APPLICATION NOTE

YMC EUROPE GMBH

Purification of monoclonal antibodies with BioPro SmartSep

Ion exchange (IEX) is widely used for the analysis and purification of bio-molecules such as proteins, peptides and monoclonal antibodies.

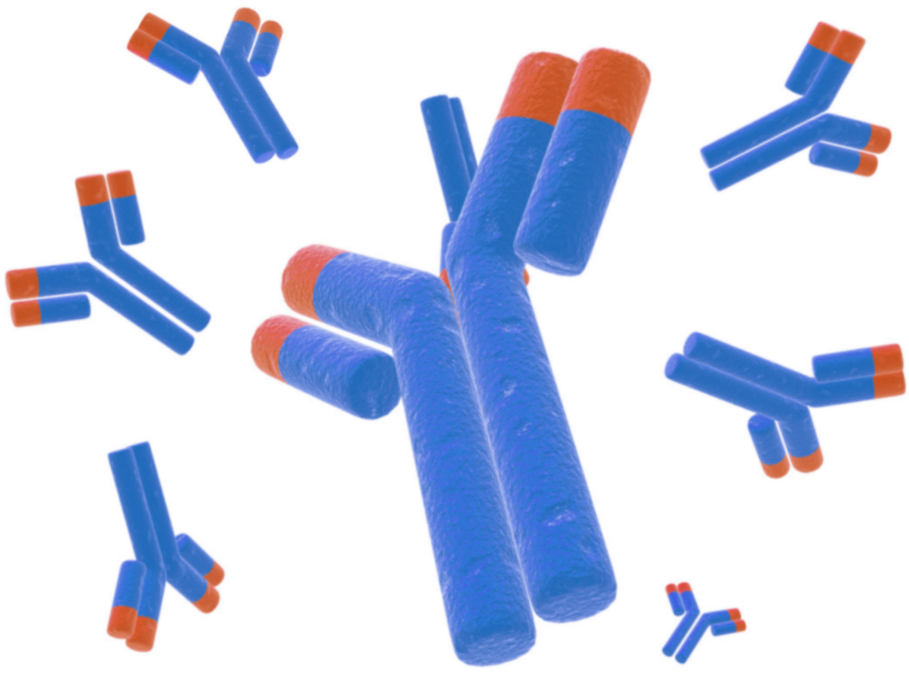
In industrial-scale production, IEX is used for initial capture, intermediate purifications or final product polishing.

Challenges during mAb purification

For purification of monoclonal antibodies, high demands are required from the separating material. Factors influencing the binding characteristics of IgG are pH, linear velocity and/or salt concentration (conductivity) at the time the sample is loaded onto the column.

Therefore, a material with highly stable performance is required with regard to all those factors. In order to demonstrate the performance of YMC-BioPro materials, several studies have been performed.

- **Purification of Adalimumab**
- **Influence of pH**
- **Influence of linear velocity**
- **Influence salt concentration**



