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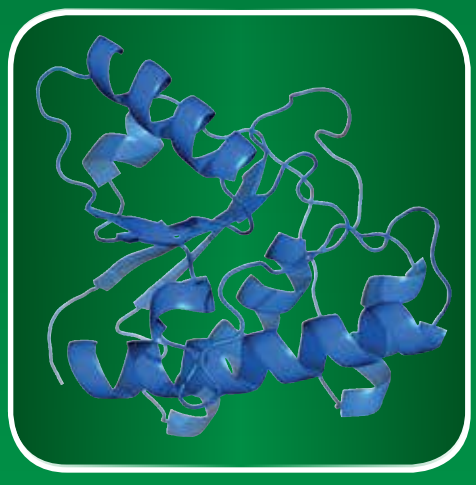
GRANDEVER BIOTECHNOLOGY CO., LTD.

YMC
EUROPE GMBH

YMC-BioPro IEX materials



Prep
Bulk
media

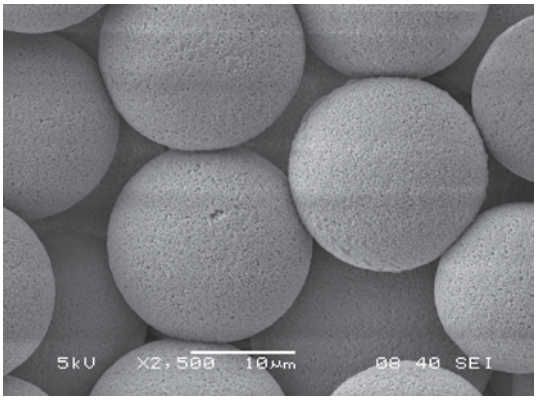
2

YMC stationary phases for preparative chromatography

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, chiral, size exclusion and normal phase/HILIC columns and bulk materials.

YMC-BioPro series products are polymeric ion exchange resins for the purification of biological molecules. They represent one part of the three-pronged technology platform of the YMC-Prep portfolio: silica-hybrid-polymer phases.



SEM picture of YMC-BioPro 30 µm resins (client data)

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YMC-BioPro

Ion exchange chromatography (IEX) is widely used in the analysis and purification of bio-molecules. Using reversible charge-charge interactions offers several advantages in comparison to other chromatographic methods, e.g. high capacity and fast throughput. Thus, IEX is often used in the capture or intermediate purification of bio-molecules.

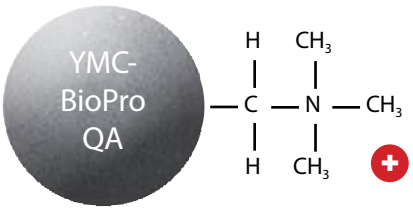
YMC-BioPro is a series of ion exchange resins specifically designed for use in bio-chromatography. This media is based on a hydrophilic polymer matrix, with a particle size of 10 µm, 30 µm or 75 µm. It is available as a strong anion exchanger (YMC-BioPro QA) or a strong cation exchanger (YMC-BioPro SP). YMC-BioPro offers a high dynamic binding capacity (DBC), together with low non-specific adsorption and excellent recovery.

Currently, YMC-BioPro is manufactured in lot sizes up to 200 l. In future, lot sizes up to 1200 l will be available.

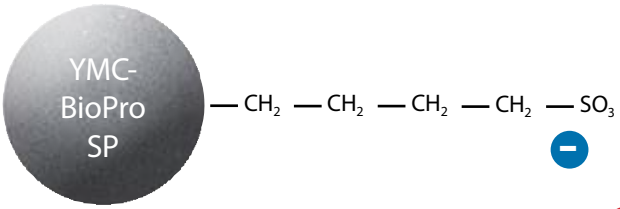
Examples of possible preparative applications of YMC-BioPro resins:

Antibody purification	Protein purification	Peptide purification
Trastuzumab **Becacizumab** **IgG, Antibody variants**	**Histones** **Interferon** **Factor VIII, Factor IX**	**Insulin**

