Chromatographic Media for Antibody / Protein Purification

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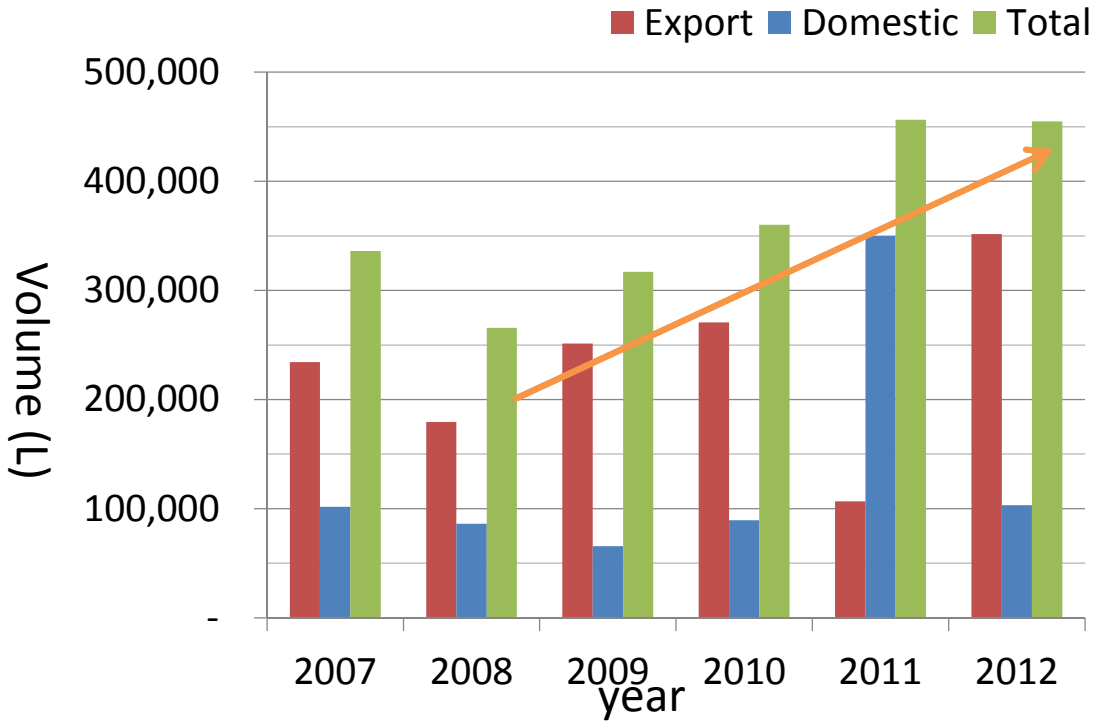
Separation Materials Department
Mitsubishi Chemical Corporation (MCC)

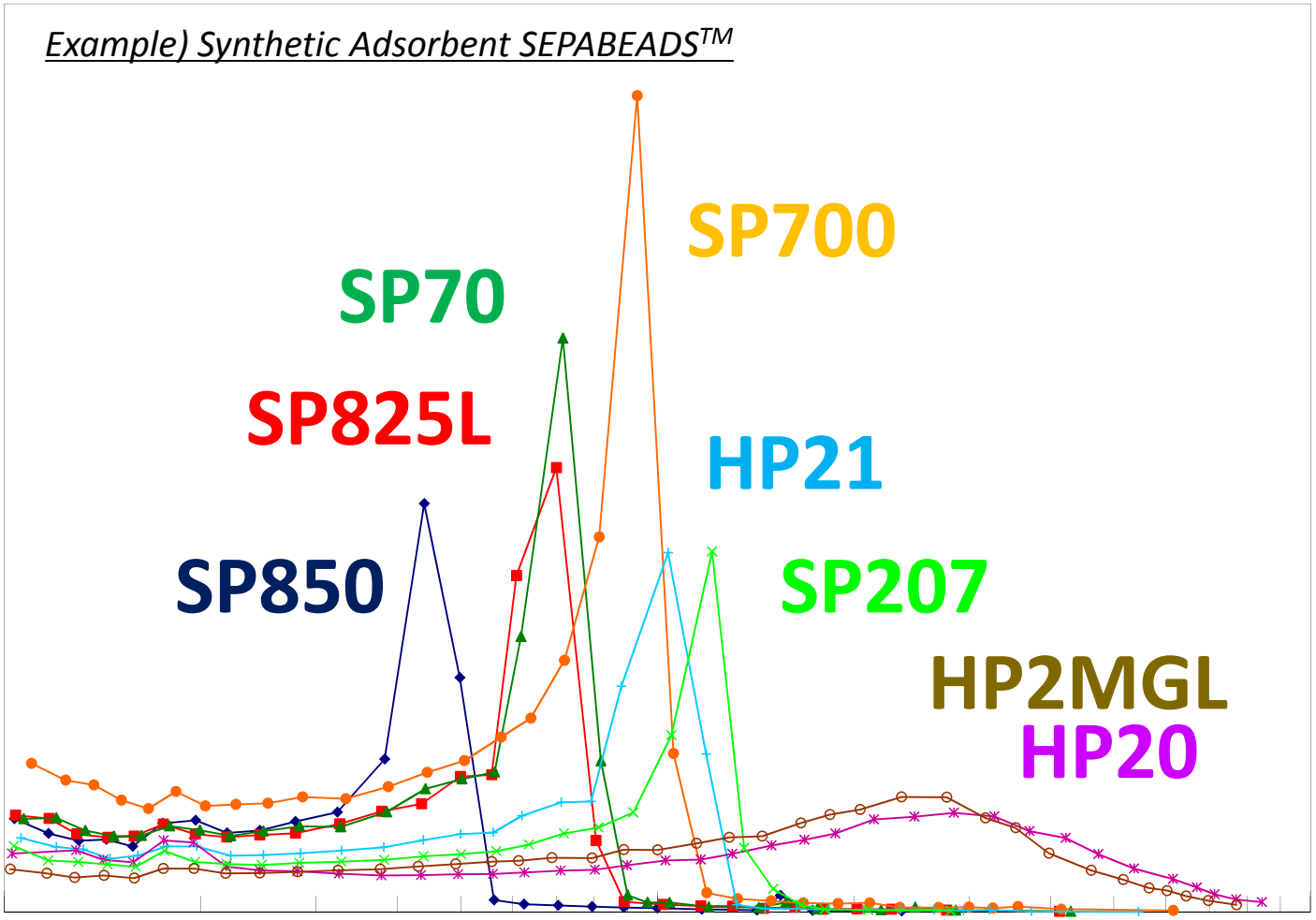
Background: MCC Bioseparation Media

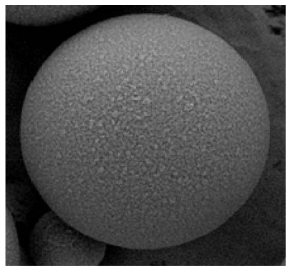
- Technology to control physical properties, such as pore radius, distribution, functionality and surface area
- Variety of Installation Records
  - Installed volume: >2,200m^3 (export 1,400m^3, domestic 800m^3, in past 5 yrs)
  - Customers: Major pharmaceutical companies (in JPN, US, India, UK, Germany, FR, Switzerland, Ireland, etc)
  - Major applications:
    - Human Insulin
    - Lisinopril
    - Cephalosporin C
    - Vancomycin
    - Daptomycin
    - Cefotaxime
    - Imipenem
  - New: Antibody/ Protein

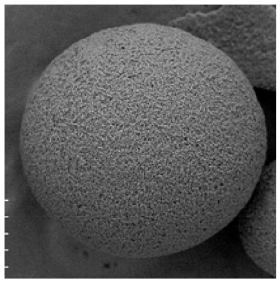


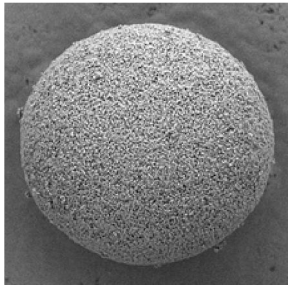
(Reference) Ability to control media properties

Example) Synthetic Adsorbent SEPABEADS™




600-800 Å


1,000-2,000 Å


4,000-5,000 Å

β-Lactums
MW ~600

Macrolides
MW 50- 1500

Peptides, Proteins
MW 1000-100,000

--- New Media for Biopharmaceutical industry ---
Highly productive & efficient purification via high throughput!

- Protein A affinity chromatography
  - MabSpeed™ rP series
- Ion-exchange chromatography
  - ChromSpeed™ series
    - type S strong cation exchanger
    - type Q strong anion exchanger
    - type CM weak cation exchanger
    - type DA weak anion exchanger

--- New Production Facility for Bio-separation Media ---

- Built in February 2013
- Prepared in clean dedicated facility
- Hygiene management by ISO9001

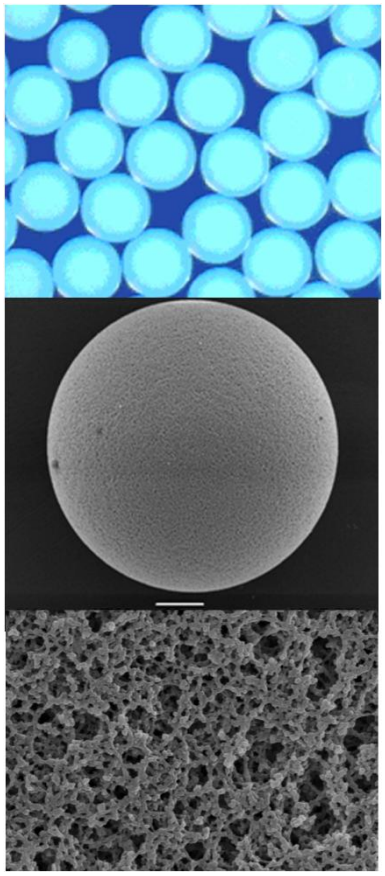
--- Product Lineup for MCC Bio-separation Media--

	Affinity	Ion Exchange			
Product	MabSpeed™	ChromSpeed™			
	rP	S	Q	CM	DA
Matrix	Crosslinked Polymethacrylate				
Ligand / Functionality	*r*Protein-A	$-SO_3^-$	$-N(CH_3)_3^+$	$-COO^-$	$-N(CH_3)_2$
Particle Size (µm)	35, 45	30, 60			

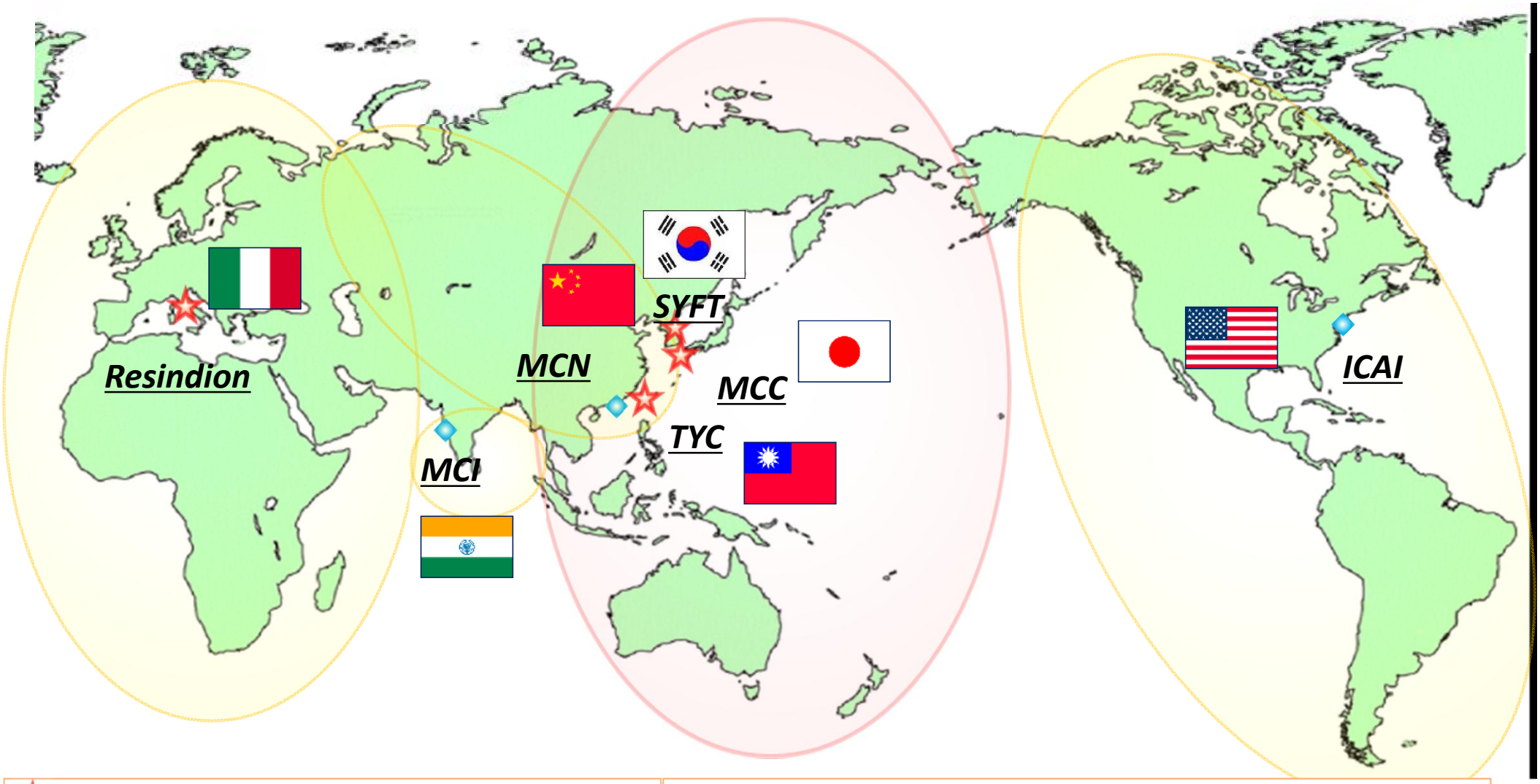
MCC's New Bio-separation Media---

High performance for achieve high throughput purification

- **Spherical and mono-disperse particles**
  - **Easy and reproducible packing**
  - **Low pressure drop**
  - **Non-compressive bed**
- **Rigid and durable matrix**
  - **No volume change**
  - **Longer life**
  - **Wide pH & solvent compatibility**



Sales/Production network



Production/Sales
- Mitsubishi Chemical Co. (Japan)
- Tai Young Chemical Co., Ltd.
- Resindion (Italy)

Sales
- Mitsubishi Chemical China Commerce Ltd.
- Mitsubishi Chemical India Pvt. Ltd.
- ITOCHU Chemicals America Inc.

