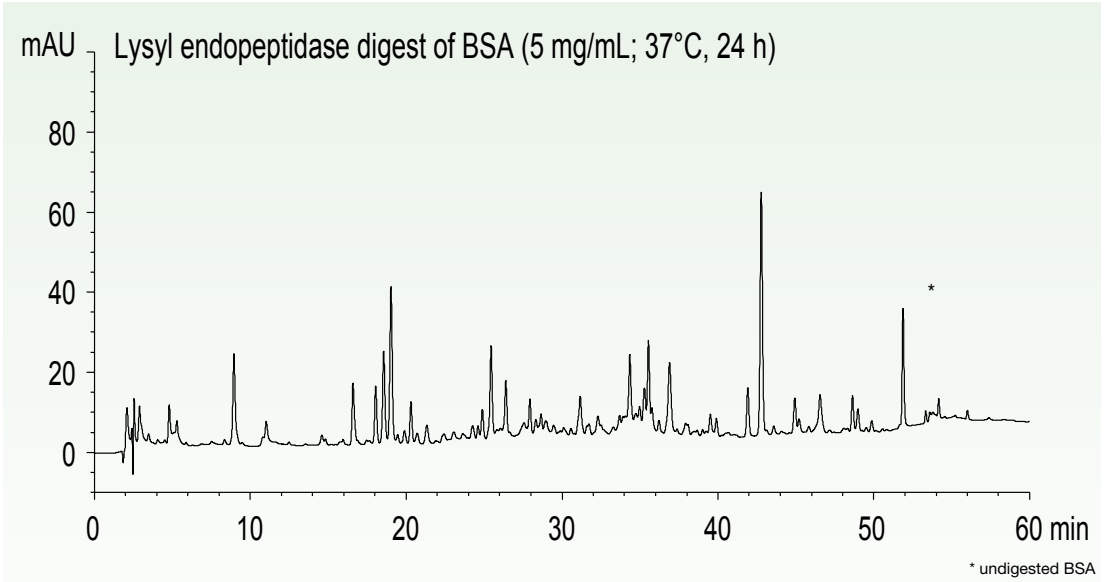
### Peptide mapping - excellent reproducibility between 5 µm and 2 µm




| | | | |
|---|---|---|---|
| Column: | YMC-UltraHT *Pro* C18 (5 µm, 12 nm) 150 × 2.0 mm ID | | |
| Part No.: | AS12S051502QT | | |
| Eluent: | A) acetonitrile/water/trifluoroacetic acid (10/90/0.1) | | |
| | B) acetonitrile/water/trifluoroacetic acid (35/65/0.1) | | |
| Gradient: | Time | A (in %) | B (in %) |
| | 0 | 100 | 0 |
| | 5 | 100 | 0 |
| | 40 | 0 | 100 |
| | 45 | 0 | 100 |
| Flow rate: | 0.2 ml/min | | |
| Temperature: | 37 °C | | |
| Detection: | UV at 220 nm | | |
| Injection: | 1 µl | | |
| Sample: | Tryptic digest of Cytochrome c | | |

| | | | |
|---|---|---|---|
| Column: | YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 × 2.0 mm ID | | |
| Part No.: | AS12S020502WT | | |
| Eluent: | A) acetonitrile/water/trifluoroacetic acid (10/90/0.1) | | |
| | B) acetonitrile/water/trifluoroacetic acid (35/65/0.1) | | |
| Gradient: | Time | A (in %) | B (in %) |
| | 0 | 100 | 0 |
| | 1.65 | 100 | 0 |
| | 13.35 | 0 | 100 |
| | 15.00 | 0 | 100 |
| Flow rate: | 0.2 ml/min | | |
| Temperature: | 37 °C | | |
| Detection: | UV at 220 nm | | |
| Injection: | 1 µl | | |
| Sample: | Tryptic digest of Cytochrome c | | |

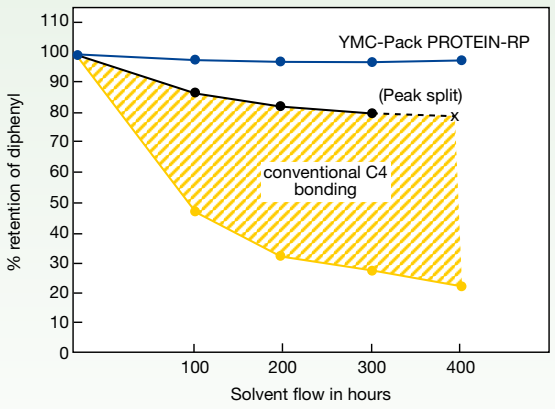
### Peptides mapping

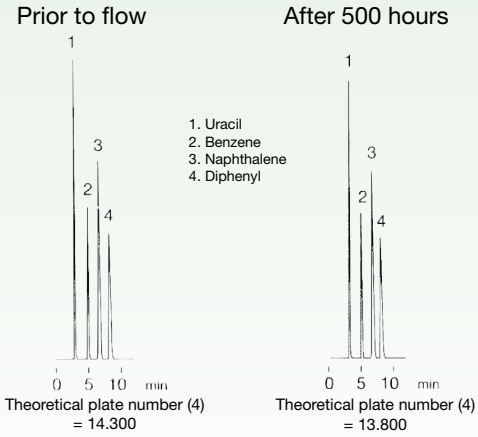



| | |
|---|---|
| Column: | YMCbasic (5 µm) 150 x 2.0 mm ID |
| Part No.: | BA99S051502WT |
| Eluent: | A) water / TFA (94/6) B) acetonitrile / TFA (100/0.1) |
| Gradient: | 5-35% B (0-50 min), 35-45% B (50-55 min), 45% B (55-60 min) |
| Flow rate: | 0.2 ml/min |
| Temperature: | 37 °C |
| Detection: | UV at 220 nm |
| Injection: | 1 µl |

# Peptide and Protein Applications

## Improved durability when used with TFA solution




Theoretical plate number (4) = 14.300

Theoretical plate number (4) = 13.800

*Flow conditions*

| | |
|---|---|
| Eluent: | water / TFA (100/0.1) |
| Flow rate: | 1.0 ml/min |
| Temperature: | ambient |

*Measurement conditions*

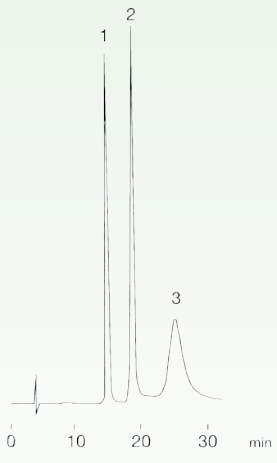
| | |
|---|---|
| Column: | YMC-Pack Protein-RP (5 µm) 250 x 4.6 mm ID |
| Part No.: | PR99S052546WT |
| Eluent: | acetonitrile / water (40/60) |
| Flow rate: | 1.0 ml/min |
| Temperature: | 30° C |
| Detection: | UV at 254 nm, 0.32 AUFS |

The selectivity of YMC-Pack Protein-RP is different from that seen with conventional wide pore butyl phases and it is specifically suited for the protein analysis.

In the applications below it effectively separates both low molecular weight compounds and high molecular weight proteins, with equally good peak shapes being obtained.

## Proteins

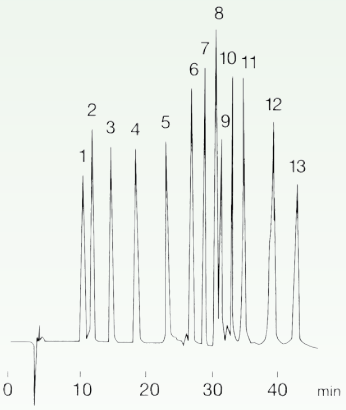
1. Cytochrome c (MW: 12.400, 5 µg)
2. BSA (MW: 66.000, 25 µg)
3. Ferritin (MW: 440.000,10 µg)




| | |
|---|---|
| Column: | YMC-Pack Protein-RP (5 µm) 250 x 4.6 mm ID |
| Part No.: | PR99S052546WT |
| Eluent: | A) water / TFA (100/0.1)<br>B) acetronitrile / TFA (100/0.1) |
| Gradient: | 30%-90% B (0-45 min., linear) |
| Flow rate: | 1.0 ml/min |
| Temperature: | ambient |
| Detection: | UV at 280 nm, 0.04 AUFS |

## Proteins and peptides

1. Met-Enkephalin
2. Leu-Enkephalin
3. Oxytocin
4. Bradykinin
5. Angiotensin I
6. Ribonuclease A
7. α-Mating factor
8. Insulin (bovine)
9. Cytochrome c
10. Lysozyme
11. BSA
12. β-Lactoglobulin
13. Ovalbumin

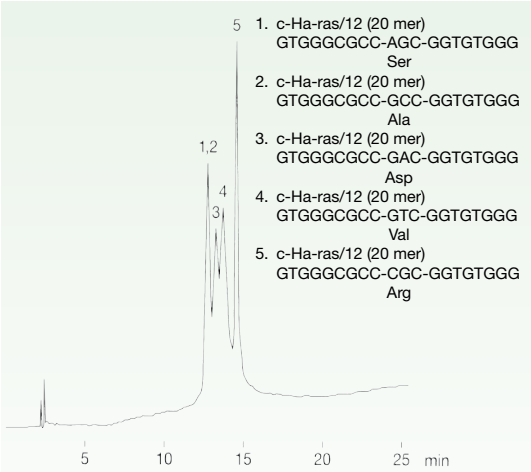



| | |
|---|---|
| Column: | YMC-Pack Protein-RP (5 µm) 250 x 4.6 mm ID |
| Part No.: | PR99S052546WT |
| Eluent: | A) water / TFA (100/0.1)<br>B) acetronitrile / TFA (100/0.1) |
| Gradient: | 10%-90% B (0-60 min., linear) |
| Flow rate: | 1.0 ml/min |
| Temperature: | ambient |
| Detection: | UV at 220 nm, 0.32 AUFS |

* Application data by courtesy YMC Co., Ltd.