Particle technology



Strong anion exchanger



Strong cation exchanger

N.B.: Fully porous particles

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Specification

	SmartSep		Capture	SmartSep		Capture
YMC-BioPro Series	**YMC-BioPro SmartSep Q10**	**YMC-BioPro SmartSep Q30**	**YMC-BioPro Q75**	**YMC-BioPro SmartSep S10**	**YMC-BioPro SmartSep S30**	**YMC-BioPro S75**
Ion exchange type	strong anion exchanger			strong cation exchanger		
Charged group	$-R-N^+(CH_3)_3$			$-R-SO_3$		
Matrix	Hydrophilic polymer beads					
Pore size	Porous					
Compression factor	1.1 - 1.4					
Particle size	10 µm	30 µm	75 µm	10 µm	30 µm	75 µm
Pressure resistance	Regular use: 3 MPa Max.: 4 MPa	Regular use: 2 MPa Max.: 3 MPa	0.3 MPa	Regular use: 3 MPa Max.: 4 MPa	Regular use: 2 MPa Max.: 3 MPa	0.3 MPa
Ion-exchange capacitiy	min. 0.08 meq/ml-resin		min. 0.10 meq/ml-resin	min. 0.08 meq/ml-resin		min. 0.10 meq/ml-resin
Dynamic binding capacity	min. 100 mg/ml-resin (BSA)		min. 160 mg/ml-resin (BSA)	min. 100 mg/ml-resin (lysozyme)		min. 160 mg/ml-resin (BSA)

Regulatory support file available under non-disclosure agreement.
Used in validated cGMP-manufacturing processes.

Customized material available on request.
DMF registered with FDA.

Advantages of YMC-BioPro

YMC-BioPro features	Your benefit
Excellent flow properties	**Low backpressure High production throughput**
Highly uniform particle and pore size distribution	**Easy and efficient column packing Improved chromatographic performance**
pH stability	**Durable against CIP-conditions Flexibility in method development**
High dynamic binding capacity at high flow rates	**High loadability Process more raw material**
Flexible production capacities	**Column filling from one lot**
Economic resin	**Cost effective**

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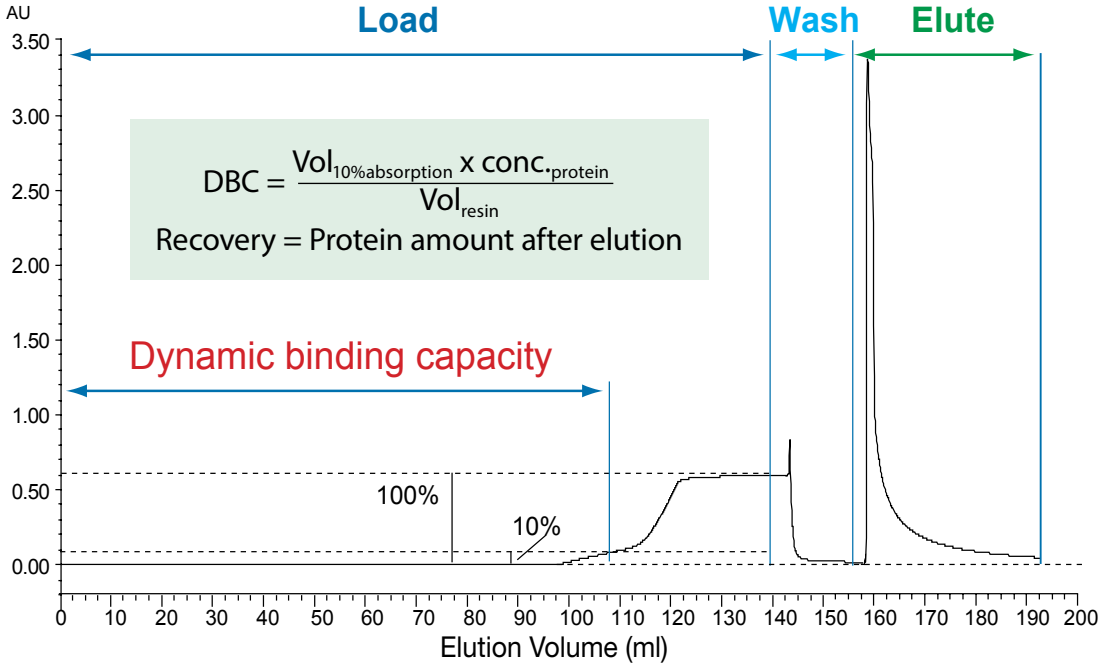
Measurement of dynamic binding capacity and recovery*