Affinity chromatography MabSpeed™

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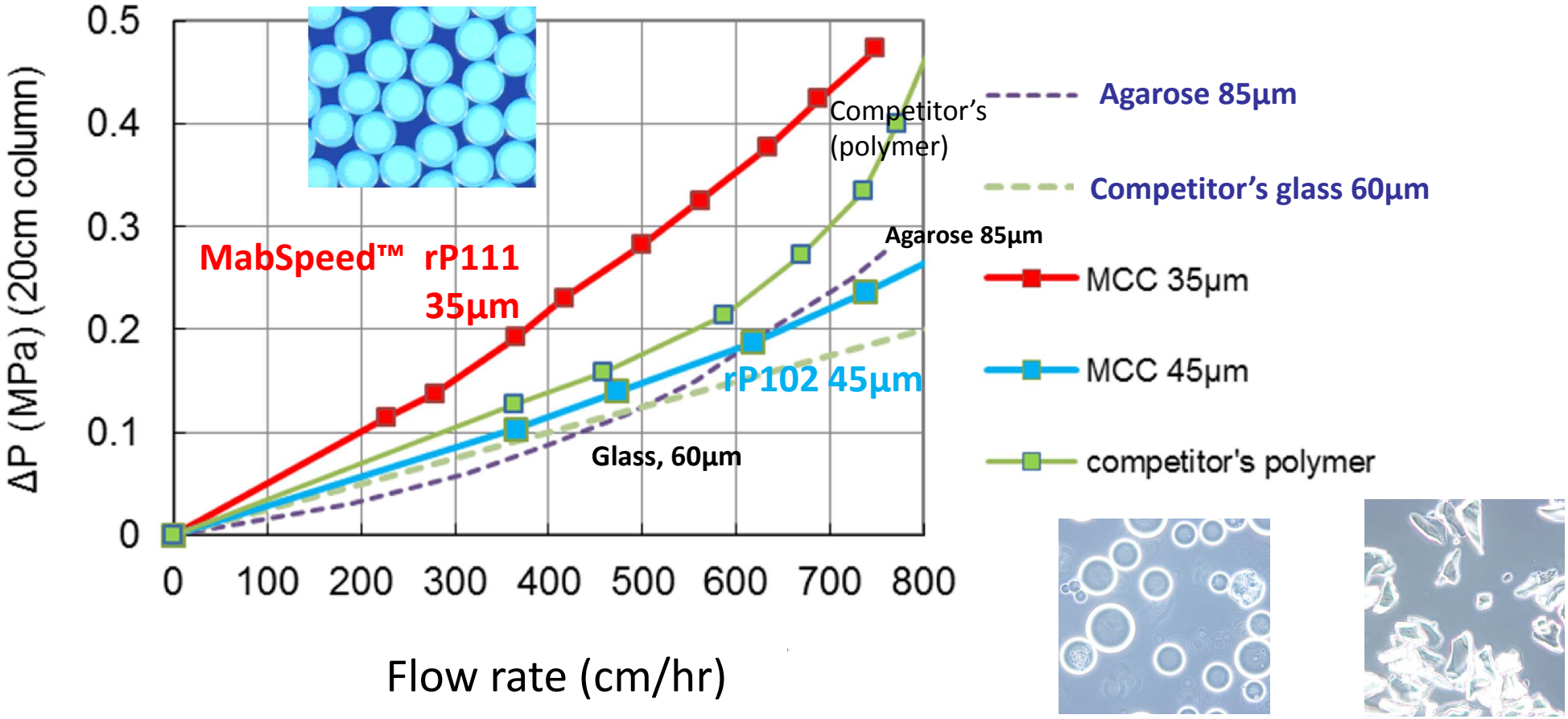
MabSpeed™ rP111 (35 µm)
rP102 (45 µm)

--- MCC *r*Protein-A affinity media --

Mode	Protein-A Affinity	
Product	MabSpeed™	MabSpeed™
	rP102	rP111
Matrix	Crosslinked Polymethacrylate	
Ligand / Functionality	*r*Protein-A	*r*Protein-A
Particle Size (µm)	45	35 (developing)*
DBC (g/L-resin) 10% breakthrough, γ-globulin, r.t. 3min)	24	33

**MabSpeed™ rP111: Samples are available. scaled-pp production began on July 2014*

Hydraulic data



Agarose 85µm

Glass, 60µm

Condition: Column 20 ID x 200 mm; Liquid: water; T@ 25 °C.

Pressure durability

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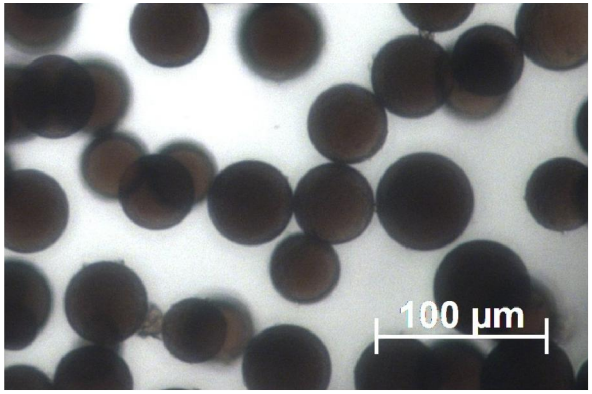
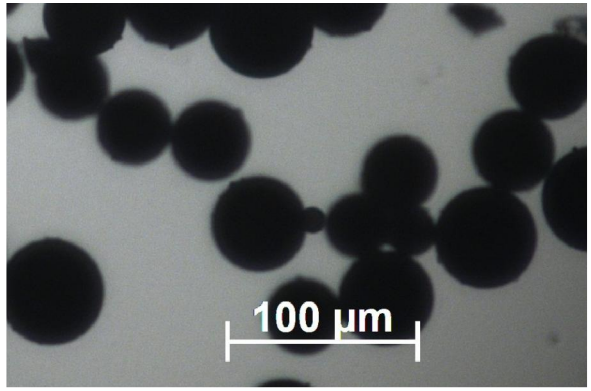
MabSpeed™ 0 G

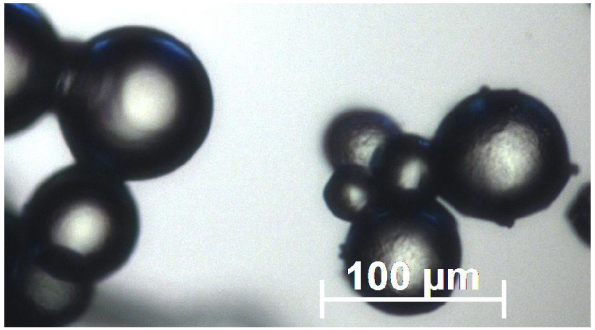


reversible

MabSpeed™ 5,000 G

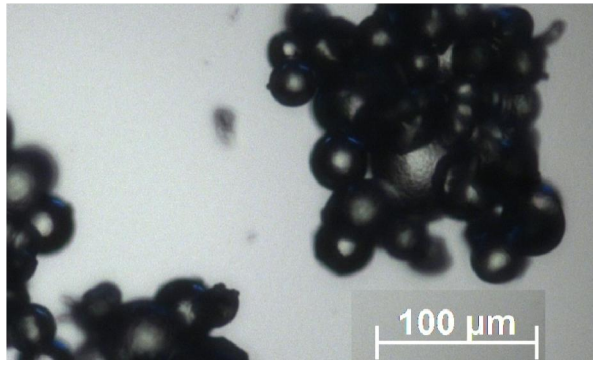


Polysaccharide 0 G



irreversible

Polysaccharide 5,000 G



Breakthrough profiles at various flow rate

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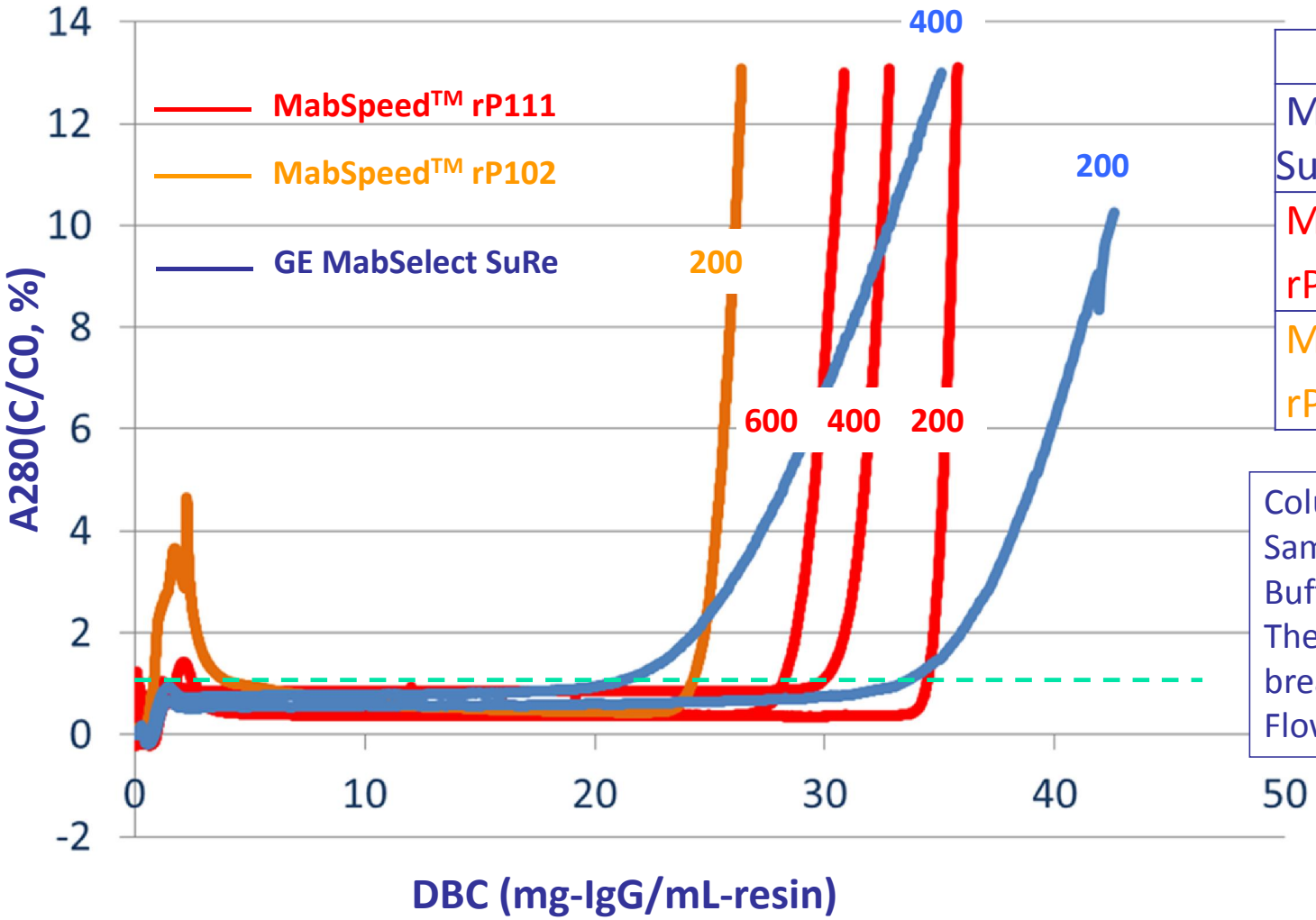

Dynamic binding capacity

(10% BTC)

(cm/h)	200	400	600
MabSelect SuRe	42.3	32.7	-
MabSpeed™ rP111	35.6	32.5	30.4
MabSpeed™ rP102	26.1	24.0	16.2