# Oligonucleotide Applications

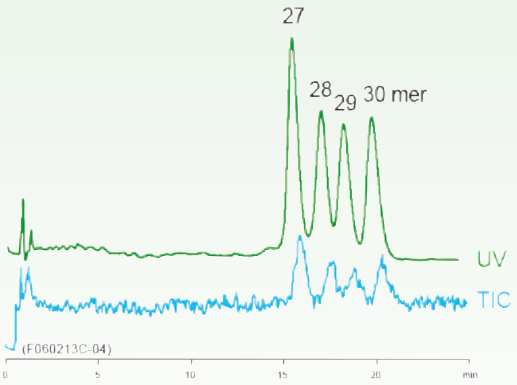
## Oligonucleotides




| | |
|---|---|
| Column: | YMC-Pack ODS-A (5 µm, 30 nm) 150 x 4.6 mm ID |
| Part No.: | AA30S051546WT |
| Eluent: | A) 100 mM Triethylamine-acetic acid (pH 6.5) / acetonitrile (94/6)<br>B) 100 mM Triethylamine-acetic acid (pH 6.5) / acetonitrile (85/15) |
| Gradient: | 0%-100% B (0-25 min, linear) |
| Flow rate: | 1.0 ml/min |
| Temperature: | 30 °C |
| Detection: | UV at 260 nm |

## LC-MS analysis of synthetic 27-30 mer oligonucleotides

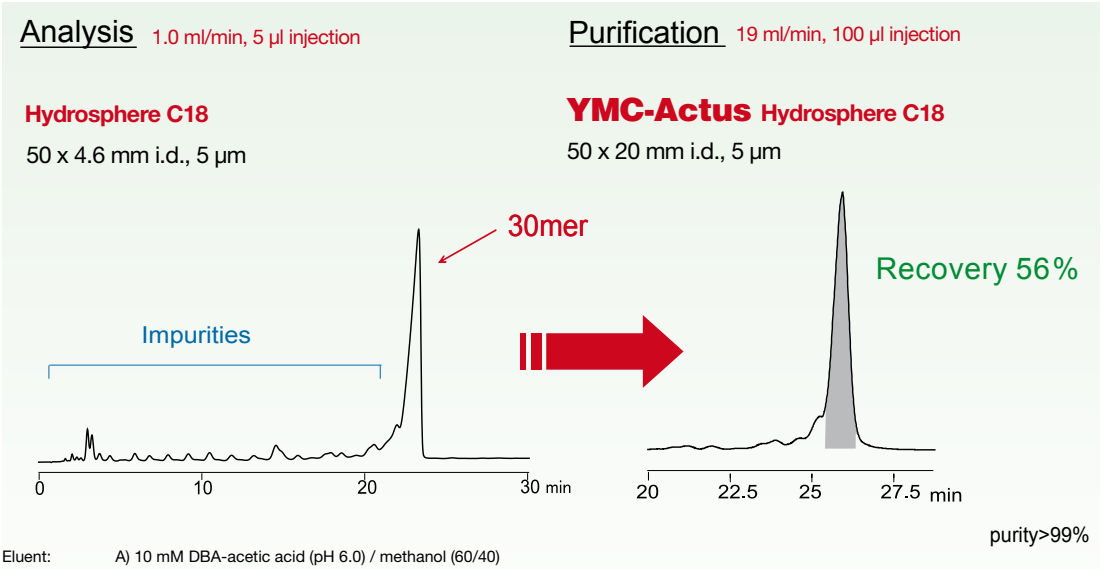
Sample: Primer of DNA sequencing
5'-CCGCTCGAGCTAAAAAAAGCCTGTGTTACC-3' (30 mer)




| | |
|---|---|
| Column: | YMC Hydrosphere C18, 50 x 2.0 mm ID, 3 µm |
| Part No.: | HS12S030502WT |
| Eluent: | A) 10 mM DBAA (pH 6.0)<br>B) Mobile phase A / acetonitrile (50/50) |
| Gradient: | 58%-62% B (0-20 min), 62% B (20-25 min) |
| Flow rate: | 0.2 ml/min |
| Temperature: | 35 °C |
| Detection: | UV at 269 nm and ESI negative-mode |
| Injection: | 1 µl (10 pmol/component) |

# Outstanding separation of highly polar compounds

## Crude synthetic 30mer oligonucleotide




| | |
|---|---|
| Eluent: | A) 10 mM DBA-acetic acid (pH 6.0) / methanol (60/40)<br>B) 10 mM DBA-acetic acid (pH 6.0) / methanol (20/80) |
| Gradient: | 10%-35% B (0-30 min.) |
| Temperature: | ambient |
| Detection: | UV at 269 nm |
| Sample: | synthetic oligonucleotide (100 µM) |

# Ordering information

## 2 µm analytical columns, 2.0 mm ID, C18

| Phase | Column dimension | | | |
|---|---|---|---|---|
| | 30 x 2.0 mm ID | 50 x 2.0 mm ID | 100 x 2.0 mm ID | 150 x 2.0 mm ID |
| YMC-UltraHT *Pro* C18 | AS12S020302WT | AS12S020502WT | AS12S021002WT | AS12S021502WT |
| YMC-UltraHT Hydrosphere C18 | HS12S020302WT | HS12S020502WT | HS12S021002WT | HS12S021502WT |

## 2 µm analytical columns, 3.0 mm ID, C18

| Phase | Column dimension | | | |
|---|---|---|---|---|
| | 30 x 3.0 mm ID | 50 x 3.0 mm ID | 100 x 3.0 mm ID | 150 x 3.0 mm ID |
| YMC-UltraHT *Pro* C18 | AS12S020303WT | AS12S020503WT | AS12S021003WT | AS12S021503WT |
| YMC-UltraHT Hydrosphere C18 | HS12S020303WT | HS12S020503WT | HS12S021003WT | HS12S021503WT |

## 3 µm analytical columns, C18

| Phase | Column dimension | | | | |
|---|---|---|---|---|---|
| | 150 x 2.0 mm ID | 150 x 3.0 mm ID | 150 x 4.6 mm ID | 250 x 3.0 mm ID | 250 x 4.6 mm ID |
| YMC-Pack ODS-A (C18) | AA12S031502WT | AA12S031503WT | AA12S031546WT | AA12S032503WT | AA12S032546WT |
| YMC-Pack ODS-AQ (C18) | AQ12S031502WT | AQ12S031503WT | AQ12S031546WT | AQ12S032503WT | AQ12S032546WT |
| YMC-Pack *Pro* C18 | AS12S031502WT | AS12S031503WT | AS12S031546WT | AS12S032503WT | AS12S032546WT |
| Hydrosphere C18 | HS12S031502WT | HS12S031503WT | HS12S031546WT | HS12S032503WT | HS12S032546WT |
| YMC-Triart C18 | TA12S031502WT | TA12S031503WT | TA12S031546WT | TA12S032503WT | TA12S032546WT |

## 3 µm analytical columns, C8 & C4

| Phase | Column dimension | | | | |
|---|---|---|---|---|---|
| | 150 x 2.0 mm ID | 150 x 3.0 mm ID | 150 x 4.6 mm ID | 250 x 3.0 mm ID | 250 x 4.6 mm ID |
| YMC-Pack Octyl (C8) | OC12S031502WT | OC12S031503WT | OC12S031546WT | OC12S032503WT | OC12S032546WT |
| YMCbasic (eq. C8) | BA12S031502WT | BA12S031503WT | BA12S031546WT | BA12S032503WT | BA12S032546WT |
| YMC-Pack Butyl (C4) | BU12S031502WT | BU12S031503WT | BU12S031546WT | BU12S032503WT | BU12S032546WT |

Other dimensions are also avialable. Please contact us: Phone +49(0)2064/427-0 or E-Mail: info@ymc.ded.