IgG

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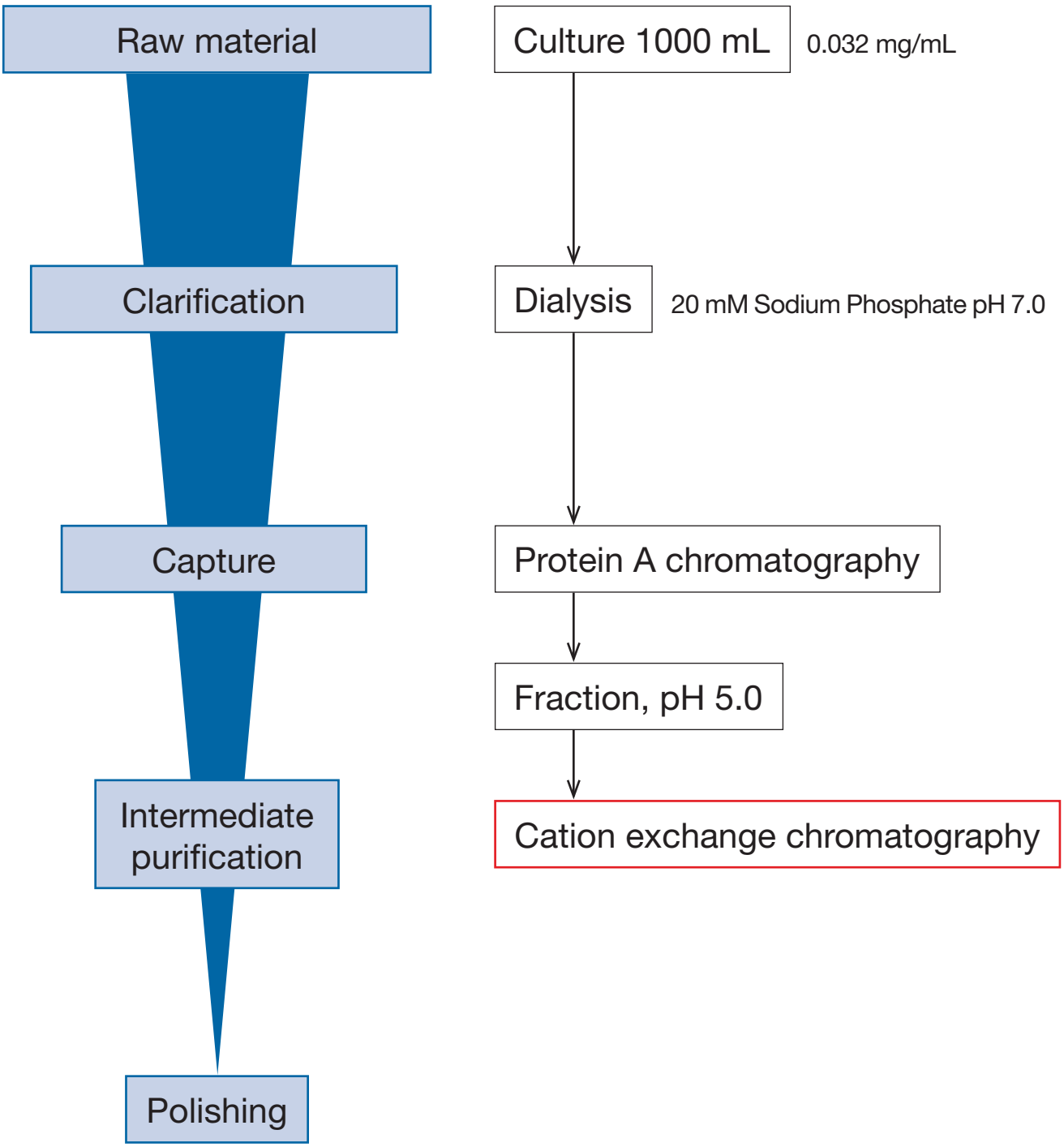
Purification Scheme for Adalimumab