Column 5mm ID x 200 cm
Sample: 1.0 mg/mL human γ-globulin
Buffer: PBS pH 7.4, Temp: 20 deg C
The DBC was determined at 10% breakthrough.
Flow: 200, 400, 600 cm/h

mAb purification from cell culture

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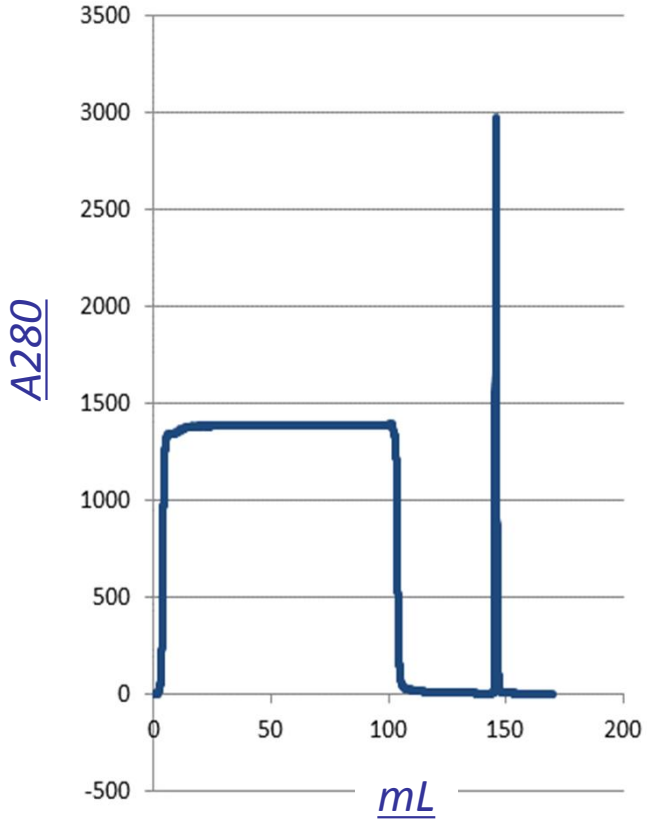
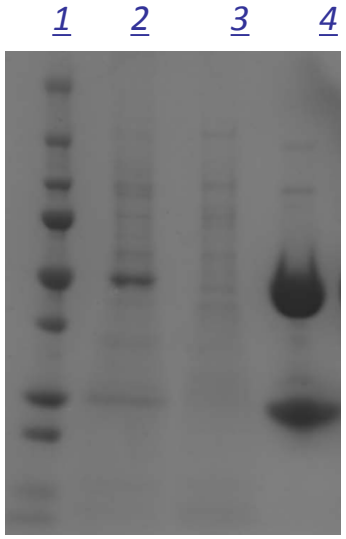


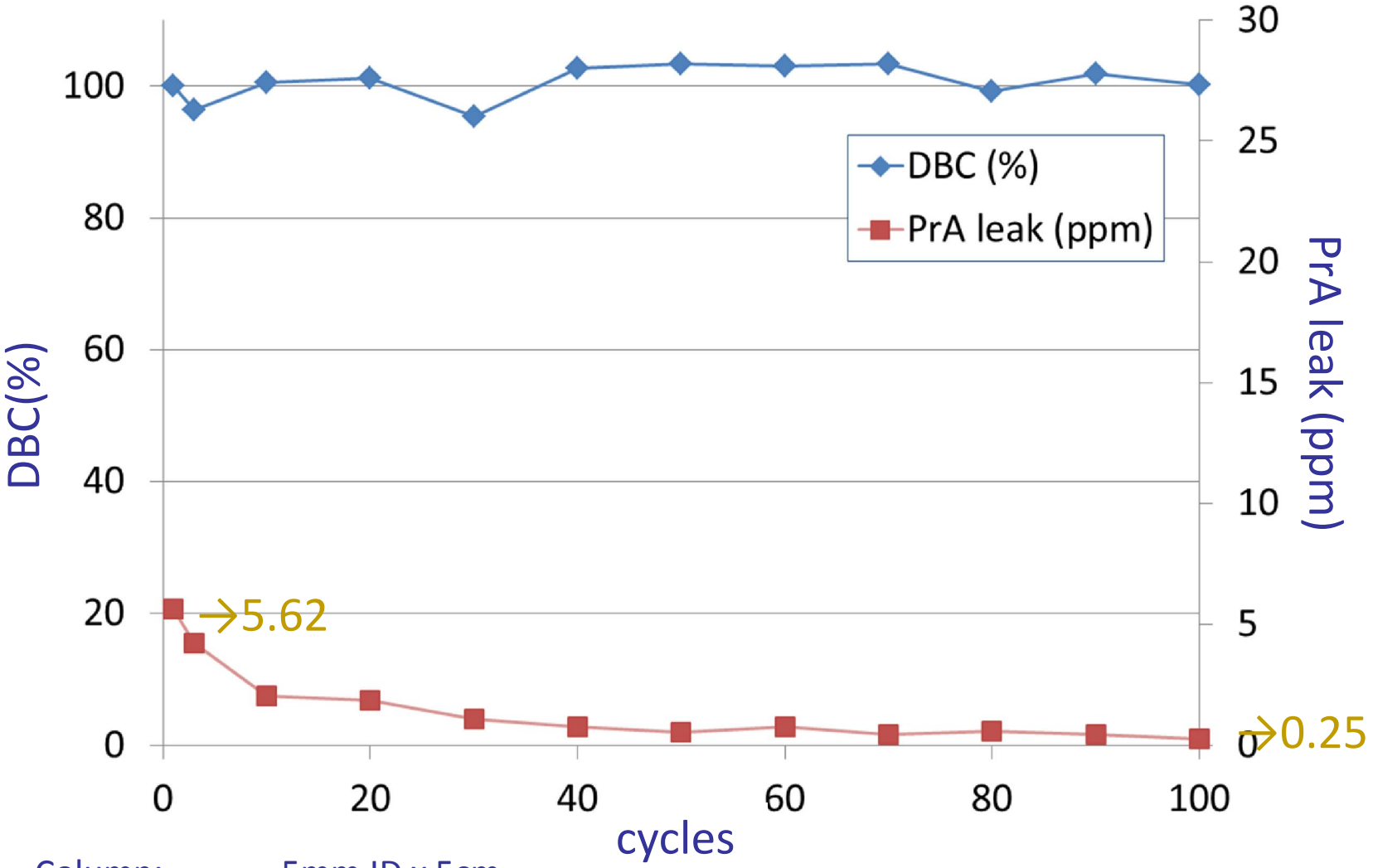


1 Molecular weight marker
2 start material
3 path through
4 mAb fraction

Conditions:
Column: MabSpeed rP111 5mm I.D. x 20cm. (BV:4.0mL)
Binding buffer PBS
Wash buffer PBS
Elution buffer 0.1M Citrate pH3.0
Flow rate 400cm/h
System AKTA avant
Sample CHO cell culture containing 0.1mg/mL mAb, 100mL

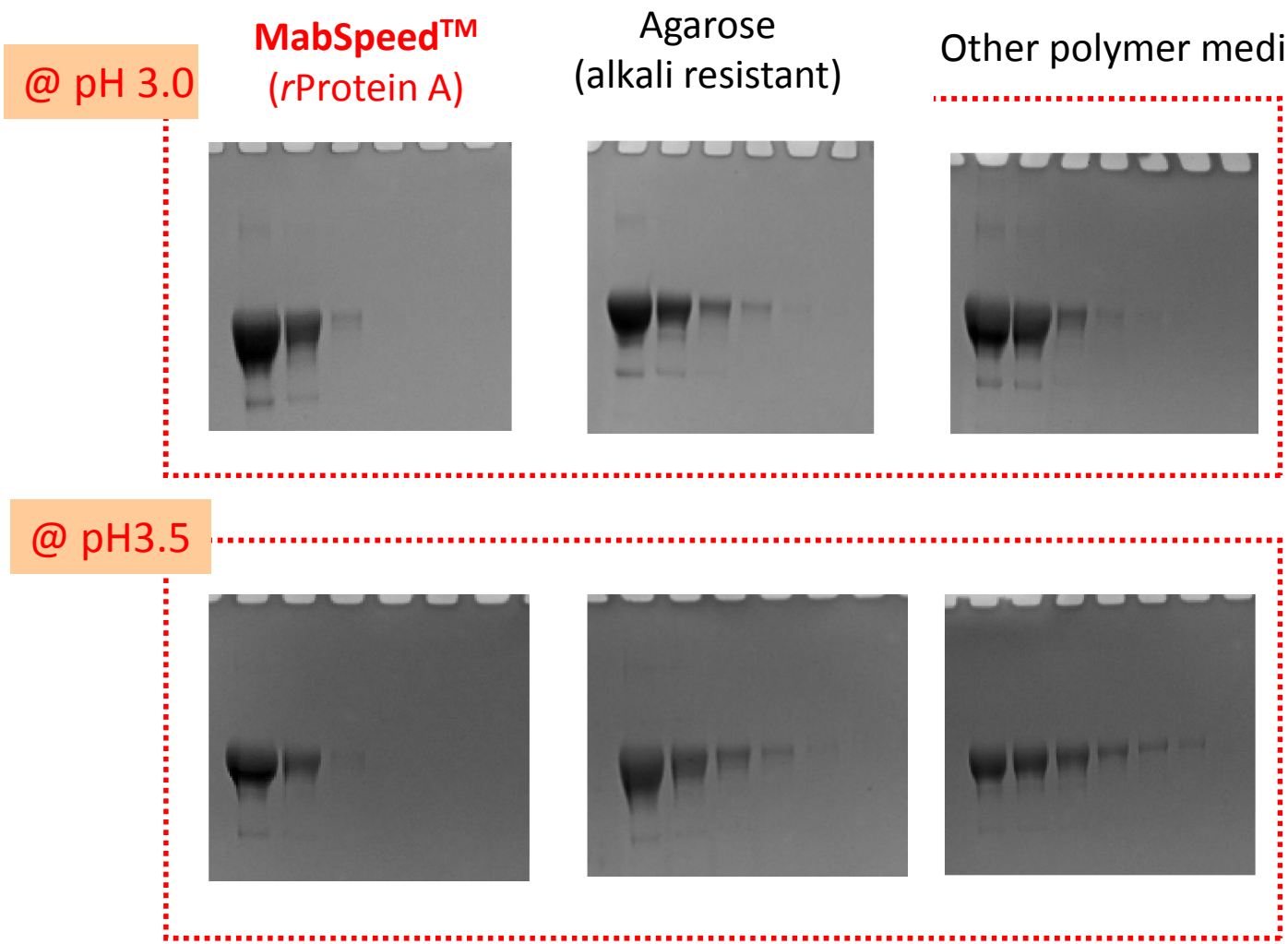
>HCP contamination
■Start material: 116,160 ppm(ng-HCP/mg-IgG)
(HCP: 11,616 ng/mL IgG: 0.1mg/mL)
■IgG fraction
- MabSpeed™ rP111: 9.2 ppm(ng-HCP/mg-IgG)
- MabSelect SuRe 63.7 ppm
>PrA ligand leakage
- MabSpeed™ rP111: 3.4 ppm (ng-PrA/mg-IgG)
>Recovery
- >98% (UV280nm)

CIP durability



Column: 5mm ID x 5cm
Binding: Phsphate bufferd saline(pH7.4)
Elution: 0.1M Sodium Citrate pH3.0
CIP: 0.1M NaOH
Contact: 15min

Effect of elution pH



rP102 data
Spin column 0.5mL resin single cycle (1-6)
1. equilibration buffer A, 6 BV
2. sample load 1mg/mL g-globulin, 2 BV
3. washout buffer A, 6 BV
4. elution buffer B, 4 BV
5. regeneration buffer C, 4 BV

Each Step 500 g x 1 min
buffer A: PBS pH7.4
buffer B: 0.1M sodium citrate pH3.0, 3.5
buffer C: 1M Tris-HCl pH9.0

- Sharp elution for MabSpeed at pH 3.5
- Elution conditions for native rProtein-A ligand can be applied for MabSpeed.