# Ordering information

## 5 µm analytical columns, C18

| Phase | Column dimension | | | | |
|---|---|---|---|---|---|
| | 150 x 2.0 mm ID | 150 x 3.0 mm ID | 150 x 4.6 mm ID | 250 x 3.0 mm ID | 250 x 4.6 mm ID |
| YMC-Pack ODS-A (C18) | AA12S051502WT | AA12S051503WT | AA12S051546WT | AA12S052503WT | AA12S052546WT |
| YMC-Pack ODS-AQ (C18) | AQ12S051502WT | AQ12S051503WT | AQ12S051546WT | AQ12S052503WT | AQ12S052546WT |
| YMC-Pack *Pro* C18 | AS12S051502WT | AS12S051503WT | AS12S051546WT | AS12S052503WT | AS12S052546WT |
| Hydrosphere C18 | HS12S051502WT | HS12S051503WT | HS12S051546WT | HS12S052503WT | HS12S052546WT |
| YMC-Triart C18 | TA12S051502WT | TA12S051503WT | TA12S051546WT | TA12S052503WT | TA12S052546WT |

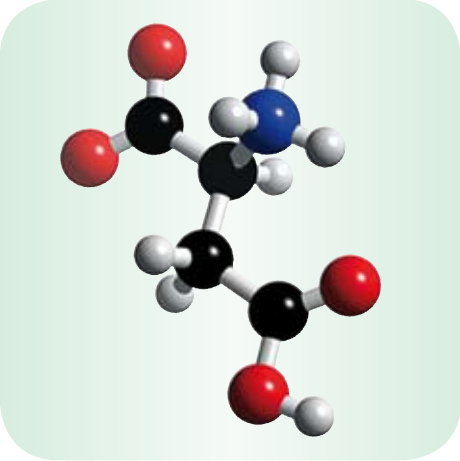
## 5 µm analytical columns, C8 & C4

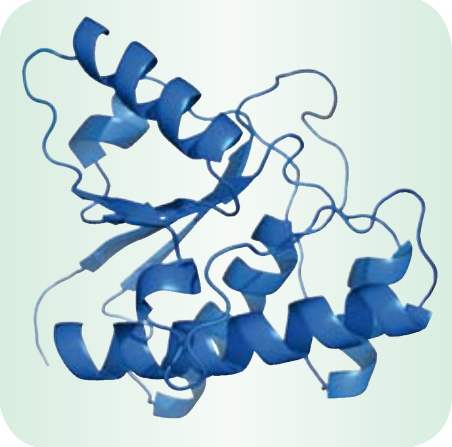
| Phase | Column dimension | | | | |
|---|---|---|---|---|---|
| | 150 x 2.0 mm ID | 150 x 3.0 mm ID | 150 x 4.6 mm ID | 250 x 3.0 mm ID | 250 x 4.6 mm ID |
| YMC-Pack Octyl (C8) | OC12S051502WT | OC12S051503WT | OC12S051546WT | OC12S052503WT | OC12S052546WT |
| YMCbasic (eq. C8) | BA12S051502WT | BA12S051503WT | BA12S051546WT | BA12S052503WT | BA12S052546WT |
| YMC-Pack Butyl (C4) | BU12S051502WT | BU12S051503WT | BU12S051546WT | BU12S052503WT | BU12S052546WT |
| YMC-Pack Protein-RP (eq. C4) | PR12S051502WT | PR12S051503WT | PR12S051546WT | PR12S052503WT | PR12S052546WT |

## 5 µm semi-preparative columns

| Phase | Column dimension | | | | |
|---|---|---|---|---|---|
| | 150 x 20 mm ID | 150 x 30 mm ID | 250 x 10 mm ID | 250 x 20 mm ID | 250 x 30 mm ID |
| YMC-Pack ODS-A (C18) | AA12S051520WT | AA12S051530WT | AA12S052510WT | AA12S052520WT | AA12S052530WT |
| YMC-Pack ODS-AQ (C18) | AQ12S051520WT | AQ12S051530WT | AQ12S052510WT | AQ12S052520WT | AQ12S052530WT |
| YMC-Pack *Pro* C18 | AS12S051520WT | AS12S051530WT | AS12S052510WT | AS12S052520WT | AS12S052530WT |
| Hydrosphere C18 | HS12S051520WT | HS12S051530WT | HS12S052510WT | HS12S052520WT | HS12S052530WT |
| YMC-Triart C18 | TA12S051520WT | TA12S051530WT | TA12S052510WT | TA12S052520WT | TA12S052530WT |
| YMC-Pack Octyl (C8) | OC12S051520WT | OC12S051530WT | OC12S052510WT | OC12S052520WT | OC12S052530WT |
| YMC-Pack Butyl (C4) | BU12S051520WT | BU12S051530WT | BU12S052510WT | BU12S052520WT | BU12S052530WT |
| YMCbasic (eq. C8) | BA12S051520WT | BA12S051530WT | BA12S052510WT | BA12S052520WT | BA12S052530WT |
| YMC-Pack Protein-RP (eq. C4) | PR12S051520WT | PR12S051530WT | PR12S052510WT | PR12S052520WT | PR12S052530WT |

Guard Columns are also available.
For more details please contact us: Phone 02064-427-0 or email info@ymc.de.





NP/
HILIC

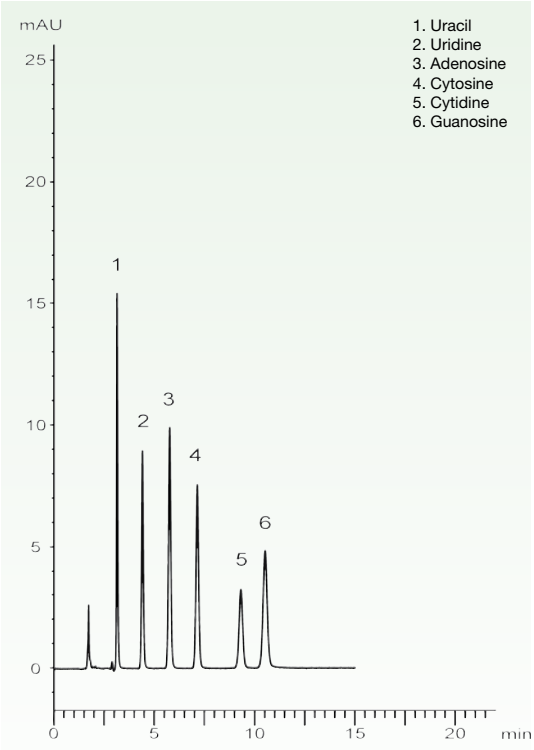
## Hydrophilic Interaction Chromatography (HILIC)

Hydrophilic Interaction Chromatography is a technique which has attracted more and more attention since it offers an alternative approach for the separation of highly polar compounds. The method itself, although it has been known for more than 25 years, has become more popular recently due to the introduction of several specialised HILIC phases. However it is not well known that HILIC separations can be accomplished using any highly polar stationary phase. This opens up a large range of materials which are suitable for HILIC separations. HILIC separations traditionally use a highly polar stationary phase and a non polar mobile phase e.g. functionalised silica with a hydrophilic coating and an acetonitrile/water mixture (90/10) for small molecules but application to larger biomolecules is becoming increasingly more popular.

## YMC Columns for HILIC:

| | YMC-Pack Silica | PVA-Sil | Polyamine II | YMC-Pack Amino | YMC-Pack Diol |
|---|---|---|---|---|---|
| **Particle size / µm** | 3; 5 | 5 | 5 | 3; 5 | 5 |
| **Pore size / nm** | 6; 12; 20; 30 | 12 | 12 | 12 | 12; 20; 30 |
| **Carbon content / %** | n/a | 4; 3 | n/a | 3 | n/a |
| **pH range** | 2.0 - 7.5 | 2.0 - 9.5 | 2.0 - 9.0 | 2.0 - 7.5 | 2.0 – 7.5 |

### Nucleosides and bases




Column: YMC-Pack Diol-NP (5µm 12nm) 150 x 2.0 mm ID)
Part No: DL12S051502WT
Eluent: water / acetonitrile (10/90) containing 10 mM $CH_3COONH_4$
Flow rate: 0.2 ml/min
Temperature: 30 °C
Detection: UV at 254 nm

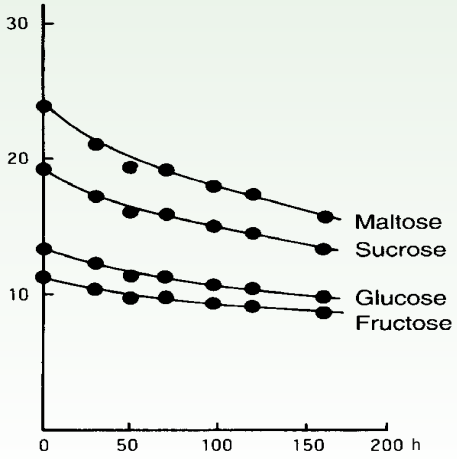
*Outstanding Retention and Resolution with YMC HILIC!*

# YMC-Pack Polyamine II

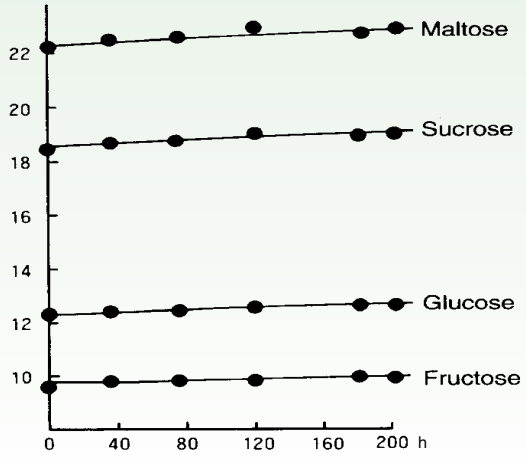
YMC-Pack Polyamine II is a unique phase, based on ultrapure YMC silica as a support material. The functionality of the stationary phase is achieved by a covalently bonded polymer layer containing secondary (2°) and tertiary (3°) amino groups. The 2° and 3° amino groups of YMC-Pack Polyamine II are only weakly nucleophilic, exhibiting a significantly reduced reactivity towards carbonyl compounds.