The steps performed in the determination of the DBC and recovery are shown in figure below. At the start of the experiment, the column has to be equilibrated, if possible using the buffer used for making the protein solution. Then the protein solution of known concentration is loaded continuously onto the column, until the UV signal reaches a plateau (i.e. 100% value). The volume needed to reach 10% of the UV absorption multiplied by the concentration of the protein solution and divided by the volume of resin in the column gives the DBC (mg/ml resin). Next the column is washed with equilibration buffer to remove all unbound protein from the column. Finally the protein is eluted with a salt (step) gradient. Assaying the resulting eluate and comparing this value to the amount loaded gives the recovery.



Column:	YMC-BioPro Q75, 50 x 5.0 mm ID
Sample:	1.5 mg/ml BSA
Equilibration buffer:	20 mM Tris-HCl (pH 8.6)
Elution buffer:	20 mM Tris-HCl (pH 8.6) cont. 0.5 NaCl
Flow rate:	0.5 ml/min
Detection:	UV at 280 nm

Excellent dynamic binding properties even at high flow rates!

* Application data by courtesy YMC Co., Ltd.

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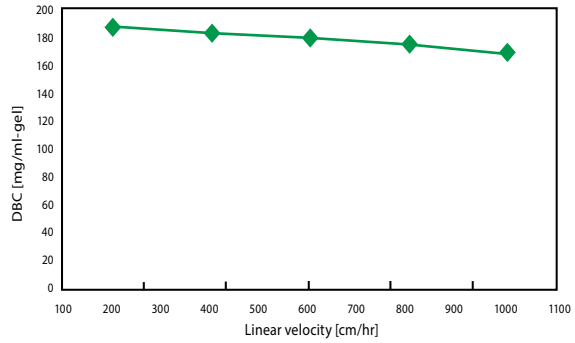
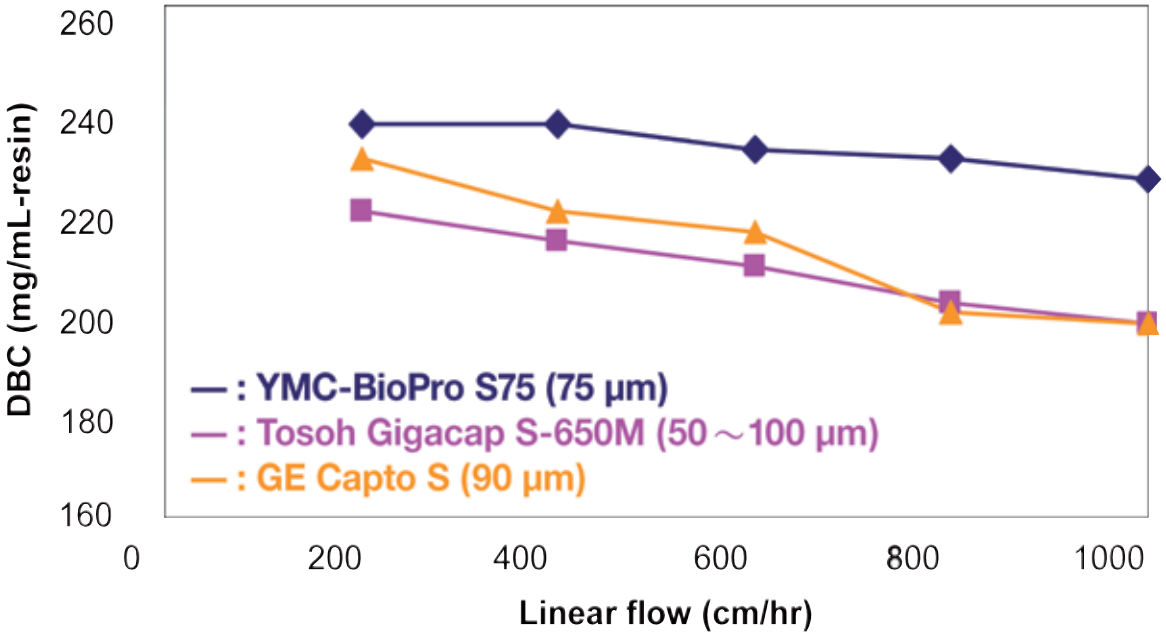
Excellent DBC at high linear flow rates*



Column: YMC-BioPro Q75, 50 x 5.0 mm ID
Sample: 1.5 mg/ml BSA
Equilibration buffer: 20 mM Tris-HCl (pH 8.6)
Elution buffer: 20 mM Tris-HCl (pH 8.6) cont. 0.5 NaCl
Detection: UV at 280 nm
Determined at 10% breakthrough.