Buffer exchanging profiles

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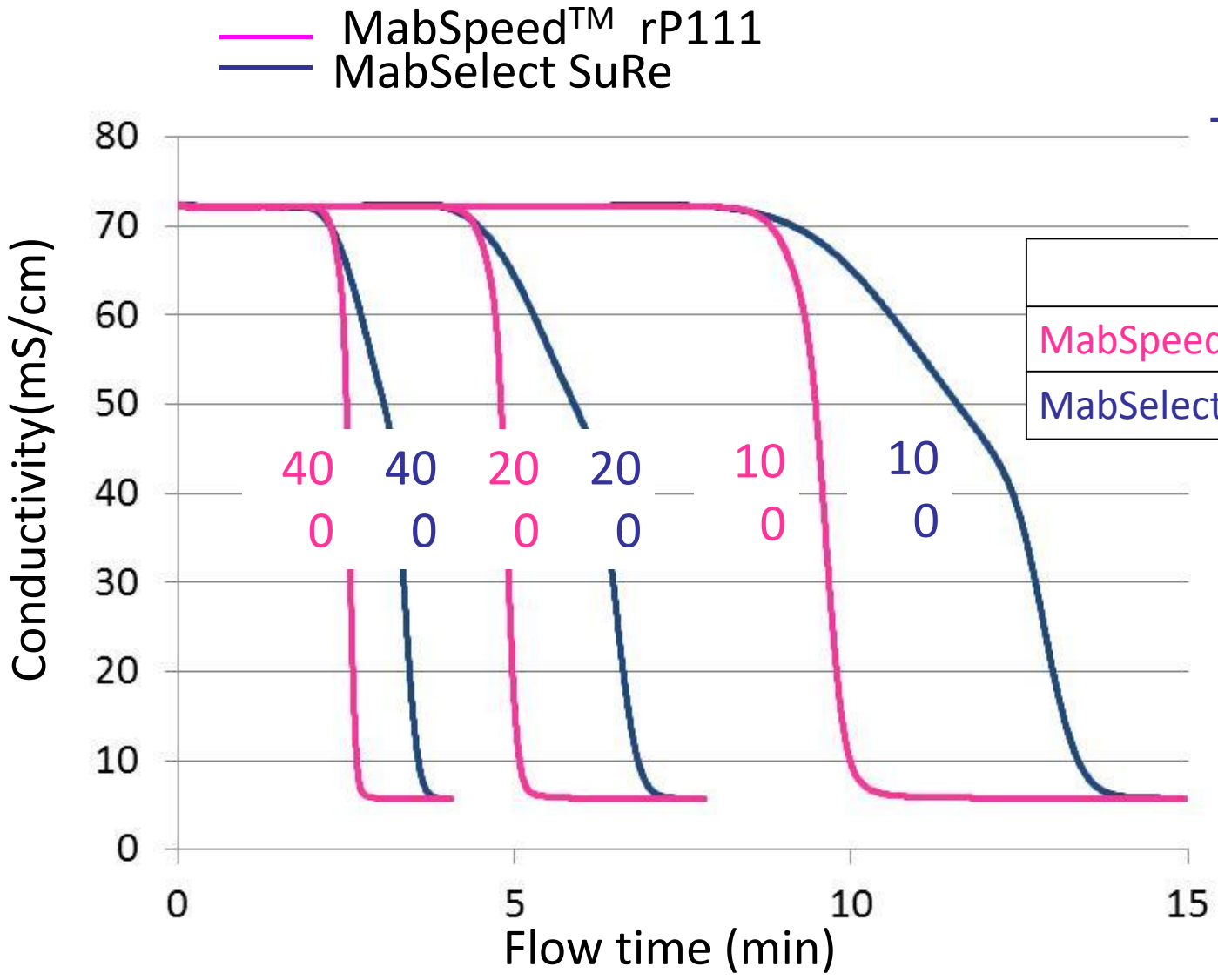
Faster equilibrium of MabSpeed contributes buffer and time savings about 30%



Time to equilibration (min, ~6.0>mS/cm)

(cm/h)	100	200	400
MabSpeed™ rP111	10.7	5.4	2.8
MabSelect SuRe	14.0	7.2	3.8

Column : 10 ID x 200 mm
Base : 0.8M NaCl
Load : 0.05M NaCl,
Temp : 20 deg-C
Flow : 100, 200, 400 cm/h

Productivity Simulation

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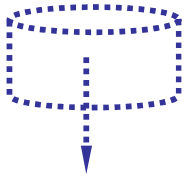
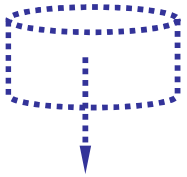
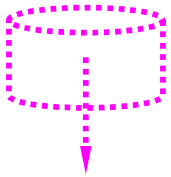
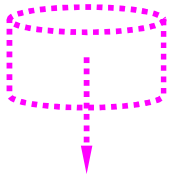
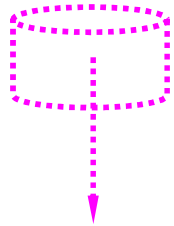
	MabSelect SuRe		MabSpeed rP111		
Flowrate (cm/h)	200cm/h	400cm/h	200cm/h	400cm/h	600cm/h
Column height (cm)					
min/cycle					
(adsorption)	200	62	206	89	56
(misc)	105	60	81	48	37
cycle/day	4.7	11.8	5.0	10.5	15.4
DBC (1% BTC,g/L-resin)	33.3	20.6	34.4	29.8	28.2
Colume volume (L)	1.57	1.57	1.57	1.57	1.57
IgG Production (g/day)	**247**	**382**	**270**	**489**	**681**

MabSpeed™ rP111 results in higher productivity than MabSelect SuRe

Mab purification Chromatography with MCC resin

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Cell culture

↓

Protein A affinity : MabSpeed™ rP111

capture,
removal of aggregate, HCP,

Column	MabSpeed™ rP111 10x150mmH(BV: 12mL)
Sample	Clarified CHO cell culture, 0.5mg/mL mab (20.8mg/mL-resin)
Equiliburation buffer	20mM Sodium Phosphate, 150mM NaCl, pH7.4
Elution buffer	20mM Sodium Citrate, pH3.4
Flow	300 cm/h, R.T. 3min
Temp.	20 deg-C

↓

Cation exchange : ChromSpeed™ S101

removal of agg, HCP, PrA

Column	ChromSpeed™ S101 5x200mmH(BV: 4mL)
Sample	mab (51.3 mg/mL-resin) after purification on MabSpeed™ rP111
Start buffer	20mM Sodium acetate, pH5.5
Elution buffer	20mM Sodium acetate, 1M NaCl, pH5.5
Flow	300 cm/h, R.T. 4min
Gradient	5% (10CV), 15% (20CV), 100% (10CV)
Temp.	20 deg-C

↓

Anion exchange : ChromSpeed™ Q101

removal of DNA, endotoxin, …

Column	ChromSpeed™ Q101 5x50mmH(BV: 1mL)
Sample	mab (192 mg/mL-resin), eluate after ChromSpeed™ S101, pH adjusted to 6.0
Start buffer	20mM Sodium acetate, pH6.0
Flow	300 cm/h, R.T. 1min
Temp.	20 deg-C

Three step process	Accumulated yield (%)	Dimers and aggregate (%)	Protein A (ppm)	HCP (ppm)
Start material	100	-	-	35717
MabSpeed™ rP111	98	<0.5%	4.4	3.1
ChromSpeed™ S101	90	<0.5%	2.6	<0.5
ChromSpeed™ Q101	88	<0.5%	2.4	<0.5

Ion exchange chromatography ChromSpeed™

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ChromSpeed™ S103, Q103, CM103, and DA103 (60 µm)
ChromSpeed™ S101, Q101, CM101, and DA101 (30 µm)

Batch adsorption capacity

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ChromSpeed	Functional group	Particle diameter (μm)	Salt-splitting capacity (eq/L-resin)	Human γ-globulin Batch adsorption capacity (g/L-resin)
S103	$-SO_3^-$	60	0.09	125
S101		30	0.08	140
Q103	$-N(CH_3)_3^+$	60	0.08	124
Q101		30	0.08	130
CM103	$-COO^-$	60	0.11	114
CM101		30	0.10	120
DA103	$-N(CH_3)_2$	60	0.13	81
DA101		30	0.11	98

SBC condition: S- and CM- type: Human γ-globulin 2.5 g/L, 20 mM citrate (pH 5.2), 25 ℃
Q- and DA- type Human γ-globulin 2.5 g/L, 20 mM Tris-HCl (pH 9.0), 25 ℃

Hydraulic properties of ChromSpeed™

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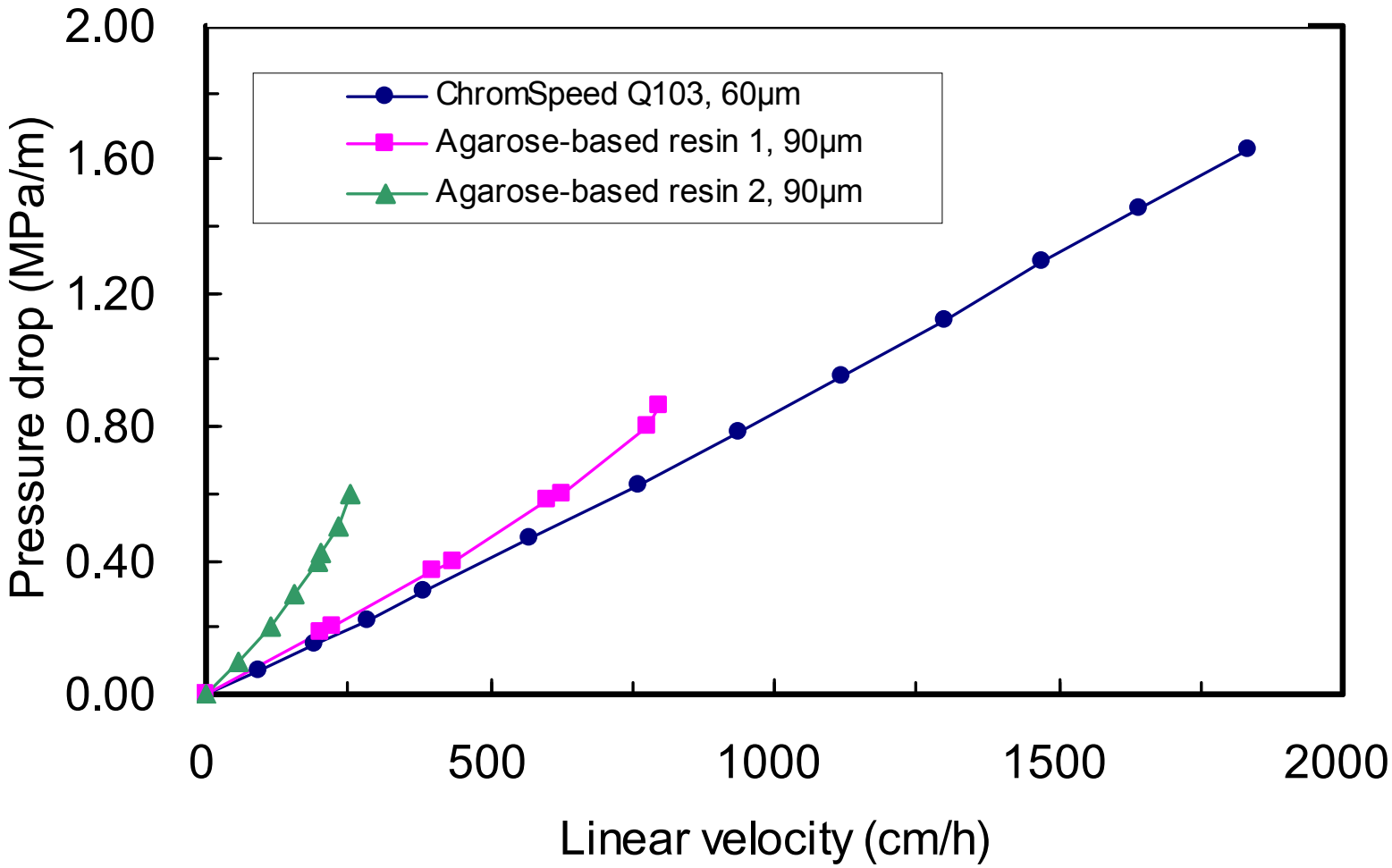
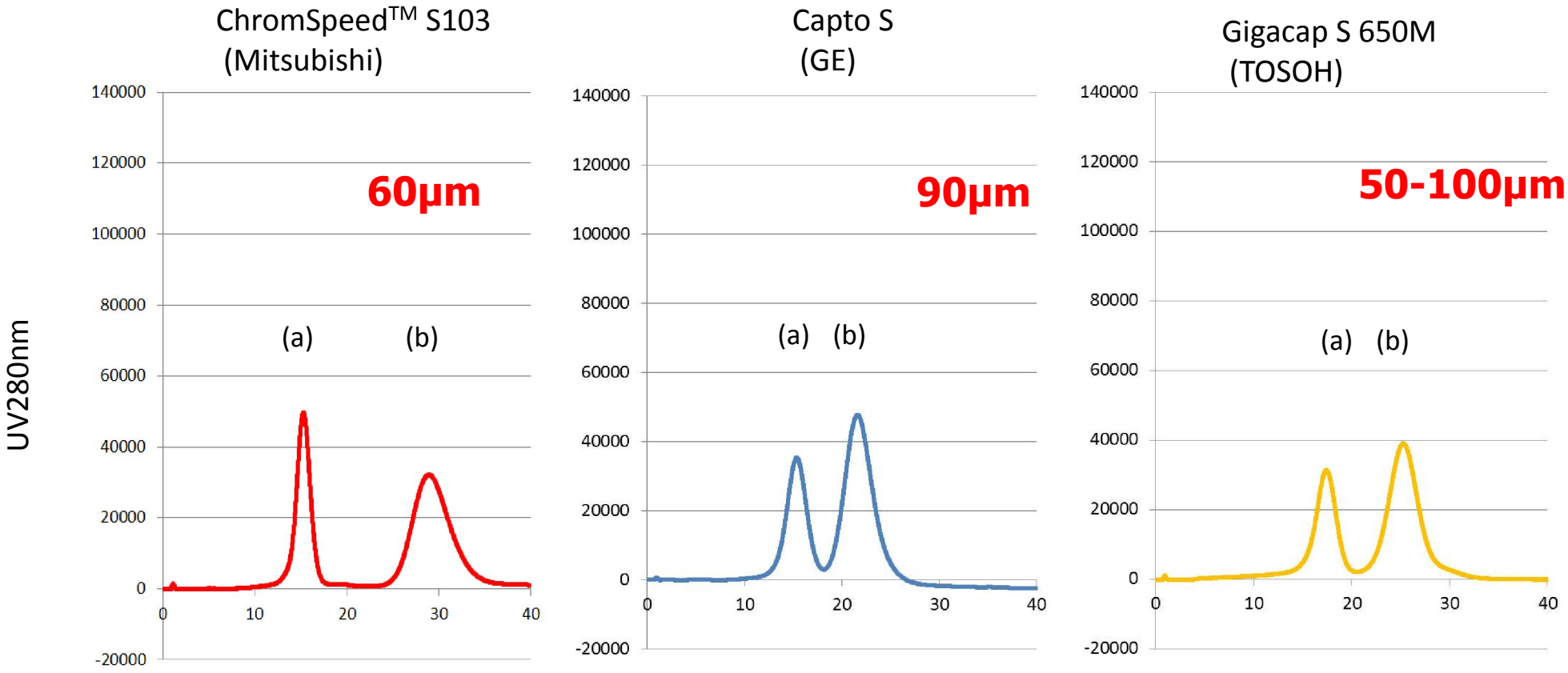
Excellent Hydraulic Property