Therefore, unlike conventional amino phases with primary n-propylamino ligands, YMC-Pack Polyamine II does not form Schiff' bases or other stable condensation products. In addition, the 2° and 3° amino groups of the polymer layer are to a large extent resistant to oxidation and hydrolysis (as shown in the figure below).

### Stability of amino type packings



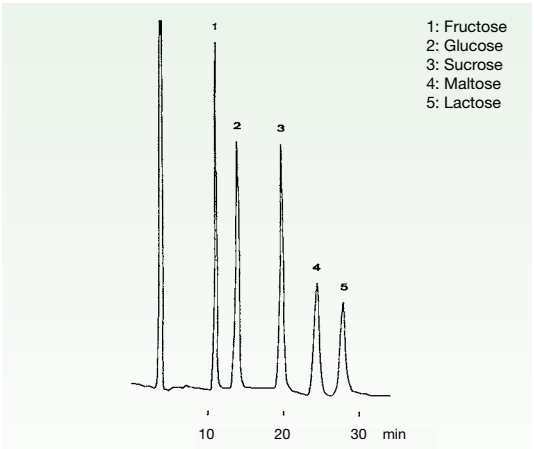

a) conventional amino phase




b) YMC-Pack Polyamine II

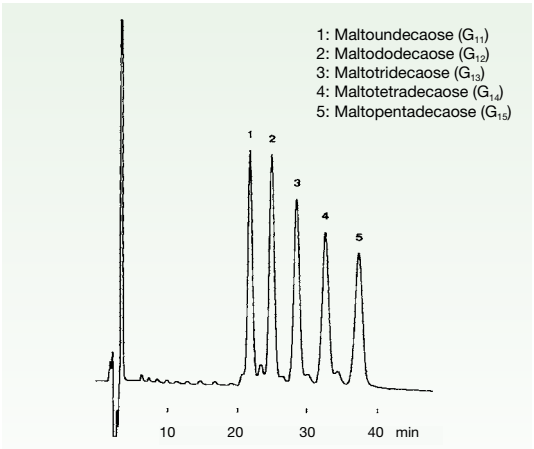
## Applications

### Mono- and Di-saccharides




Column: YMC-Pack Polyamine II (5 µm) 250 x 4.6 mm ID
Part No.: PB12S052546WT
Eluent: ACN / $H_2O$ = 75/25
Flow rate: 1.0 ml/min
Detection: RI, 32x$10^{-6}$ RIU/FS
Temperature: 26 °C

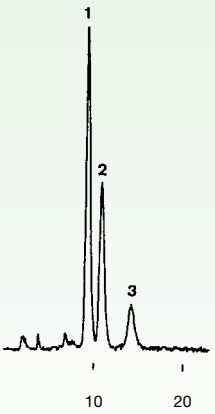
### Malto-oligosaccharides




Column: YMC-Pack Polyamine II (5 µm) 250 x 4.6 mm ID
Part No.: PB12S052546WT
Eluent: ACN / $H_2O$ = 55/45
Flow rate: 1.0 ml/min
Detection: RI, 32 x $10^{-6}$ RIU/FS
Temperature: 26 °C

* Application data by courtesy YMC Co., Ltd.

### Pyridylamino (PA)-Sugar chains




G: Galactose
GN: N-Acetylglucosamine
M: Mannose
F: Fructose
PA: Pyridylamino

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

GN(β1♦6)
GN(β1♦2)
M(α1♦6)
GN(β1♦4)
GN(β1♦2)
M(α1♦3)
M(β1♦4)GN(β1♦4)GN-PA

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

G(β1♦4)GN(β1♦2)M(α1♦6)
G(β1♦4)GN(β1♦2)M(α1♦3)
M(β1♦4)GN(β1♦4)GN-PA

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

G(β1♦4)GN(β1♦2)
G(β1♦4)GN(β1♦4)
G(β1♦4)GN(β1♦2)
M(α1♦6)
M(α1♦3)
M(β1♦4)GN(β1♦4)GN-PA

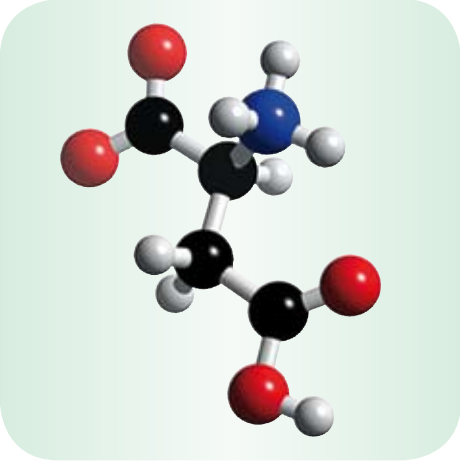
| | |
|---|---|
| Column: | YMC-Pack Polyamine II (5 µm) 150 x 4.6 mm ID |
| Part No.: | PB12S051546WT |
| Eluent: | methanol / $NH_4H_2PO_4$ (20 mM) = 80/20 |
| Flow rate: | 1.0 ml/min |
| Detection: | fluoresence, Ex.320 nm, Em. 400 nm, 4 mV/FS |
| Temperature: | 37 °C |

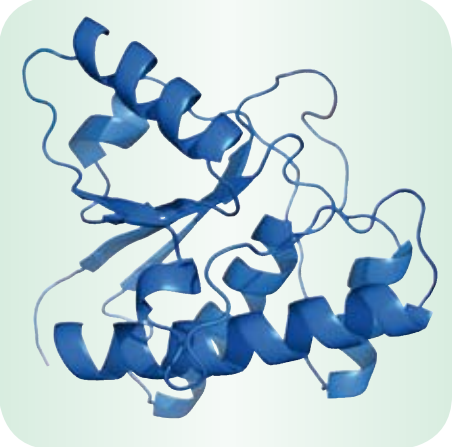
# Ordering Information

## YMC-Pack Polyamine II, 12 nm, 5 µm

| Column length | Column ID (mm) | | | |
|---|---|---|---|---|
| | 2.1 | 3.0 | 4.0 | 4.6 |
| 33 mm | PB12S050302QT | PB12S050303QT | PB12S050304QT | PB12S050346WT |
| 50 mm | PB12S050502QT | PB12S050503QT | PB12S050504QT | PB12S050546WT |
| 100 mm | PB12S051002QT | PB12S051003QT | PB12S051004QT | PB12S051046WT |
| 150 mm | PB12S051502QT | PB12S051503QT | PB12S051504QT | PB12S051546WT |
| 250 mm | PB12S052502QT | PB12S052503QT | PB12S052504QT | PB12S052546WT |

Guard Columns are also available.
For more details please contact us: Phone 02064-427-0 or email info@ymc.de.

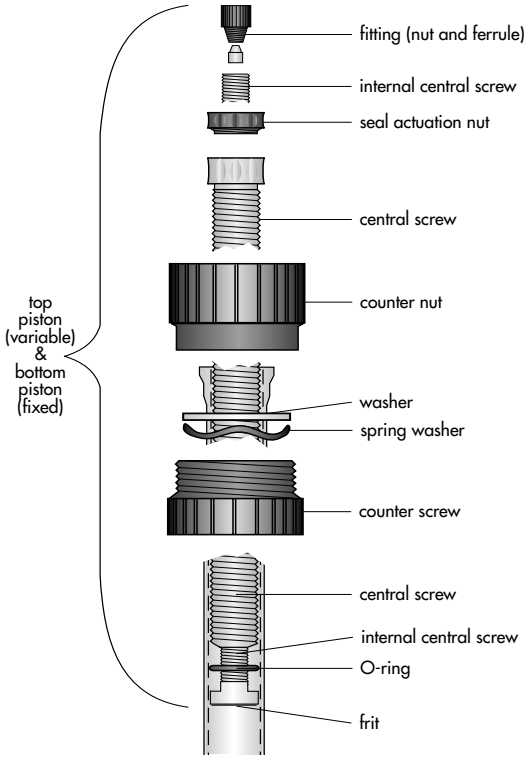




GLASS
COLUMNS

# ECO Glass Columns

- **biocompatible**
- **solvent resistant version (optional)**
- **easy to use**
- **low temperature application**
- **2 adjustable length plungers (Multivario) supplied on request**
- **compatible with any LC system**
- **water-jacketed version available on request**




**ECO Column ID 10-50 mm**

## General

ECO columns are glass columns for almost all types of soft gel and low pressure (pressure limit 5 - 30 bar) liquid chromatography applications. With a choice of one or two adjustable length plungers, they are available in two forms: AB (aqueous buffer) for use with aqueous buffers and cold room applications and SR (solvent resistant) for all forms of normal and reversed phase chromatography.