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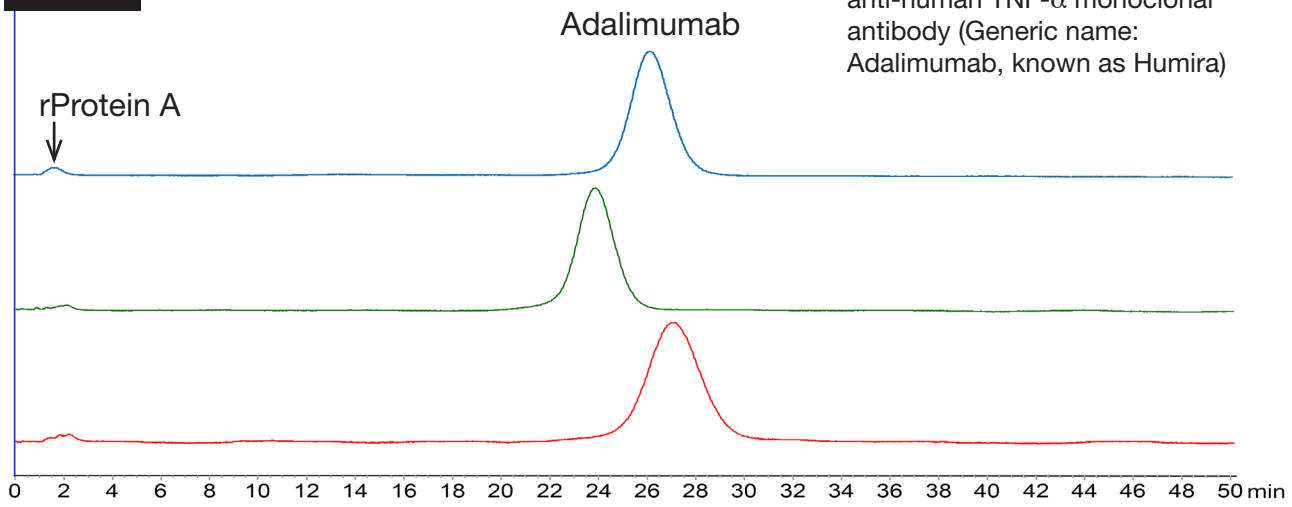
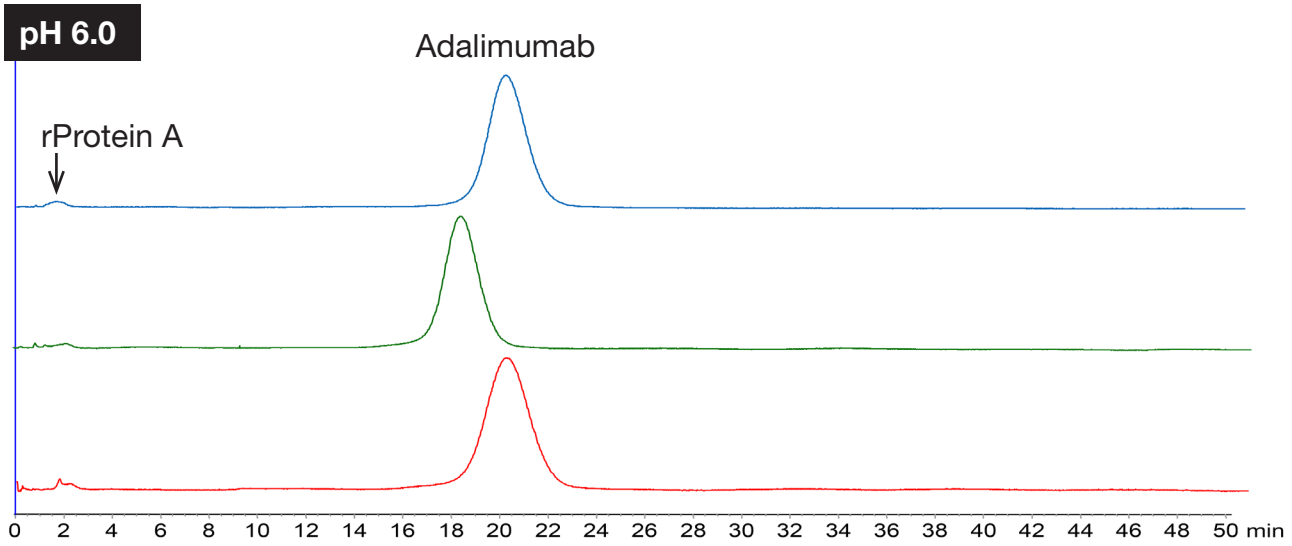
pH 5.3



Formulation of the humanized anti-human TNF-α monoclonal antibody (Generic name: Adalimumab, known as Humira)

pH 6.0



Column:	50 x 5.0 mm ID
Eluent:	A) 20 mM citric acid-NaOH (pH 5.3) B) Eluent A containing 0.5 M NaCl
Gradient:	0-100% B (0-30 CV)
Flow rate:	180 cm/hr (0.59 mL/min)
Temperature:	ambient
Detection:	UV at 280 nm
Sample:	Anti-hTNFalpha Adalimumab (after affinity chromatography)
IgG Load:	0.1 mg
Injection:	0.25 mL