Column: YMC-BioPro S75, 50 x 5.0 mm ID
Sample: 1.0 mg/ml Lysozym in equilibration buffer
Equilibration buffer: 20 mM Glycine-NaOH (pH 9.0)
Elution buffer: 20 mM Glycine-NaOH (pH 9.0) with 0.5 M NaCl
Detection: UV at 300 nm

High sample loading at high flow rates is determined by the dynamic binding capacity of an ion exchange resin.
The dynamic binding capacity of YMC-BioPro is excellent even at high flow rates. When compared to similar competitor products it consistently exhibits a higher dynamic binding capacity. This results in higher sample loading in your preparative processes.

* Application data by courtesy YMC Co., Ltd.

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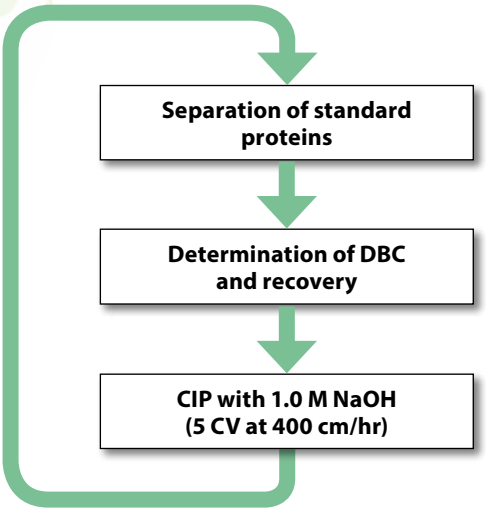
Cleaning-in-place (CIP) performance of YMC-BioPro

Test protocol



Column: YMC-BioPro S75, 50 x 5.0 mm ID

Conditions of standard protein separation

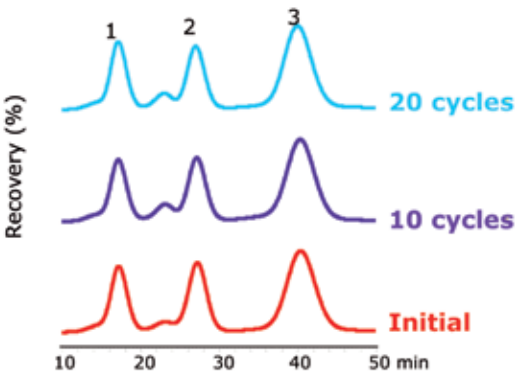
Eluent: A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8)
B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 0.5 M NaCl
Gradient: 0-100% B (0-60 min, Linear)
Flow rate: 0.59 ml/min (180 cm/hr)
Temperature: 25 °C
Detection: UV at 220 nm
Injection: 24 µl

Conditions of DBC determination

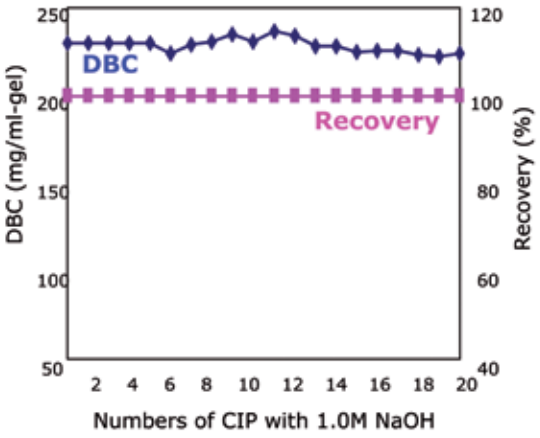
Equilibration buffer: 20 mM Glycine-NaOH(pH 9.0)
Elution buffer: 0.5 M NaCl in equilibration buffer
Flow rate: 2.62 ml/min (800 cm/hr)
Sample: 1.0 mg/ml Lysozymein equilibration buffer
Temperature: ambient (25 °C)
Detection: UV at 300 nm
DBC is determined at 10% breakthrough.