ECO columns are produced by high-precision CNC manufacturing. They are competitively priced and equipped with a screw-lock system which makes it possible to open and seal the column simply and quickly. Each column passes a quality control pressure test. A water-jacketed option can be supplied on request.

## Versions available

| Version | Temperature Range [°C] | Plunger | Seal | Frit |
|---|---|---|---|---|
| **ECO AB (Aqueous Buffer)** | 4 - 40 | POM | Viton® O-ring EPDM | Porous glass (ID 10 - 50 mm) Polyethylene (ID 70 - 80 mm) |
| **ECO SR (Solvent Resistant)** | 16 - 40 | PVDF | Kalrez® O-ring | Porous glass (ID 10 - 50 mm) Stainless steel (ID 70 - 80 mm) |

## Characteristics

Pressure limit: 5 - 30 bar (depending on diameter)

Temperature range: aqueous buffer version (AB): 4 - 40 °C
solvent resistant version (SR): 16 - 40 °C

Wetted parts: borosilicate glass
aqueous buffer version (AB): POM, sintered glass frit and Viton® seal (ID 10 - 50 mm)
POM, polyethylene frit and EPDM seal (ID 70 - 80 mm)
solvent resistant version (SR): PVDF, sintered glass frit and Kalrez® seal (ID 10 - 50 mm)
PVDF, stainless steel frit and Karlez® seal (ID 70 - 80 mm)

Diameters: 10 mm, 15 mm, 25 mm, 50 mm, 70 mm, 80 mm

Bed lengths: 120 mm, 200 mm, 450 mm, 750 mm, 1000 mm

Height adjustment: Adjustable plunger provides up to 120 mm bed height adjustment (2 adjustable plungers supplied on request).

Connections: The columns are supplied with different adaptors to allow direct connection to any LC-system.

## Specifications

| | | Vario | | Multivario | |
|---|---|---|---|---|---|
| ID [mm]* | Pressure limit [bar] | Bed length [mm] | Volume [ml] | Bed length [mm] | Volume [ml] |
| 10 | 30 | 0-120<br>80-200<br>330-450<br>630-750<br>880-1000 | 0-9.4<br>6.3-16<br>26-35<br>50-59<br>69-79 | 0-120<br>0-200<br>210-450<br>510-750<br>760-1000 | 0-9.4<br>0-16<br>17-35<br>40-59<br>60-79 |
| 15 | 25 | 0-120<br>80-200<br>330-450<br>630-750<br>880-1000 | 0-21<br>14-35<br>58-80<br>111-133<br>156-177 | 0-120<br>0-200<br>210-450<br>510-750<br>760-1000 | 0-21<br>0-35<br>37-80<br>90-133<br>134-177 |
| 25 | 15 | 0-120<br>80-200<br>330-450<br>630-750<br>880-1000 | 0-59<br>39-98<br>162-221<br>309-368<br>432-491 | 0-120<br>0-200<br>210-450<br>510-750<br>760-1000 | 0-59<br>0-98<br>103-221<br>250-368<br>373-491 |
| 50 | 10 | 0-120<br>80-200<br>330-450<br>630-750<br>880-1000 | 0-236<br>157-393<br>648-884<br>1237-1473<br>1728-1964 | 0-120<br>0-200<br>210-450<br>510-750<br>760-1000 | 0-236<br>0-393<br>412-884<br>1001-1473<br>1492-1964 |
| 70 | 5 | — | — | 0-120<br>0-200<br>90-450<br>390-750<br>640-1000 | 0-462<br>0-770<br>356-1732<br>1501-2886<br>2463-3848 |
| 80 | 5 | — | — | 0-120<br>0-200<br>90-450<br>390-750<br>640-1000 | 0-603<br>0-1005<br>452-2262<br>1960-3770<br>3217-5026 |

* Columns with inner diameter of 20 and 32 mm are available upon request

## Accessories supplied

All columns: 1x frit removal tool / 2x plugs, PTFE (1/4“-28G)

and for each specific diameter the following tubing, nuts and ferrules:

ID 10–15 mm: 1x 1m FEP-tubing (0.8 x 1.6 mm); 4x 1/4“-28G nuts and ferrules (collapsible) for 1/16“ tubing; 2x M6 nuts and ferrules for 1/16“ tubing and 2x 10-32 nuts and ferrules for 1/16“ tubing

ID 25–80 mm: 1x 1m FEP-tubing (1.6 or 2.4 x 3.2 mm); 4x 1/4“-28G nuts and ferrules (collapsible) for 1/8“ tubing and 2x M6 nuts and ferrules for 1/8“ tubing

* Application data by courtesy YMC Co., Ltd.

# Ordering guide

The part numbers for the columns contain information on the column type, inner diameter, column length, plunger type, frit porosity and seal (O-ring) material.

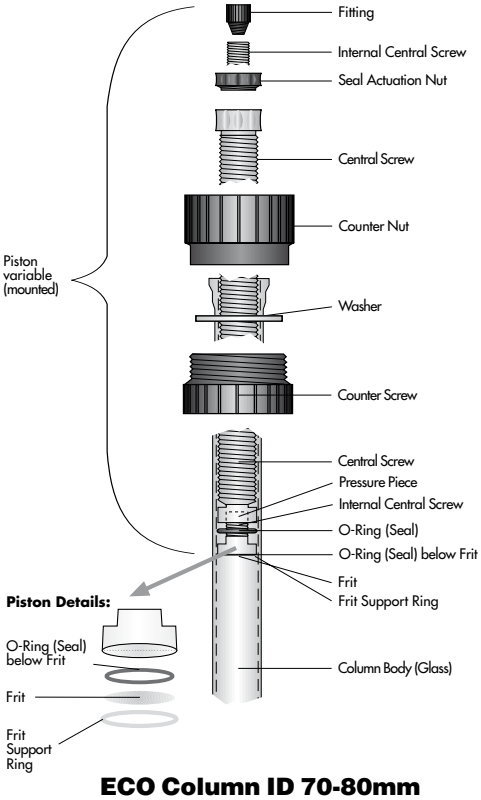
| Inner diameter ID [mm]* | Pressure limit [bar] | Order code |
|---|---|---|
| **10** | 30 | ECO10/ |
| **15** | 25 | ECO15/ |
| **25** | 15 | ECO25/ |
| **50** | 10 | ECO50/ |
| **70** | 5 | ECO70/ |
| **80** | 5 | ECO80/ |

* Columns with inner diameter of 20 and 32 mm are available upon request

| Max. bed length [mm] | Order code |
|---|---|
| **120** | 120 |
| **200** | 200 |
| **450** | 450 |
| **750** | 750 |
| **1000** | 999 |
| Longer columns are available on request. | |

| No. of adjustable plungers | Order code |
|---|---|
| **1 adjustable plunger (Vario)** | V |
| **2 adjustable plungers (Multivario)** | M |
| up to 120 mm bed variability each | |

| Frit porosity [µm] | Order code |
|---|---|
| **10** | 0 |
| **16 - 40** | 3 |
| **40 - 100** | 4 |
| Other frit porosities are available on request. | |




**ECO Column ID 70-80mm**

| Column version | Seal O-ring material | Order code |
|---|---|---|
| **ECO AB (Aqueous Buffer)** | Viton® / EPDM | V |
| **ECO SR (Solvent Resistant)** | Kalrez® | K |

| Option | Order code |
|---|---|
| **Water-jacket version*** | -K |
| *(only in combination with Multi Vario plunger) | |

# Ordering guide

## Examples

To order an aqueous buffer ECO column with an inner diameter of 15 mm, a column length of 120 mm, with 1 adjustable plunger and a frit porosity of 16 - 40 µm, a Viton O-ring and without the water-jacket option, please use the corresponding part number ECO15/120V3V.

**Part Number: ECO15/120V3V**

| Example | ECO | 15/ | 120 | V | 3 | V |
|---|---|---|---|---|---|---|
| Column type | ECO | | | | | |
| Inner diameter | | 15/ | | | | |
| Max. bed length | | | 120 | | | |
| Plunger type | | | | V | | |
| Frit porosity | | | | | 3 | |
| Seal O-ring material | | | | | | V |

To order a solvent resistant ECO column with an inner diameter of 10 mm, a column length of 120 mm, with 2 adjustable plungers and a frit porosity of 10 µm, a Kalrez O-ring and with the water-jacket option, please use the corresponding part number ECO10/120M0K-K.