--- No bed compression at 1,800cm/hr linear velocity ---



1-1) S103 (60µm) vs competitors'



Conditions:
Column 100 x 5mm I.D. (BV:2.0mL)
Eluent A 20mM Sodium Phosphate (pH6.5)
Eluent B A + 1.0M NaCl
Flow rate 1.0mL/min (R.T. 2min)
Gradient 0-100% B over 60min
Sample (a) Cytochrome C + (b) lysozyme = 125 / 125ug / 50uL
(pI) (9.3) (11.0)

Effect of IgG Concentration

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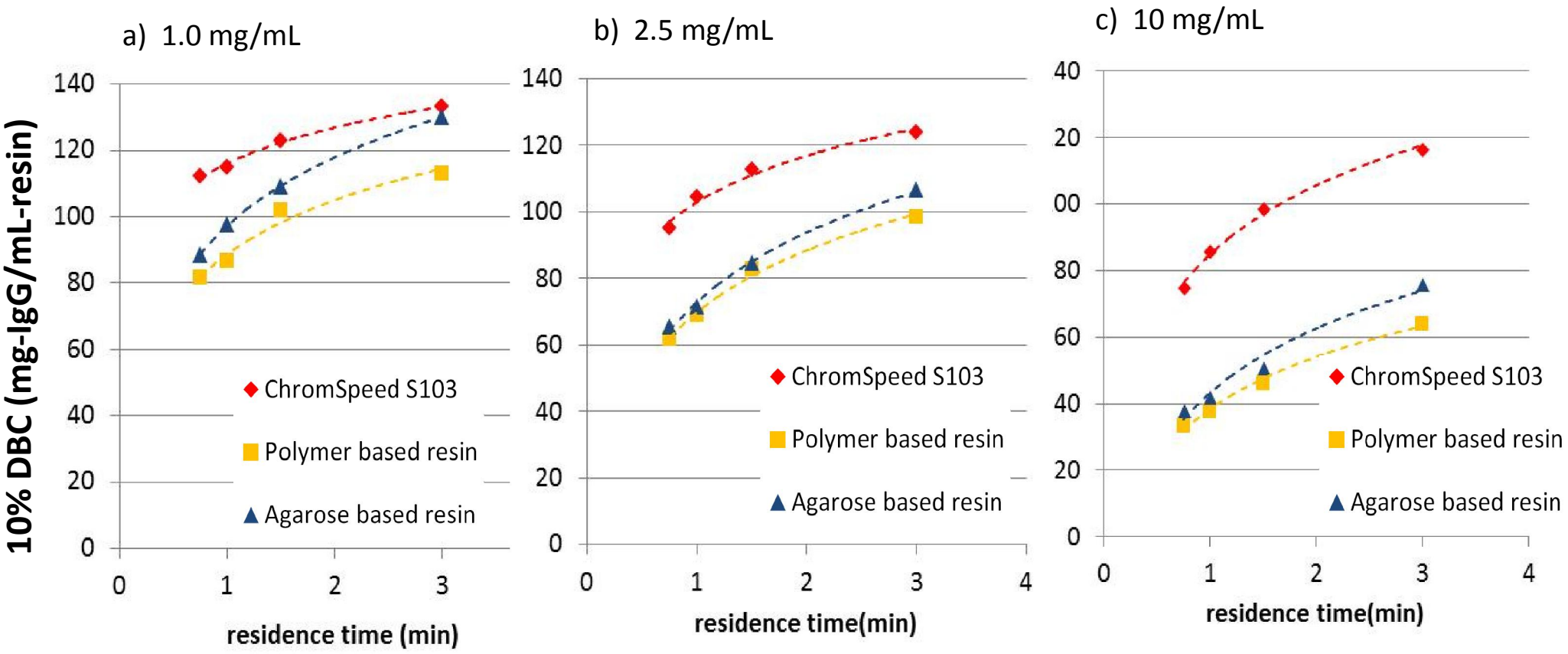
Column	5 x 100 mmH (BV:2.0mL)
Buffer	20mM Sodium Acetate (pH5.5)
Flow rate	200, 400, 600, 800 cm/h (R.T. 3.0, 1.5, 1.0, 0.75 min)
Sample	a) 1.0, b) 2.5, c) 10 mg/mL gamma-globulin (IgG)



- The highest dynamic binding capacity was observed for ChromSpeed™ S103.
- Moreover, DBC showed less dependence on the IgG concentration for ChromSpeed™ S103.

Durability of ChromSpeed™ S103

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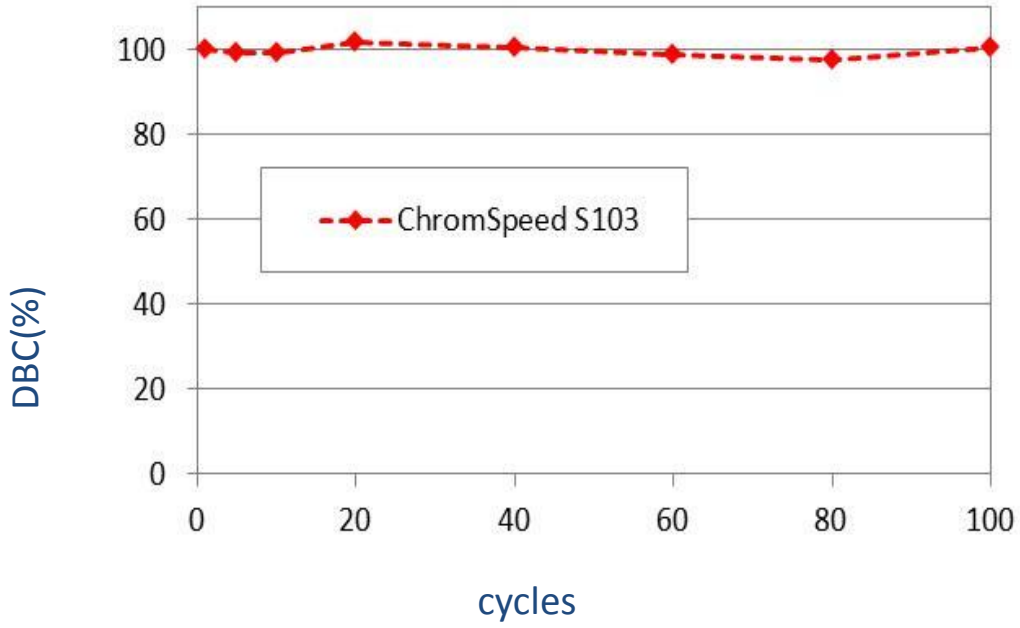
THE KAITEKI COMPANY
Mitsubishi Chemical Holdings Group

- SBC *1 >120 mg-IgG/mL-resin
- Recovery*2 >99% (IgG)
- Pressure Drop*3 < 1.5MPa/m at 1,000 cm/h
- Alkaline tolerance*4 100cycles of 0.5M NaOH exposure O.K.



*1 2.5 g/L, 20 mM Sodium acetate (pH 5.5), 20 deg-C
*2 1.0 g/L, 20 mM Sodium acetate (pH 5.5), 20 deg-C
*3 10x200mmH, 150mM NaCl, room temperature
*4 0.5M NaOH, 15min/cycle, 20deg-C, DBC: IgG at 10 % breakthrough

1-2) S103 (60µm) vs S101 (30µm)

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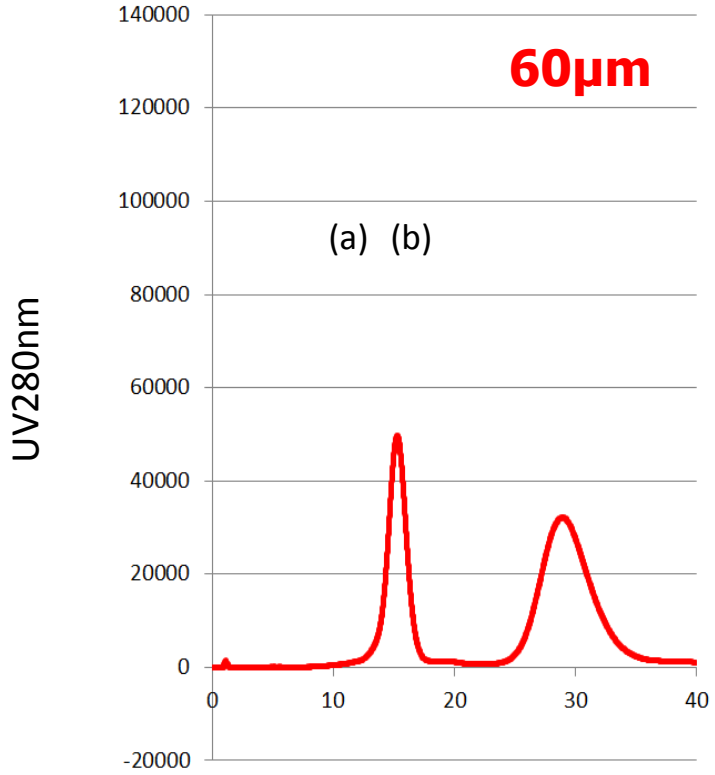
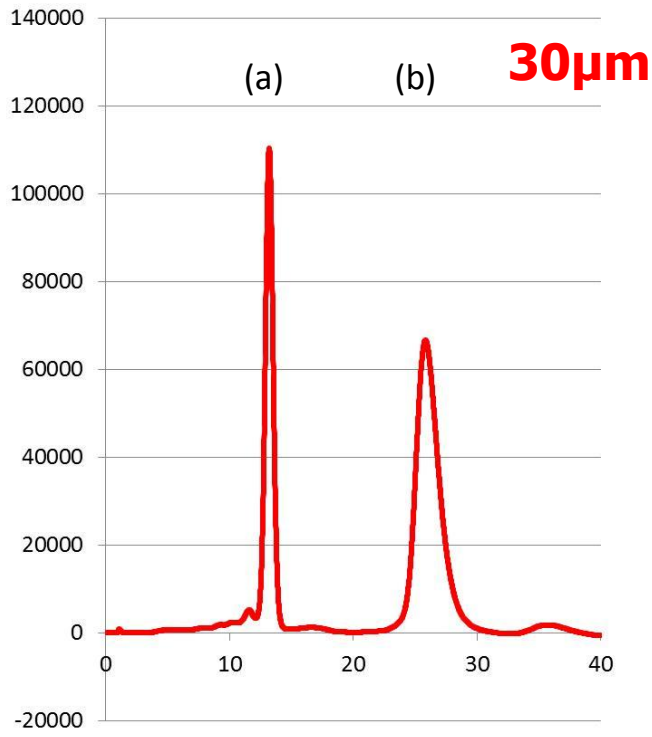
ChromSpeed™ S103
(Mitsubishi)



ChromSpeed™ S101
(Mitsubishi)



Conditions:
Column 100 x 5mm I.D. (BV:2.0mL)
Eluent A 20mM Sodium Phosphate (pH6.5)
Eluent B A + 1.0M NaCl
Flow rate 1.0mL/min (R.T. 2min)
Gradient 0-100% B over 60min
Sample (a) Cytochrome C + (b) lysozyme = 125 / 125ug / 50uL
(pI) (9.3) (11.0)

1-3) S101 (30µm) vs competitors'