CIP performance of YMC-BioPro

Separation of standard proteins*



DBC and recovery*



YMC-BioPro is well suited for alkaline CIP procedures.
The dynamic binding capacity (DBC) and the selectivity are unaffected by 20 cycles of CIP with 1.0 M NaOH. The recovery of proteins is maintained at 100%, which demonstrates the absence of nonspecific adsorption of proteins of this hydrophilic resin.

* Application data by courtesy YMC Co., Ltd.

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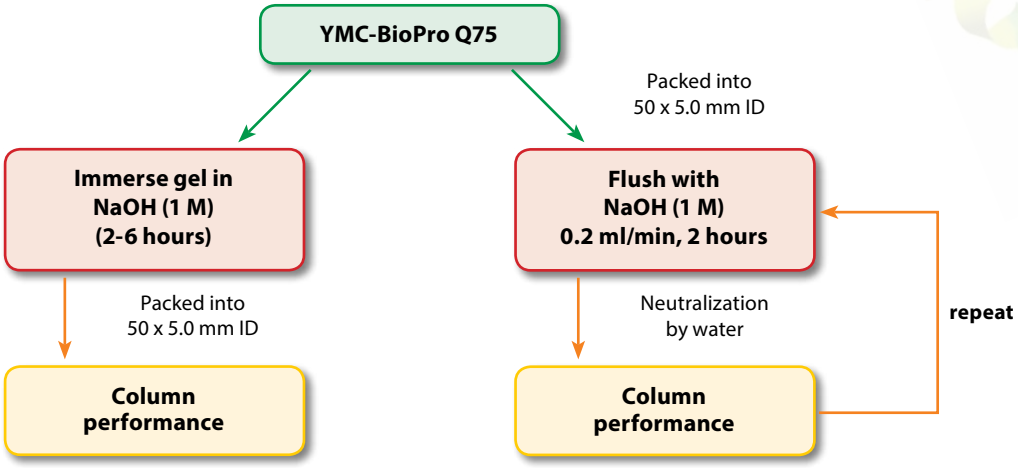
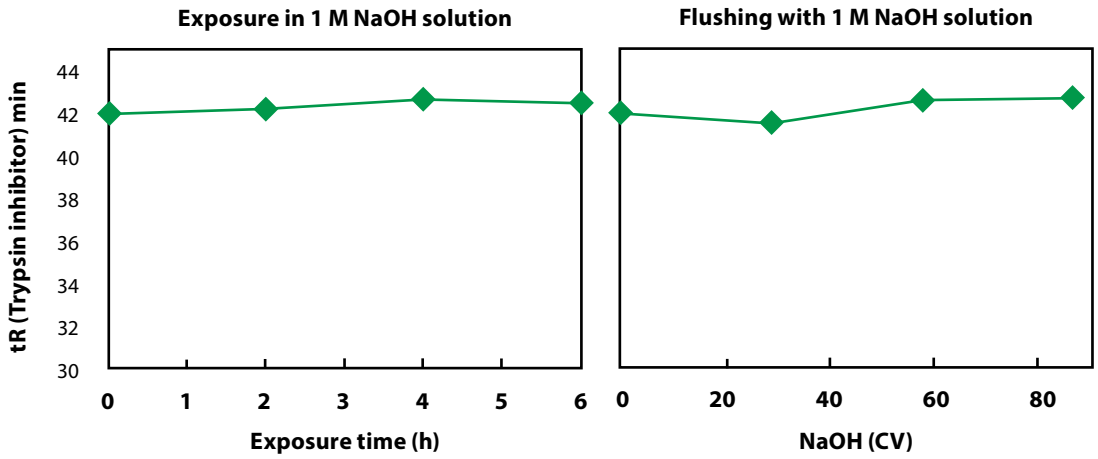
Stability against alkaline conditions*





Column performance test conditions

Eluent:	A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl 10 - 80% B (0-60 min)	Temperature: Detection: Sample: Injection:	25 °C UV at 220 nm Trypsin inhibitor, Transferrin 40 µl
Flow rate:	0.5 ml/min		

Stability against alkaline conditions is essential for efficient sanitizing procedures in a highly regulated environment.
YMC-BioPro demonstrates excellent tolerance against challenging alkaline conditions. Stability testing shows that 0.1M NaOH is suitable for long term storage of YMC-BioPro. The high chemical stability of YMC-BioPro resins allows effective cleaning with alkaline solution and the longevity this material ensures efficient and economic purifications.