**Part Number: ECO10/120M0K-K**

| Example | ECO | 10/ | 120 | M | 0 | K | -K |
|---|---|---|---|---|---|---|---|
| Column type | ECO | | | | | | |
| Inner diameter | | 10/ | | | | | |
| Column length | | | 120 | | | | |
| Plunger type | | | | M | | | |
| Frit porosity | | | | | 0 | | |
| Seal O-ring material | | | | | | K | |
| Water-jacket version | | | | | | | -K |

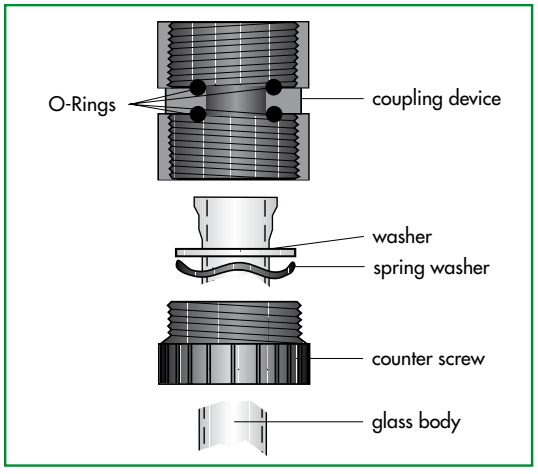
## Packing Adaptors

The ECO series packing adaptors consist of a column coupler and an empty glass body. These must be of the same diameter as the column to be packed and must be used as packing adaptors, not for extending the length of a column body. The length of the additional glass body used should be selected to allow:

- Double the volume of slurry compared to the required packed bed volume if packing silica materials
- Triple the volume of slurry for softer packing materials

The product manual supplied with each column contains detailed examples of dry packing and slurry packing techniques.

For ordering information please see next page.




*column coupler*

* Application data by courtesy YMC Co., Ltd.

# Ordering information for ECO packing adaptors

| Inner Diameter* | Description | Length | Part Number |
|---|---|---|---|
| **10** | Column Coupler (no Glass Body) including Viton® O-Ring | | ECO10KU/V |
| **15** | Column Coupler (no Glass Body) including Viton® O-Ring | | ECO15KU/V |
| **25** | Column Coupler (no Glass Body) including Viton® O-Ring | | ECO25KU/V |
| **50** | Column Coupler (no Glass Body) including Viton® O-Ring | | ECO50KU/V |
| **70** | Column Coupler (no Glass Body) including EPDM O-Ring | | ECO70KU/V |
| **80** | Column Coupler (no Glass Body) including EPDM O-Ring | | ECO80KU/V |
| **10** | Glass Body for use with Column Coupler | 120<br>200<br>450<br>750<br>1000 | ECO10/120<br>ECO10/200<br>ECO10/450<br>ECO10/750<br>ECO10/999 |
| **15** | Glass Body for use with Column Coupler | 120<br>200<br>450<br>750<br>1000 | ECO15/120<br>ECO15/200<br>ECO15/450<br>ECO15/750<br>ECO15/999 |
| **25** | Glass Body for use with Column Coupler | 120<br>200<br>450<br>750<br>1000 | ECO25/120<br>ECO25/200<br>ECO25/450<br>ECO25/750<br>ECO25/999 |
| **50** | Glass Body for use with Column Coupler | 120<br>200<br>450<br>750<br>1000 | ECO50/120<br>ECO50/200<br>ECO50/450<br>ECO50/750<br>ECO50/999 |
| **70** | Glass Body for use with Column Coupler | 120<br>200<br>450<br>750<br>1000 | ECO70/120<br>ECO70/200<br>ECO70/450<br>ECO70/750<br>ECO70/999 |
| **80** | Glass Body for use with Column Coupler | 120<br>200<br>450<br>750<br>1000 | ECO80/120<br>ECO80/200<br>ECO80/450<br>ECO80/750<br>ECO80/999 |

* Column Couplers for columns with inner diameter of 20 and 32mm are available upon request

# ECO$^{PLUS}$ Glass Columns

- **Suitable for universal use**
- **Biocompatible**
- **Simple to use**
- **Problem-free connection to any LC system**
- **Height-adjustable pistons at both ends**
- **Suitable for cold rooms from 4 - 40 °C (with polyethylene piston and EPDM O ring)**
- **SR-Version resistant to organic solvents (SR = Solvent Resistant)**

## General

Biochromatography is widely applied in high-performance downstream processing techniques that can be used for a range of compounds, such as proteins, peptides or nucleic acids. When using various chromatographic techniques such as ion exchange, affinity or gel permeation chromatography, increasingly high-performance separation media are used and, as a result, higher demands are made on the quality of the column hardware.
ECO$^{PLUS}$ glass columns meet the highest criteria for professional laboratory use. Particular attention has been paid to the column volume ranges that are as wide as possible (0.4 - 982 ml) and to the high pressure resistance (up to 80 bar / 1160 psi), so that high flow rates and performance/efficiency can be achieved.
We have selected high-quality, inert materials to make sure ECO$^{PLUS}$ glass columns are biocompatible and offer the best conditions for high recovery with no loss of bio-activity of your biomolecules.
Thanks to the “Quick-Lock” seal and the two adjustable pistons, the columns are fully adjustable and easy to use.
Given the wide range of diameters, frit porosities and lengths available, you can use ECO$^{PLUS}$ glass columns for the most diverse of applications.

**„Quick-Lock“ Fitting**

No more than a quarter turn is needed to seal the column.
Piston height adjustment is done by turning the locked “Quick-Lock” fitting.

* Application data by courtesy YMC Co., Ltd.

## “Connectivity and compatibility”

Two of the most frequently asked questions about using Kronlab glass columns are:

***Question 1:***

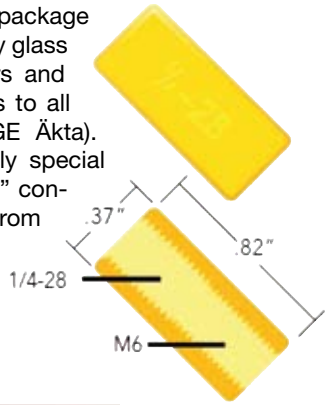
*What packing materials can I use in Kronlab glass columns?*

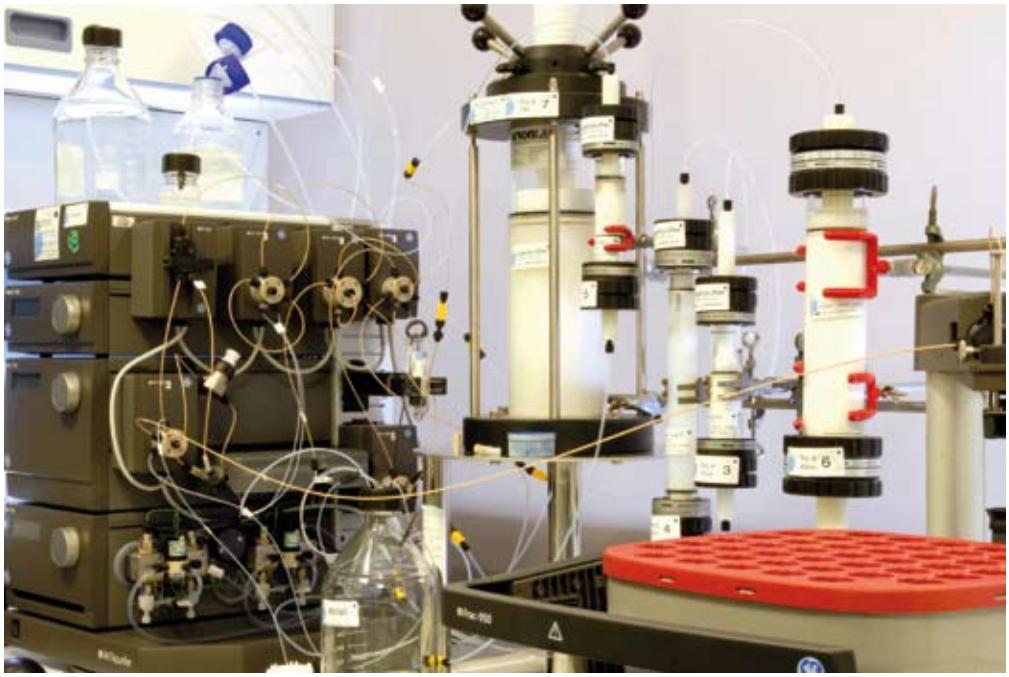
**Answer:**
In theory you can use any packing material from any manufacturer of your choice!
The only restrictions are those linked to the conditions of use with materials that come into contact with the media, such as the pressure limits of the column and packing material. To accommodate this, Kronlab offers a wide range of variants and different column versions. Our application laboratory has examples of applications in the fields of ion exchange, size exclusion, gel permeation, normal/reversed phase and affinity chromatography, etc. with an enormous range of phases from various manufacturers, including YMC, GE, Pall, Bio-Rad, Tosoh and others.