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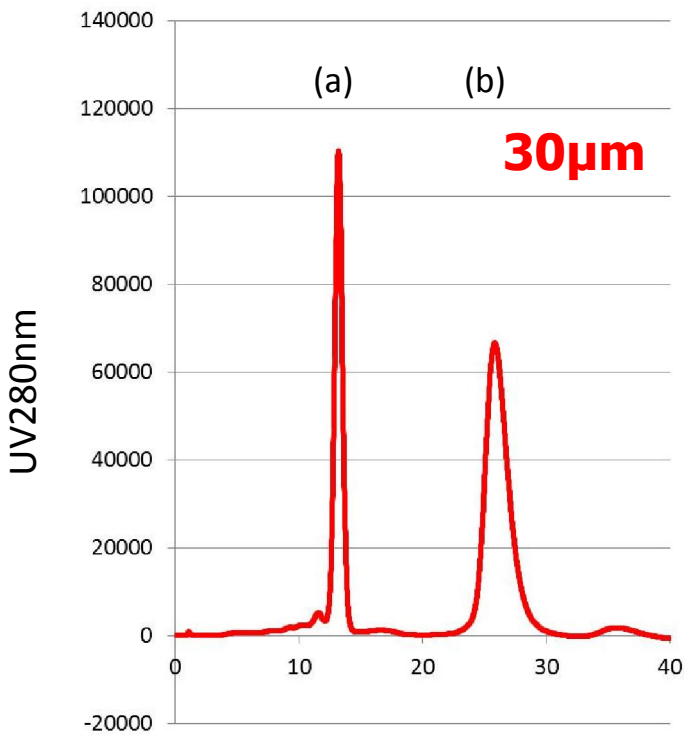
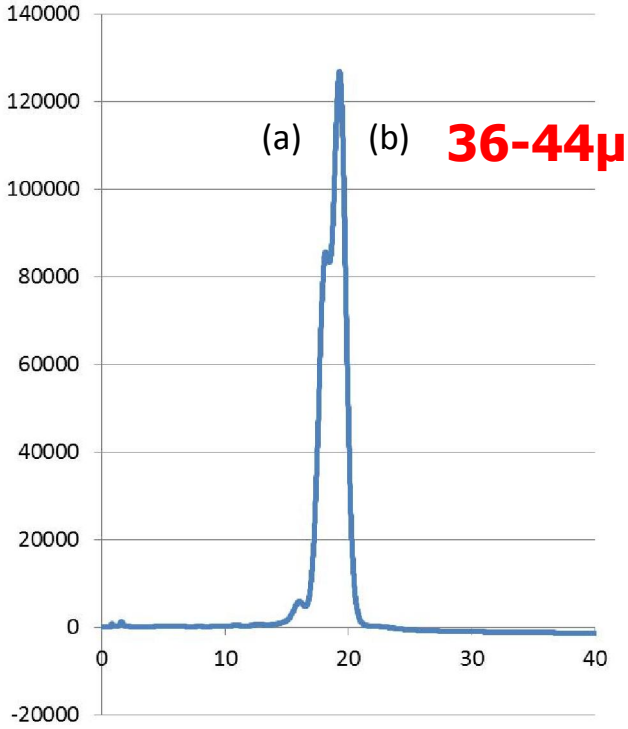
ChromSpeed™ S101
(Mitsubishi)



Capto SP ImpRes
(GE)



Conditions:

Column	100 x 5mm I.D. (BV:2.0mL)
Eluent A	20mM Sodium Phosphate (pH6.5)
Eluent B	A + 1.0M NaCl
Flow rate	1.0mL/min (R.T. 2min)
Gradient	0-100% B over 60min
Sample	(a) Cytochrome C + (b) lysozyme = 125 / 125ug / 50uL
(pI)	(9.3) (11.0)

2-1) Q103 (60µm) vs competitors'

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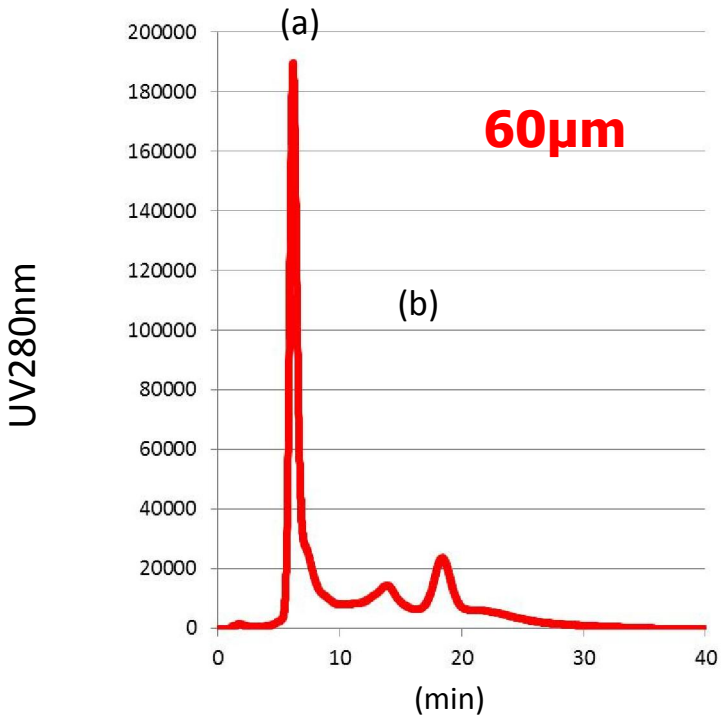
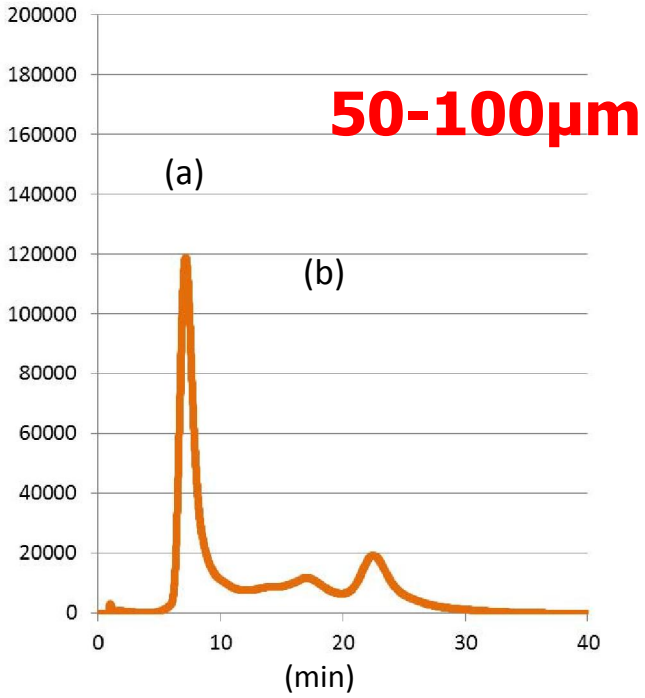
ChromSpeed™ Q103
(Mitsubishi)



Gigacap Q
(TOSOH)



Conditions:
Column 100 x 5mm I.D. (BV 2mL)
Eluent A 50mM Tris-HCl (pH8.5)
Eluent B A + 1.0M NaCl
Flow rate 1.0mL/min (R.T. 2min)
Gradient 0-100% B over 60min
Sample (a) Myoglobin + (b) Trypsin inhibitor = 250/50ug / 25uL
(pI) (7.5) (4.5)

2-2) Q103 (60µm) vs Q101 (30µm)

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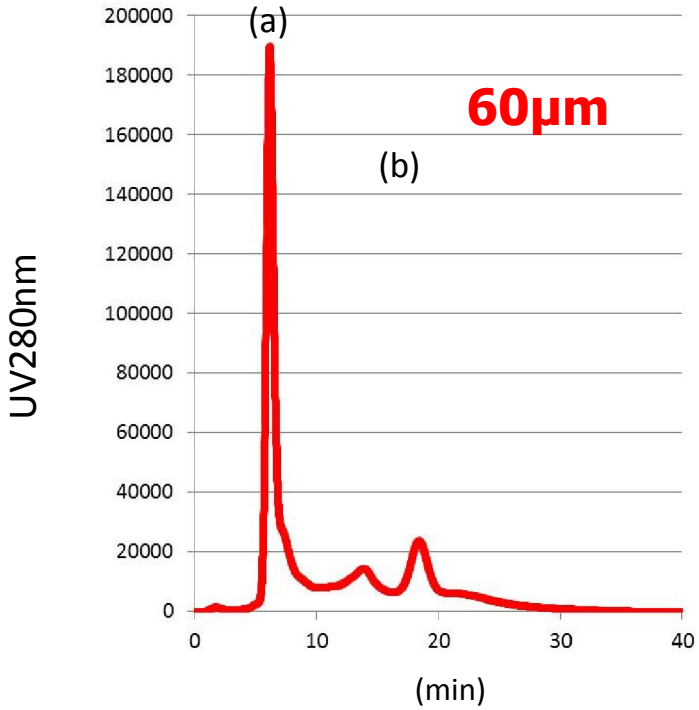
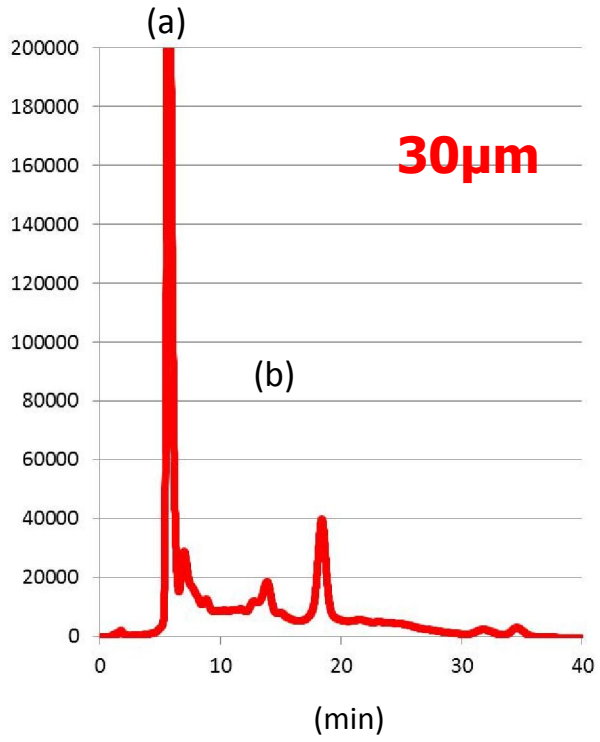
ChromSpeed™ Q103
(Mitsubishi)



ChromSpeed™ Q101
(Mitsubishi)

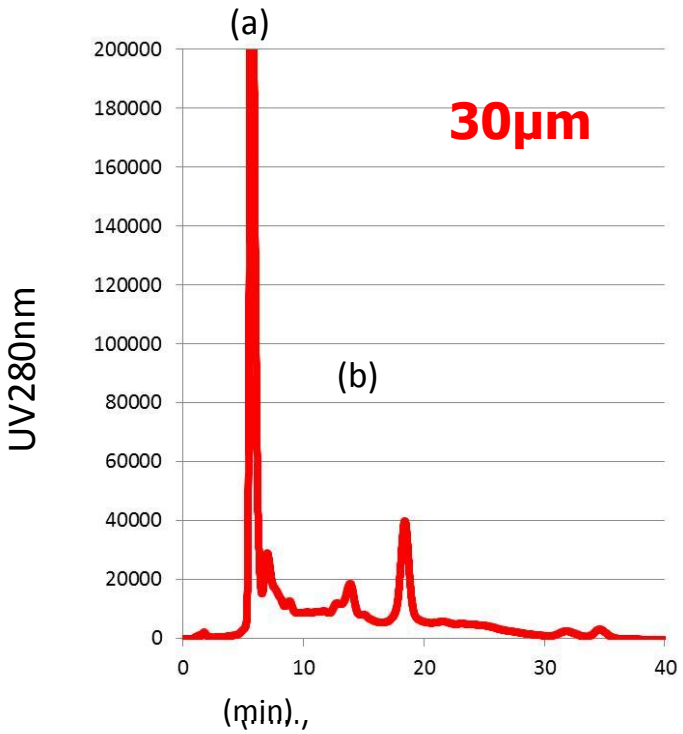


Conditions:

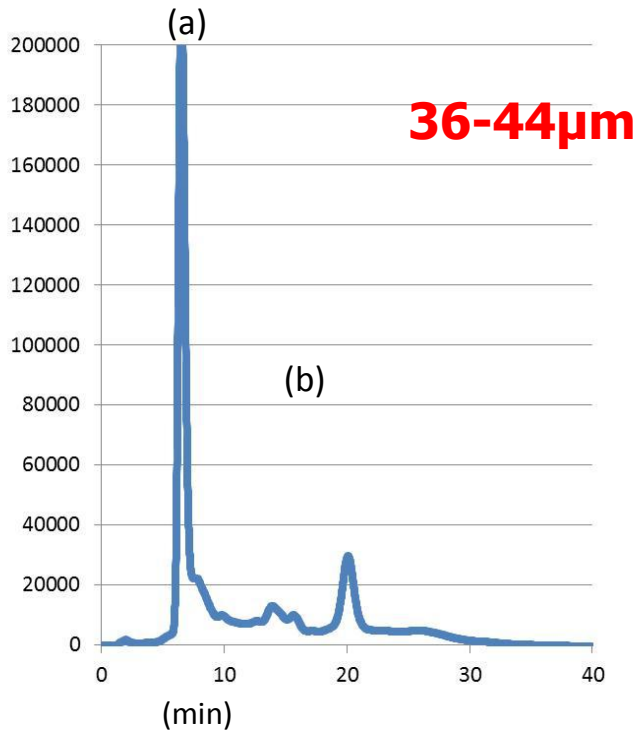
Column 100 x 5mm I.D. (BV 2mL)
Eluent A 50mM Tris-HCl (pH8.5)
Eluent B A + 1.0M NaCl
Flow rate 1.0mL/min (R.T. 2min)
Gradient 0-100% B over 60min
Sample (a) Myoglobin + (b) Trypsin inhibitor = 250/50ug / 25uL
(pI) (7.5) (4.5)

2-3) Q101 (30µm) vs competitors'