* Application data by courtesy YMC Co., Ltd.

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Applications of YMC-BioPro

High binding capacity and high recovery

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[1] (%)
YMC-BioPro QA	126	120	95
Mono Q (GE Healthcare)	100	35	35
BioAssist Q (Tosoh Bioscience)	73	58	79

[1] Recovery: (Eluted amount/Dynamic binding capacity) x 100

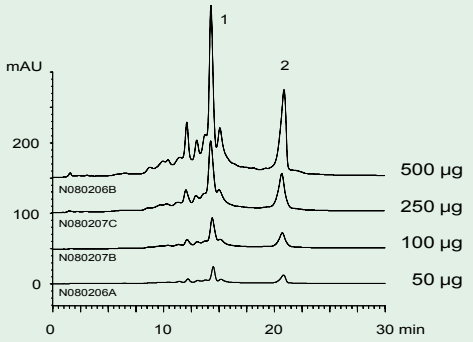
High recovery rates for YMC-BioPro

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.

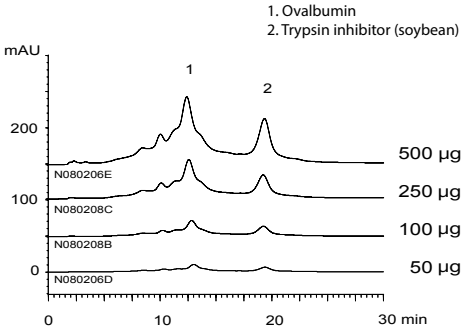
YMC-BioPro Q75 is ideally suited for DNA removal.

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) 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

* Application data by courtesy YMC Co., Ltd.

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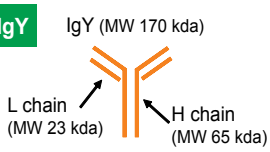
Applications of YMC-BioPro

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

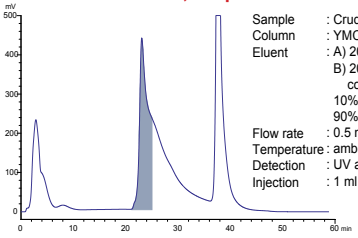
Crude extract from egg yolk containing IgY

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



Step 1: Capture purification by IEX

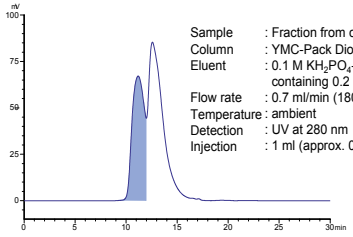
YMC-BioPro Q75, 75 µm



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)