***Question 2:***

*Can I link Kronlab glass columns to any LC system from other manufacturers, or can I only use Kronlab systems?*

**Answer:**
Of course we would love you to use Kronlab glass columns as a “package” with Kronlab LC systems – but of course there are no restrictions! The accessory package included in Kronlab laboratory glass columns contains connectors and adaptors to link our columns to all current LC systems (e.g. GE Äkta). As an option, we can supply special components such as “yellow” connection adaptors to convert from metric to imperial systems (M6 at 1/4-28).



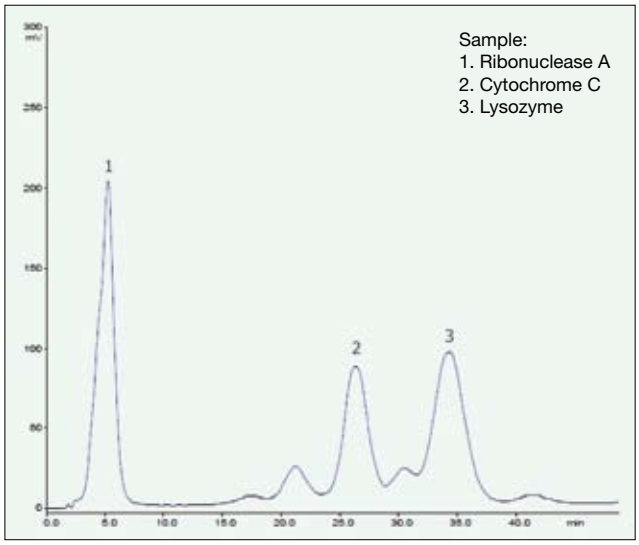



**Kronlab laboratory glass columns in a “multi-purpose” application laboratory**
(with the kind permission of InVivo BioTech Service GmbH)

## Application Examples

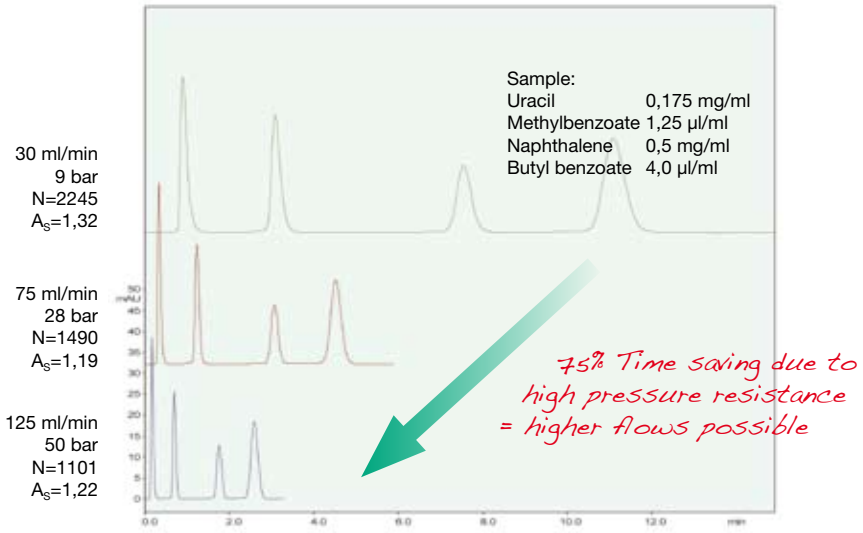
In reversed phase and adsorption chromatography, the possibilities for using glass columns are often limited due to high back pressures generated by small particles. The high pressure resistance of the ECO$^{PLUS}$ glass columns allow you to achieve high flow rates for demanding separations. The example shows that this enables a considerable acceleration of the separation, which means that you can achieve significant time savings.

### Separation of a standard test mixture of proteins




| | |
|---|---|
| Column: | Kronlab ECO$^{PLUS}$ 250 x 15 mm ID |
| Stationary phase: | YMC-BioPro SP, 30 µm (bed length 170 mm) |
| Mobile phase: | 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 |
| Gradient: | 40-80% B |
| Flow rate: | 6 ml/min |
| Temperature: | 25°C |
| Detection: | UV at 220 nm |
| Injection: | 100 µl |

### Example of application with reversed phase media




| | |
|---|---|
| Column: | Kronlab ECO$^{PLUS}$ 125 x 25 mm ID |
| Stationary phase: | YMC-Pack ODS-AQ, 10 µm, 12 nm, 8 cm bed length |
| Flow rate: | 30 ml/min - 75 ml/min - 125 ml/min |
| Mobile phase: | ACN/$H_2O$ (50:50) |
| Injection: | 500 µl |
| Detection: | UV at 254 nm |

* Application data by courtesy YMC Co., Ltd.

## Product options

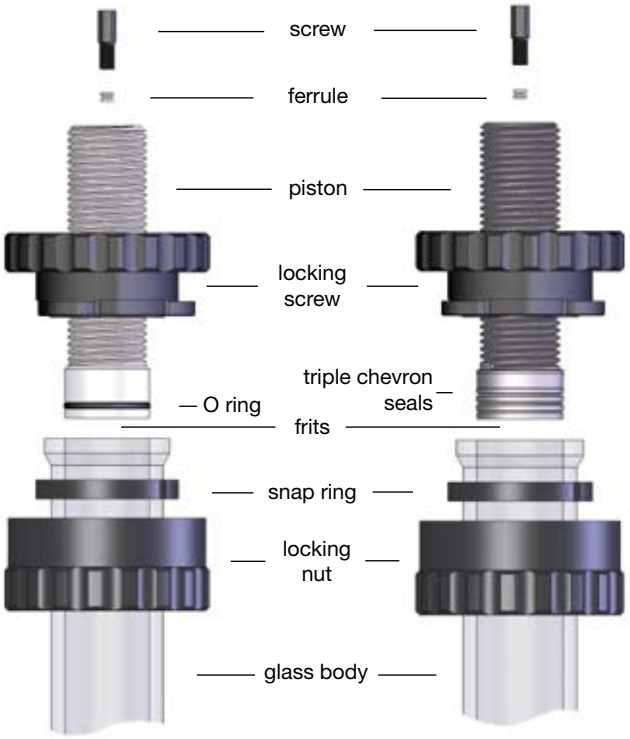
ECO$^{PLUS}$ laboratory glass columns are routinely available in three different lengths (125, 250, 500 mm) and three combinations of pistons (Short, Short/long, Long) in order to accommodate different volume configurations.

5 mm ID | 10 mm ID | 15 mm ID | 25 mm ID | 35 mm ID | 50 mm ID

The modular construction allows for a range of piston variations to provide the ideal column volume.

**Standard Version**
**for aqueous buffers (AB)**

**Optional Version**
**Solvant Resistant (SR)**




ECO$^{PLUS}$ glass columns are multi-purpose columns for all liquid chromatography applications (with pressure limits of 30 to 80 bar (435 to 1160 psi) - depending on column diameter as shown in the table on page 6). ECO$^{PLUS}$ glass columns are available in two versions:

**Standard-Version**

(AB = Aqueous Buffer) for aqueous buffers and applications in cold rooms.

**Optional-Version**

(SR = Solvent Resistant) for normal and reversed phase chromatography.

The height-adjustable pistons (standard) at each end of the ECO$^{PLUS}$ glass column with Teflon ribs (SR-version) are suitable for the entire spectrum of normal phase and reversed phase chromatography as well as biochromatography above ambient temperature.

All ECO$^{PLUS}$ glass columns are made with high-precision CNC machines and undergo several rigorous quality controls before they are delivered.

## Versions available / materials used / components in contact with media

All the columns are made of borosilicate glass 3.3 (calibrated precision glass - KPG®)

| Version* | Temperature range [°C] | max. Pressure [bar] | Piston | Seal | Frits |
|---|---|---|---|---|---|
| ECO$^{PLUS}$ AB (Aqueous buffer) | 4 - 40 | ID 05 mm: 80<br>ID 10 mm: 80<br>ID 15 mm: 70<br>ID 25 mm: 50<br>ID 35 mm: 40<br>ID 50 mm: 30 | Polyethylene | O ring EPDM (ethylene-propylene-diene-M-class rubber) | Column ID 5-50 mm: Polyethylene |
| ECO$^{PLUS}$ SR (solvent resistant) | 16 - 40 | ID 05 mm: 80<br>ID 10 mm: 50<br>ID 15 mm: 50<br>ID 25 mm: 50<br>ID 35 mm: 40<br>ID 50 mm: 25 | PTFE (Teflon) | PTFE (Teflon) | Column ID 5-15 mm: sintered glass<br>Column ID 25-50 mm: Stainless steel (SS) |

* Material certificates for wetted parts are available upon request.