ChromSpeed™ Q101
(Mitsubishi)



Capto Q ImpRes
(GE)

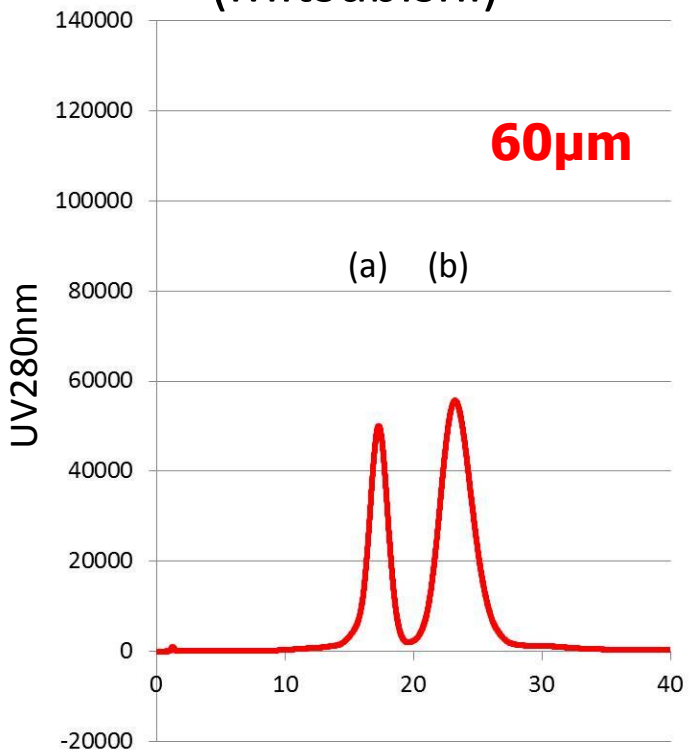


Conditions:
Column 100 x 5mm I.D. (BV 2mL)
Eluent A 50mM Tris-HCl (pH8.5)
Eluent B A + 1.0M NaCl
Flow rate 1.0mL/min (R.T. 2min)
Gradient 0-100% B over 60min
Sample (a) Myoglobin + (b) Trypsin inhibitor = 250/50ug / 25uL
(pI) (7.5) (4.5)

3-1) CM103 (60µm) vs competitors'

MITSUBISHI CHEMICAL

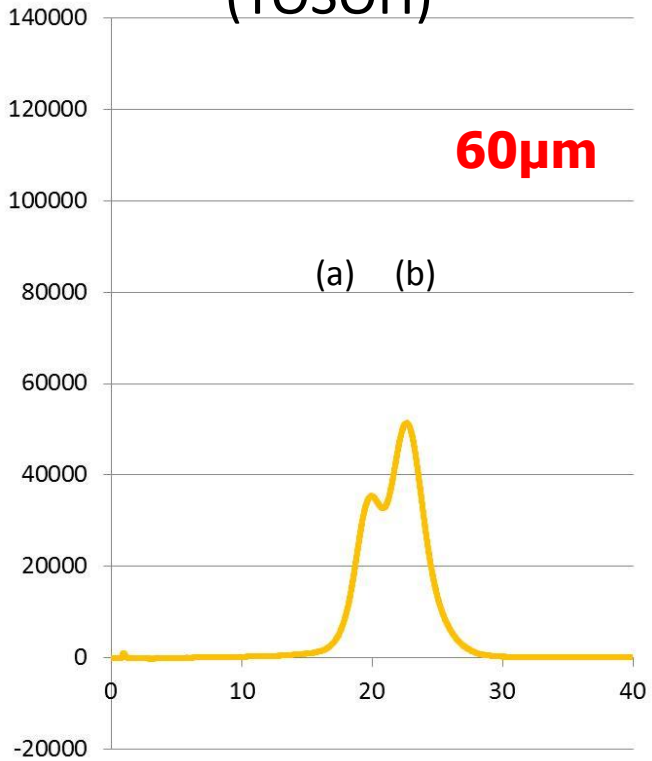
ChromSpeed™ CM103 (Mitsubishi)



Capto CM (GE)

Comparable product unavailable

GigaCap CM 650M (TOSOH)

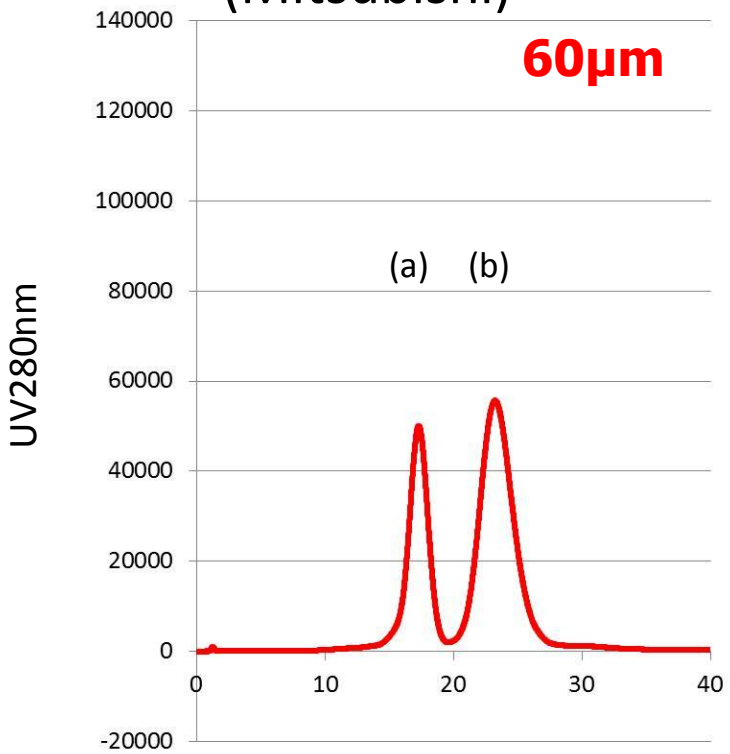


Conditions:
Column 100 x 5mm I.D. (BV:2.0mL)
Eluent A 20mM Sodium Phosphate (pH6.5)
Eluent B A + 1.0M NaCl
Flow rate 1.0mL/min (R.T. 2min)
Gradient 0-100% B over 60min
Sample (a) Cytochrome C + (b) lysozyme = 125 / 125ug / 50uL
(pI) (9.3) (11.0)

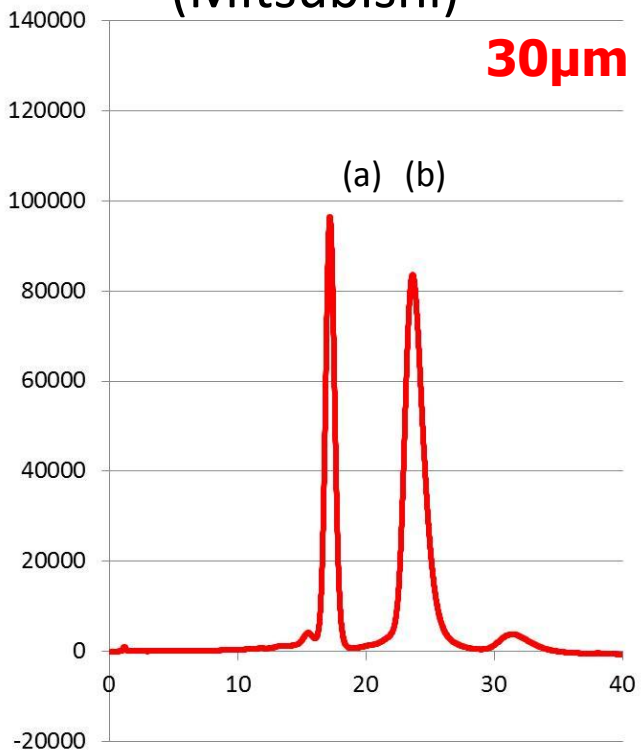
3-2) CM103 (60µm) vs CM101 (30µm)

MITSUBISHI CHEMICAL

ChromSpeed™ CM103 (Mitsubishi)



ChromSpeed™ CM101 (Mitsubishi)



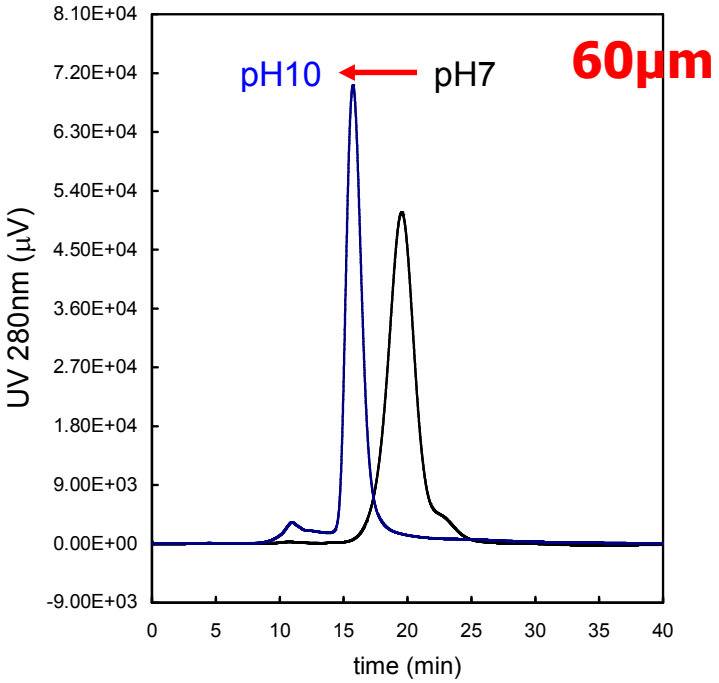
Conditions:

Column 100 x 5mm I.D. (BV:2.0mL)
Eluent A 20mM Sodium Phosphate (pH6.5)
Eluent B A + 1.0M NaCl
Flow rate 1.0mL/min (R.T. 2min)
Gradient 0-100% B over 60min
Sample (a) Cytochrome C + (b) lysozyme = 125 / 125ug / 50uL
(pI) (9.3) (11.0)

4-1) DA103 (60μm) vs competitors'

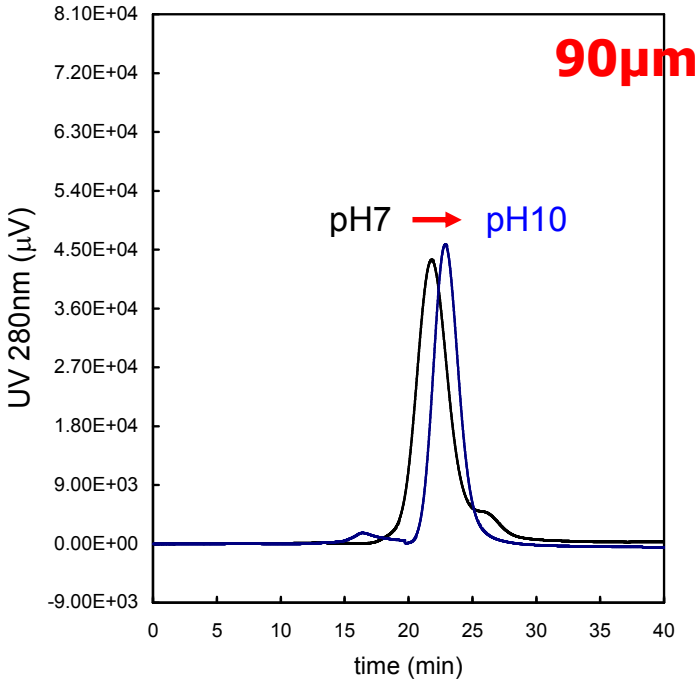
MITSUBISHI CHEMICAL

ChromSpeed DA103
(Mitsubishi)



Conditions: Column, 100 x 9mmI.D. (6.4ml);
Black Eluent A, 20mM sodium phosphate (pH7.0);
Eluent B, A + 1.0M NaCl;
Blue Eluent A, 20mM Glycine-NaOH (pH10.0);
Eluent B, A + 1.0M NaCl;
Flow rate, 1.0ml/min (94cm/h); Gradient, 0-50% B over 30min.
Samples: α-Lactalbumin, 160μg / 160μl

Capto DEAE
(GE)



Conditions: Column, 5ml;
Black Eluent A, 20mM sodium phosphate (pH7.0);
Eluent B, A + 1.0M NaCl;
Blue Eluent A, 20mM Glycine-NaOH (pH10.0);
Eluent B, A + 1.0M NaCl;
Flow rate, 0.786ml/min; Gradient, 0-50% B over 30min.
Samples: α-Lactalbumin, 125μg / 125μl

4-2) DA103 (60µm) vs DA101 (30µm)