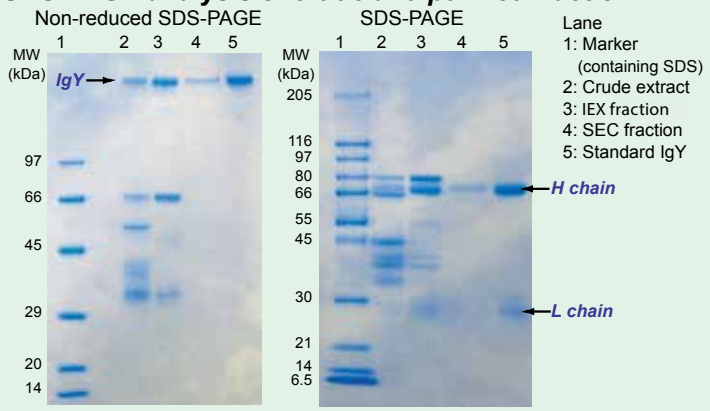
Step 2: Final purification by SEC

YMC-Pack Diol-200, 5 µm

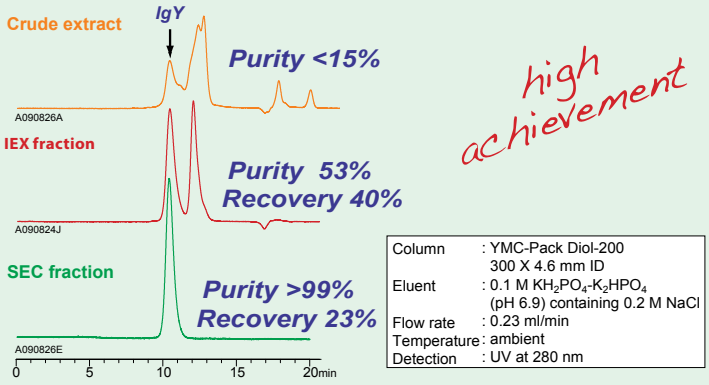


Sample : Fraction from capture purification by IEC
Column : YMC-Pack Diol-200, 5 µm, 300 X 8.0 mm ID
Eluent : 0.1 M KH_2PO_4-K_2HPO_4 (pH 6.9) containing 0.2 M NaCl
Flow rate : 0.7 ml/min (180 cm/hr)
Temperature : ambient
Detection : UV at 280 nm
Injection : 1 ml (approx. 0.45 mg IgY)

SDS-PAGE analysis of crude and purified fraction



SEC analysis of crude and purified fraction



high achievement

Column : YMC-Pack Diol-200 300 X 4.6 mm ID
Eluent : 0.1 M KH_2PO_4-K_2HPO_4 (pH 6.9) containing 0.2 M NaCl
Flow rate : 0.23 ml/min
Temperature : ambient
Detection : UV at 280 nm

* Application data by courtesy YMC Co., Ltd.

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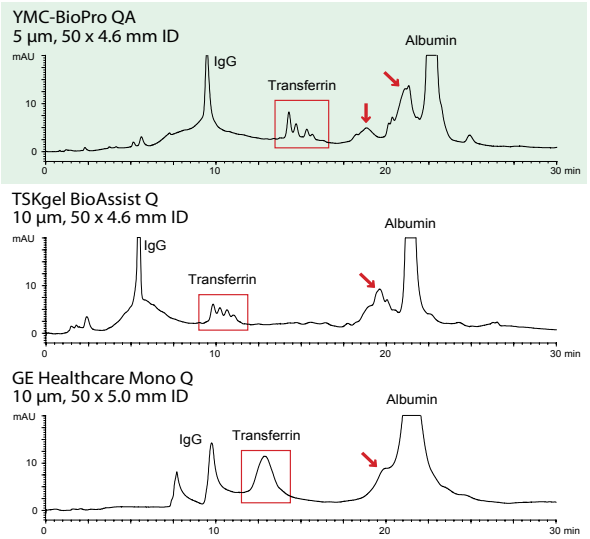
Applications of YMC-BioPro

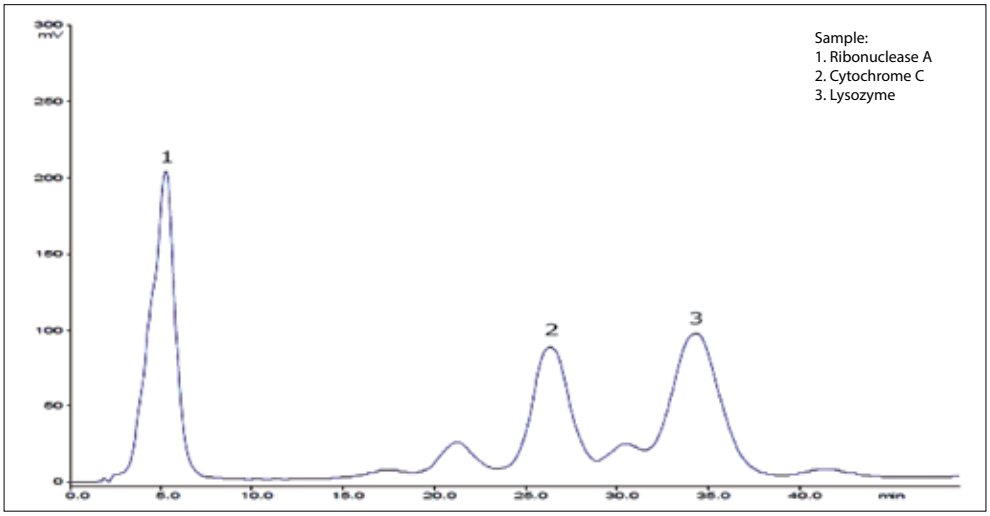
Separation of proteins in human serum on YMC-BioPro QA and commercial Q type products*



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)

Separation of a standard test mixture of proteins*



Column:	YMC 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

* Application data by courtesy YMC Co., Ltd.