### Specifications

| ID [mm] | Short pistons | | Short/long piston | | Long pistons | |
|---|---|---|---|---|---|---|
| | Bed length [mm] | Volume [ml] | Bed length [mm] | Volume [ml] | Bed length [mm] | Volume [ml] |
| 5 | 22 - 125<br>147 - 250<br>397 - 500 | 0.4 - 2.5<br>2.9 - 4.9<br>7.8 - 9.8 | 0 - 125<br>67 - 250<br>317 - 500 | 0 - 2.5<br>1.3 - 4.9<br>6.2 - 9.8 | 0 - 125<br>0 - 250<br>237 - 500 | 0 - 2.5<br>0 - 4.9<br>4.7 - 9.8 |
| 10 | 32 - 125<br>157 - 250<br>407 - 500 | 2.5 - 9.8<br>12 - 20<br>32 - 39 | 0 - 125<br>77 - 250<br>327 - 500 | 0 - 9.8<br>6.0 - 20<br>26 - 39 | 0 - 125<br>0 - 250<br>247 - 500 | 0 - 9.8<br>0 - 20<br>19 - 39 |
| 15 | 24 - 125<br>149 - 250<br>399 - 500 | 4.2 - 22<br>26 - 44<br>71 - 88 | 0 - 125<br>69 - 250<br>319 - 500 | 0 - 22<br>12 - 44<br>56 - 88 | 0 - 125<br>0 - 250<br>239 - 500 | 0 - 22<br>0 - 44<br>42 - 88 |
| 25 | 28 - 125<br>153 - 250<br>403 - 500 | 14 - 61<br>75 - 123<br>198 - 245 | 0 - 125<br>73 - 250<br>323 - 500 | 0 - 61<br>36 - 123<br>159 - 245 | 0 - 125<br>0 - 250<br>243 - 500 | 0 - 61<br>0 - 123<br>119 - 245 |
| 35 | 30 - 125<br>155 - 250<br>405 - 500 | 29 - 120<br>149 - 241<br>390 - 481 | 0 - 125<br>75 - 250<br>325 - 500 | 0 - 120<br>72 - 241<br>313 - 481 | 0 - 125<br>0 - 250<br>245 - 500 | 0 - 120<br>0 - 241<br>236 - 481 |
| 50 | 36 - 125<br>161 - 250<br>410 - 500 | 71 - 245<br>316 - 491<br>805 - 982 | 0 - 125<br>81 - 250<br>331 - 500 | 0 - 245<br>159 - 491<br>650 - 982 | 0 - 125<br>0 - 250<br>250 - 500 | 0 - 245<br>0 - 491<br>491 - 982 |

**Standard accessories** (included with the column)

All glass columns include: 1x frit ejector / 2x plugs, PTFE (1/4“-28G),

plus the screws, ferrules and capillaries required to link up to any LC system, depending on the tube ID:

ID 5 mm: 1x 1m ETFE 1/16” capillary tubing pre-assembled / 4x ¼”-28G screws / ferrules for 1/16“ tubing / 2x M6 screws / ferrules for 1/16” tubing / 2 x 10-32 screws / ferrules for 1/16“ tubing.

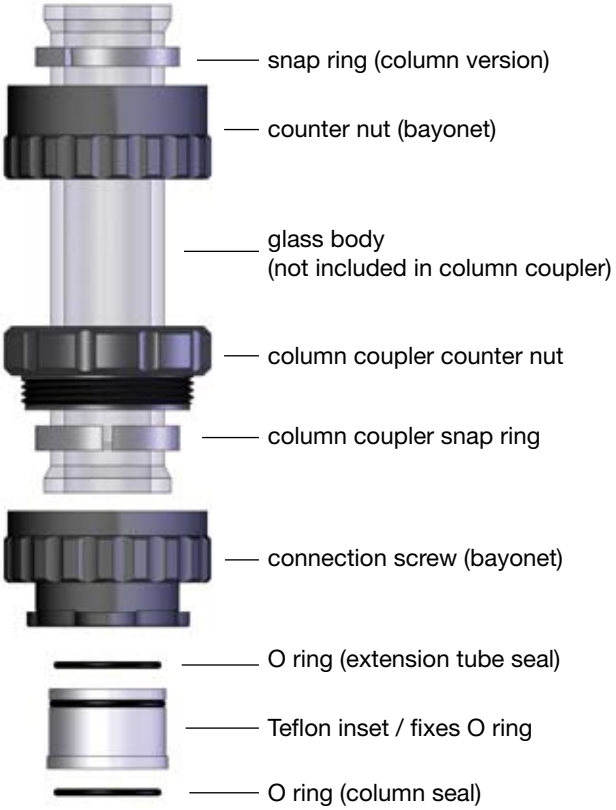
ID 10 –15 mm: 1x 1m FEP 1/16” capillary tubing (0.8 x 1.6 mm) / 4x ¼”-28G screws / ferrules for 1/16” tubing / 2x M6 screws / ferrules for 1/16” tubing / 2x 10-32 screws / ferrules for 1/16” tubing.

ID 25 – 50 mm: 1x 1m FEP 1/8” capillary tubing (1.6 x 3.2 mm) / 4x ¼”-28G screws / ferrules for 1/8” tubing / 2x M6 screws / ferrules for 1/8” tubing.

* Application data by courtesy YMC Co., Ltd.

## Accessories / Replacement parts

To allow packing of a glass column, you can use the ECO$^{PLUS}$ column coupler to join a glass body to a column. For this to work, it must have the same inside diameter as the column.
The length of the extra glass body required for column packing depends on the packing material being used and can be calculated from the packing instructions for the chosen packing material.




Column couplers consist of:

- ECO$^{PLUS}$ connection screw with Teflon insert (assembled)
- Column coupler counter screw with snap ring
- Counter nut (bayonet) with snap ring
- AB-version has two sets (4 items) of Viton® O rings
- SR-version has two Kalrez® O rings

### ECO$^{PLUS}$ column coupler

| Column ID [mm] | AB-Version column couplers* Part-No. | SR-Version column couplers* Part-No. |
|---|---|---|
| 5 | TAC05KU-AB | TAC05KU-SR |
| 10 | TAC10KU-AB | TAC10KU-SR |
| 15 | TAC15KU-AB | TAC15KU-SR |
| 25 | TAC25KU-AB | TAC25KU-SR |
| 35 | TAC35KU-AB | TAC35KU-SR |
| 50 | TAC50KU-AB | TAC50KU-SR |

* the connection piece does not include a glass body: please order this separately

### ECO$^{PLUS}$ glass bodies

| Column ID [mm] | max. bed length 125 mm Part-No. | max. bed length 250 mm Part-No. | max. bed length 500 mm Part-No. |
|---|---|---|---|
| 5 | TAC05/125-2 | TAC05/250-2 | TAC05/500-2 |
| 10 | TAC10/125-2 | TAC10/250-2 | TAC10/500-2 |
| 15 | TAC15/125-2 | TAC15/250-2 | TAC15/500-2 |
| 25 | TAC25/125-2 | TAC25/250-2 | TAC25/500-2 |
| 35 | TAC35/125-2 | TAC35/250-2 | TAC35/500-2 |
| 50 | TAC50/125-2 | TAC50/250-2 | TAC50/500-2 |

## Part numbers

The part number for an ECO$^{PLUS}$ glass column consists the identification of the inner diameter, maximum bed length, piston set type, frit porosity and material, plus the model type (SR or AB version).

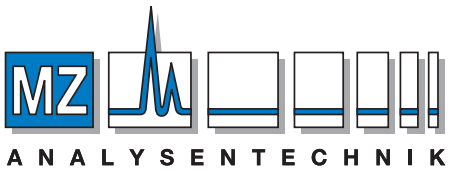
To order a solvent-resistant ECO$^{PLUS}$ glass column with an inner diameter of 25 mm, a maximum bed length of 500 mm, 2 short pistons and stainless steel frits with a porosity of 2 µm, please use the following order number: TAC25/500S2-SR-2 (see example).

| | | | | | | |
|---|---|---|---|---|---|---|
| Combination options | TAC05/ (5 mm ID)<br>TAC10/ (10 mm ID)<br>TAC15/ (15 mm ID)<br>TAC25/ (25 mm ID)<br>TAC35/ (35 mm ID)<br>TAC50/ (50 mm ID) | 125 (125 mm max. bed length)<br>250 (250 mm max. bed length)<br>500 (500 mm max. bed length) | —<br>Standard version has 2 short pistons: no product coding required;<br>For other versions, please insert either:<br>SL (1 short/1 long piston)<br>or<br>L (2 long pistons) | PE Polyethylene (AB-Version)<br>G Sintered glass (SR-Version with ≤ 15 mm ID)<br>S Stainless steel (SS) (SR-Version with ≥ 25 mm ID) | 2 (2 µm)<br>5 (5 µm only poly-ethylene frits available)<br>0 (10 µm) | -AB-2 (aqueaous buffer)<br>-SR-2 (solvent restistant) |
| | | | | | | |
| Example | TAC25/ | 500 | | S | 2 | -SR-2 |
| Inner diameter | 25 mm | | | | | |
| max. bed length | | 500 mm | | | | |
| Piston type | | | | | | |
| Frit material | | | | Stainless steel | | |
| Frit porosity | | | | | 2 µm | |
| Version | | | | | | SR-Version |



* Application data by courtesy YMC Co., Ltd.

Your local distributor:

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