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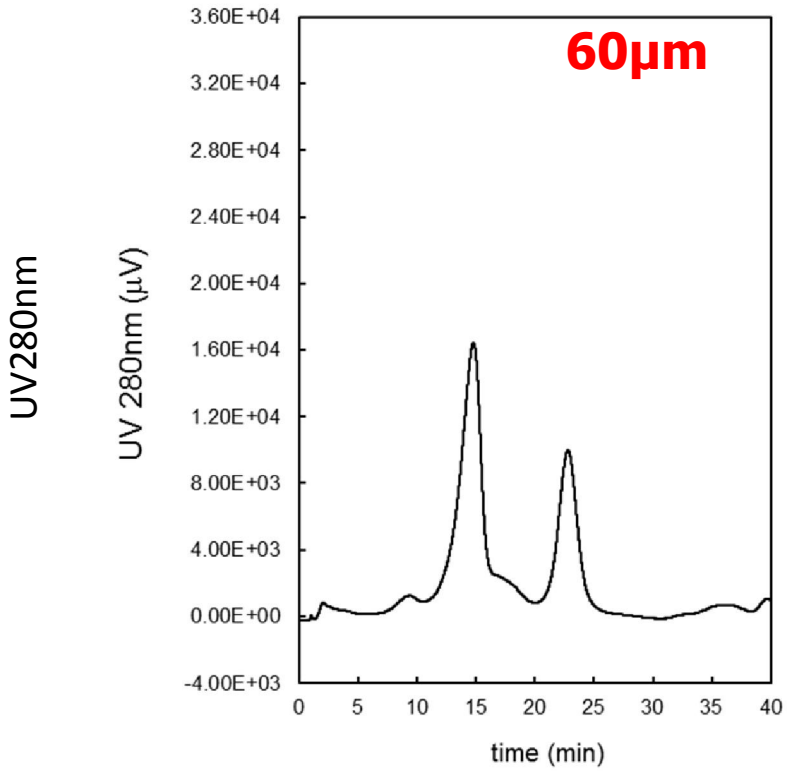
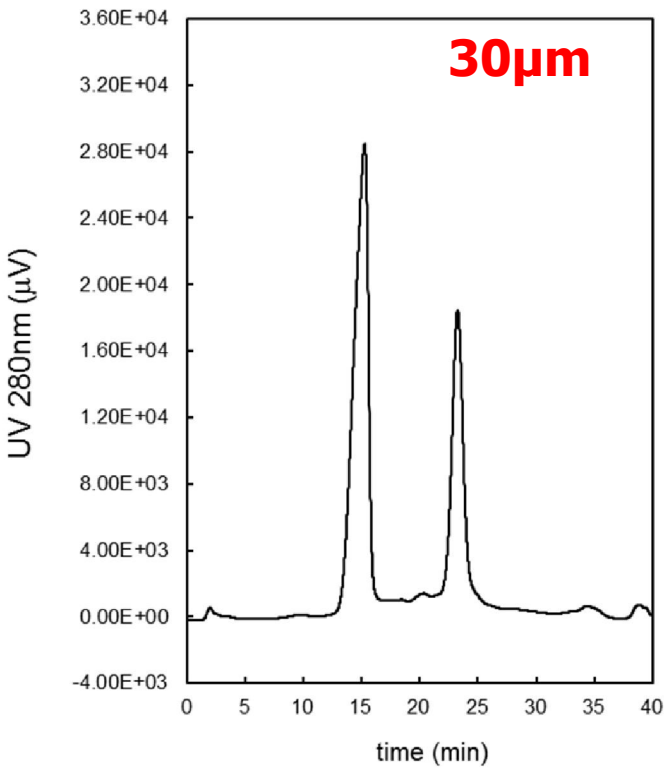
ChromSpeed™ DA103
(Mitsubishi)



ChromSpeed™ DA101
(Mitsubishi)



Conditions: Column, 50 x 5mmI.D. (I1301, 60mm);
Eluent A, 20mM sodium phosphate (pH7.0);
Eluent B, A + 1.0M NaCl;
Flow rate, 0.5ml/min (150cm/h); Gradient, 0-50% B over 30min.
Samples: a-Lactalbumin / Trypsin inhibitor = 25mg / 50mg / 25ml

Product Details

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- Standard package size: 25 / 100 / 1000 mL
- Slurry in 20%-Ethanol

- Documentations
  - COA
  - MSDS
  - RSF (NDA required)

- Screening columns available for ChromSpeed™ 103 Series
- 1 mL columns
- 5 mL columns (soon to be on market)

Thank you

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Reference

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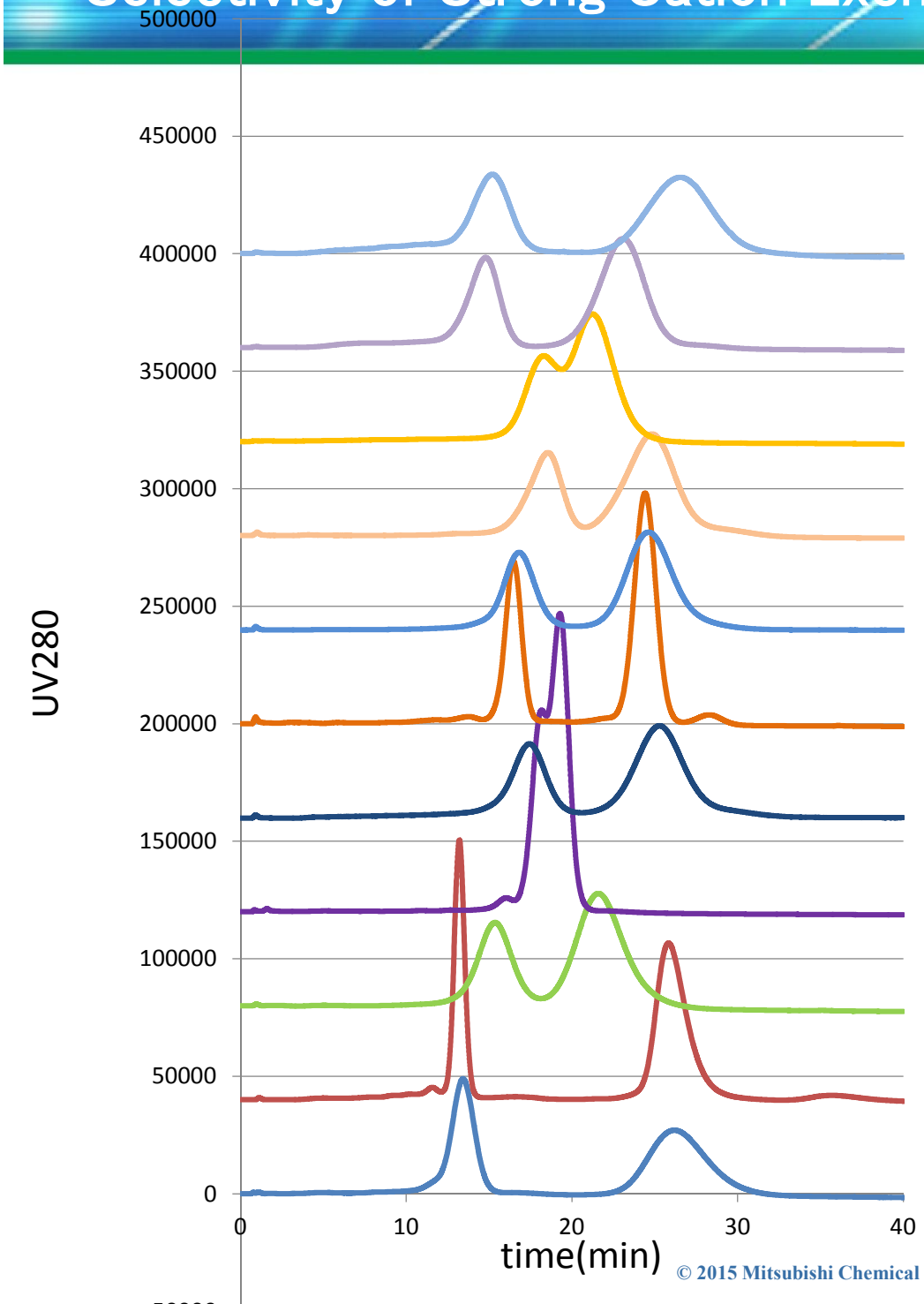
Selectivity of Strong Cation Exchange

Conditions:	
Column	100 x 5mm I.D. (BV:2.0mL)
Eluent A	20mM Sodium Phosphate (pH6.5)
Eluent B	A + 1.0M NaCl
Flow rate	1.0mL/min (R.T. 2min)
Gradient	0-100% B over 60min
Sample	(a) Cytochrome C + (b) lysozyme = 125 / 125ug / 50uL
(pI)	(9.3) (11.0)



Eshmuno S (75-95um, Merck Millipore)

BioPro S75 (75um, YMC)

SP Sepharose FF (90um, GE healthcare)

Nuvia S (85um, Bio-Rad)

CellufineMax S (40-130 um, JNC)

GigaCap S650S (20-50um, TOSOH)

GigaCap S650M (50-100um, TOSOH)

Capto SP ImpRes (40um, GE healthcare)

Capto S (90um, GE healthcare)

ChromSpeed™ S101 (30um, Mitsubishi)

ChromSpeed™ S103 (60um, Mitsubishi)

Selectivity of Strong Cation

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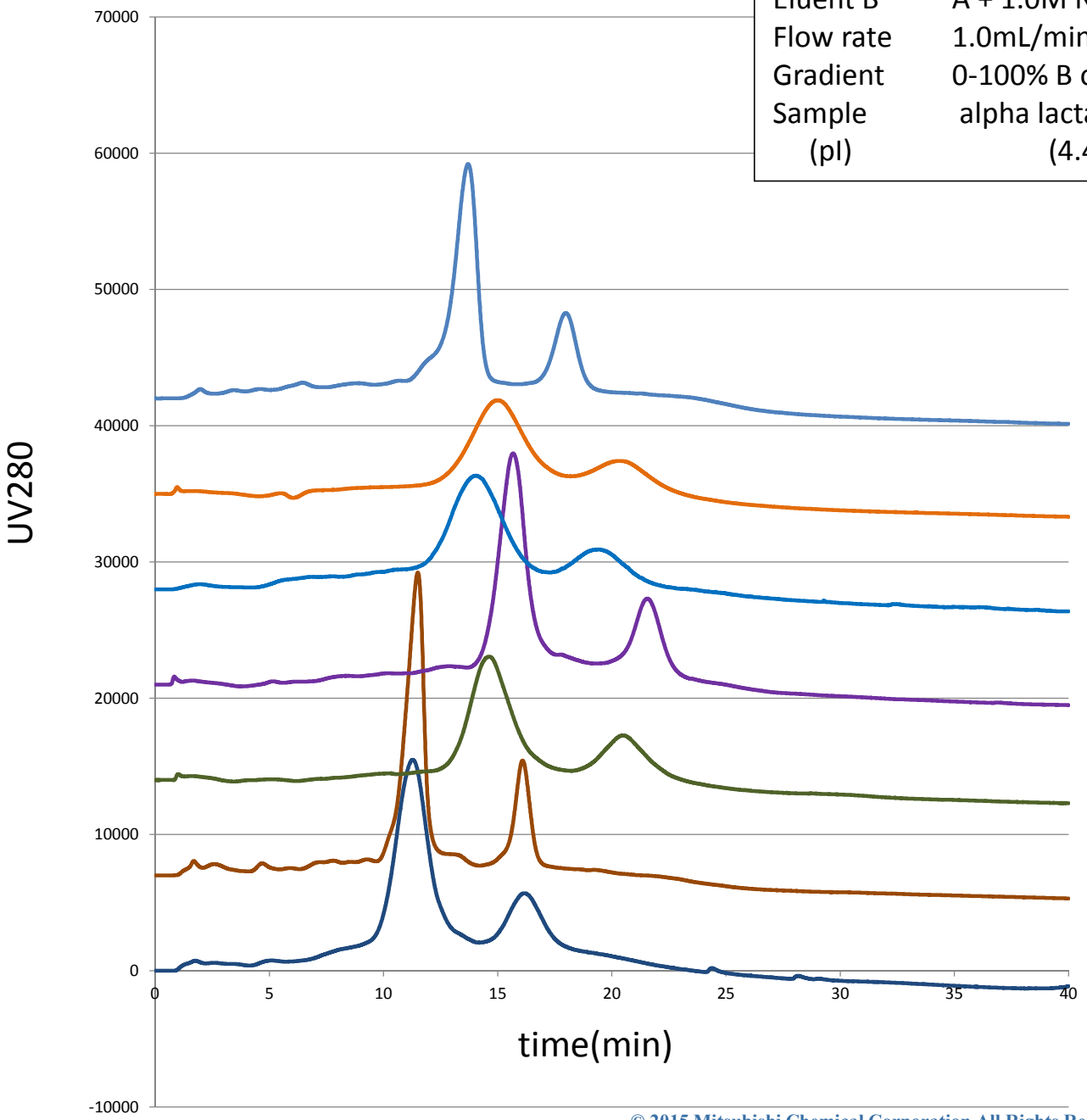
Conditions:	
Column	100 x 5mm I.D. (BV:2.0mL)
Eluent A	50mM Tris-HCl (pH8.5)
Eluent B	A + 1.0M NaCl
Flow rate	1.0mL/min (R.T. 2min)
Gradient	0-100% B over 60min
Sample	alpha lactalbumin + Trypsin inhibitor = 25 / 125ug / 25uL
(pI)	(4.4) (4.5)



CaptoQ ImpRes (40um, GE healthcare)

Capto Q (90um, GE healthcare)

Q Sepharose FF (90um, GE healthcare)

Gigacap Q650S (20-50um, TOSOH)

Gigacap Q650M (50-100um, TOSOH)

ChromSpeed Q101 (30um, Mitsubishi)

ChromSpeed Q103 (60um, Mitsubishi)