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YMC glass columns

Ideally suited for use with YMC-BioPro Prep stationary phases:
Glass columns by YMC for biochromatography

YMC ECO column

- Pressure resistance: 5 - 30 bar
- Inner diameter: 10 - 80 mm
- Column length: 120 - 1000 mm
- Variable bed length
- Column volumes: 0 - 5 L
- Available as AB (aqueous solutions) SR (solvent resistant)

YMC ECO^{PLUS} column

- Pressure resistance: 30 - 80 bar
- Inner diameter: 5 - 50 mm
- Column length: 125 - 500 mm
- Variable bed length
- Column volumes: 0 - 950 mL
- Available as AB (aqueous solutions) SR (solvent resistant)

YMC Pilot columns

- Inner diameter 100 mm, 140 mm and 200 mm, others on request
- Two standard glass lengths 500 / 850 mm, others on request
- Variable bed length (standard 100-400 mm or 450-750 mm)
- Column volumes: up to 24 L
- Pressure resistance: 5 - 10 bar
- Optional accessories, e.g. manometer, bubble traps, stop valves, packing devices
- Manufactured to customer's demands
- Also available as SR version = **S**olvent **R**esistant

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Ordering information YMC-BioPro

Strong anion exchanger
YMC-BioPro Q

Product	Particle Size	Code	Pack Sizes*				
			50 ml	250 ml	1 L	5 L	25 L
YMC-BioPro SmartSep Q10	10 µm	QSA0S10	✔	✔	✔	✔	✔
YMC-BioPro SmartSep Q30	30 µm	QSA0S30	✔	✔	✔	✔	✔
YMC-BioPro Q75	75 µm	QAA0S75	✔	✔	✔	✔	✔

Strong cation exchanger
YMC-BioPro S

Product	Particle Size	Code	Pack Sizes*				
			50 ml	250 ml	1 L	5 L	25 L
YMC-BioPro SmartSep S10	10 µm	SSA0S10	✔	✔	✔	✔	✔
YMC-BioPro SmartSep S30	30 µm	SSA0S30	✔	✔	✔	✔	✔
YMC-BioPro S75	75 µm	SPA0S75	✔	✔	✔	✔	✔

* Larger or customised pack sizes are available on request.
** Conventional YMC-BioPro Q30/S30 (QAA0S30/SPA0S30) available on request.

PE container

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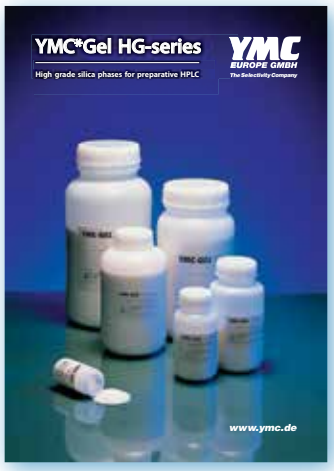
YMC stationary phases for preparative chromatography

YMC has more than 30 years experience in the manufacture of stationary phases for preparative liquid chromatography.
YMC-BioPro series products are polymeric ion exchange resins for the purification of biological molecules. They represent one part of the three-pronged technology platform of the YMC-Prep portfolio: silica-hybrid-polymer phases.

Further information about YMC*Gel silica and YMC-Triart Prep products can be found in the following brochures:



YMC*Gel HG-series brochure

General purpose preparative phases on high grade silica base.

Available as silica or with C18, C8, C4, C1, phenyl, cyano, amino or diol bonding.

Particle size: 10 µm, 15 µm, 20 µm, 50 µm
Pore size: 8 nm, 12 nm, 20 nm, 30 nm

YMC-Triart Prep

Hybrid silica phase, durable against pH 2.0 - 10.0.

Available in C18 and C8 modification.

Particle size: 10 µm, 15 µm, 20 µm
Pore size: 12 nm, 20 nm

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