BioPro SmartSep S30

GE Source 30S

Tosoh TSKgel SP-3PW

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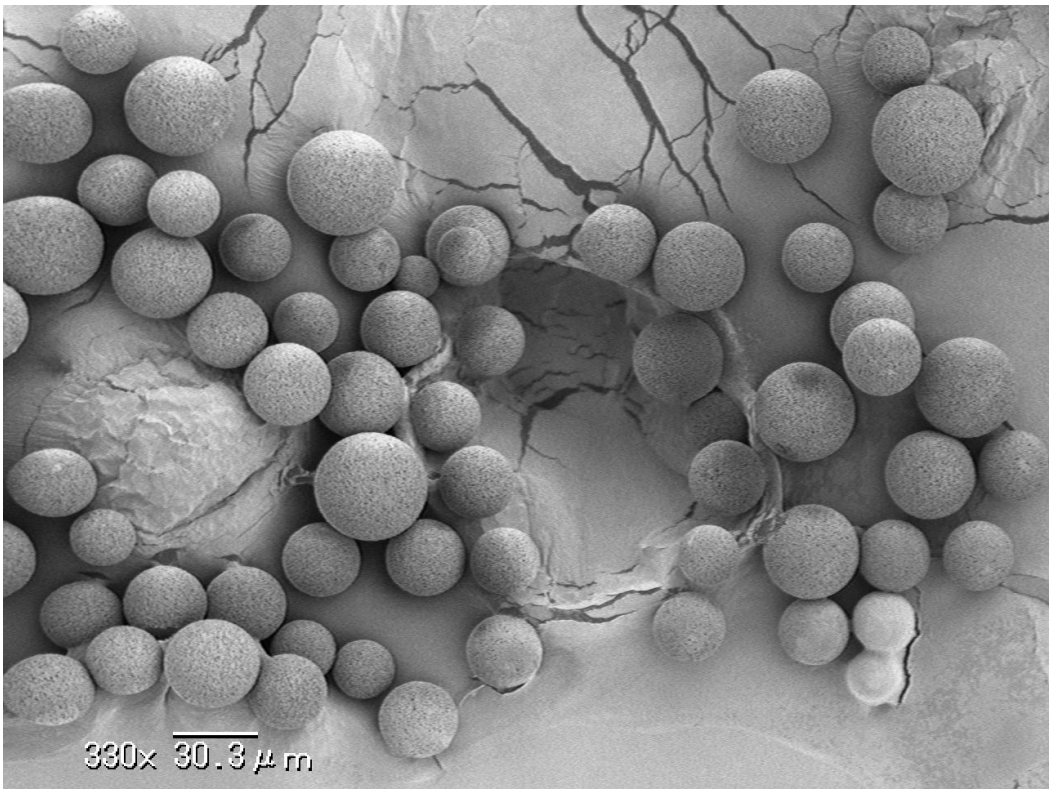
Purification of monoclonal antibodies with BioPro SmartSep

In order to demonstrate the behaviour of BioPro SmartSep under different elution conditions, experiments with different values of pH, linear velocity and salt concentration were performed and the dynamic binding capacity (DBC) recorded.
The parameters changed were:

Experimental conditions

pH	: 6.0 vs. 5.3
Linear velocity	: 200 - 800 cm/hr
Salt concentration	: 0 - 50 mM NaCl

Column:	50 x 5.0 mm ID
Equilibration buffer:	20 mM citric acid-NaOH buffer (pH 5.3 or 6.0)
Elution buffer:	Equilibration buffer containing 0.5 M NaCl
Flow rate:	200 - 800 cm/hr (0.66-2.62 mL/min)
Temperature:	ambient (25°C)
Detection:	UV at 280nm
Sample:	**1.5 mg/mL human polyclonal Adalimumab** in equilibration buffer



BioPro SmartSep S30 particles

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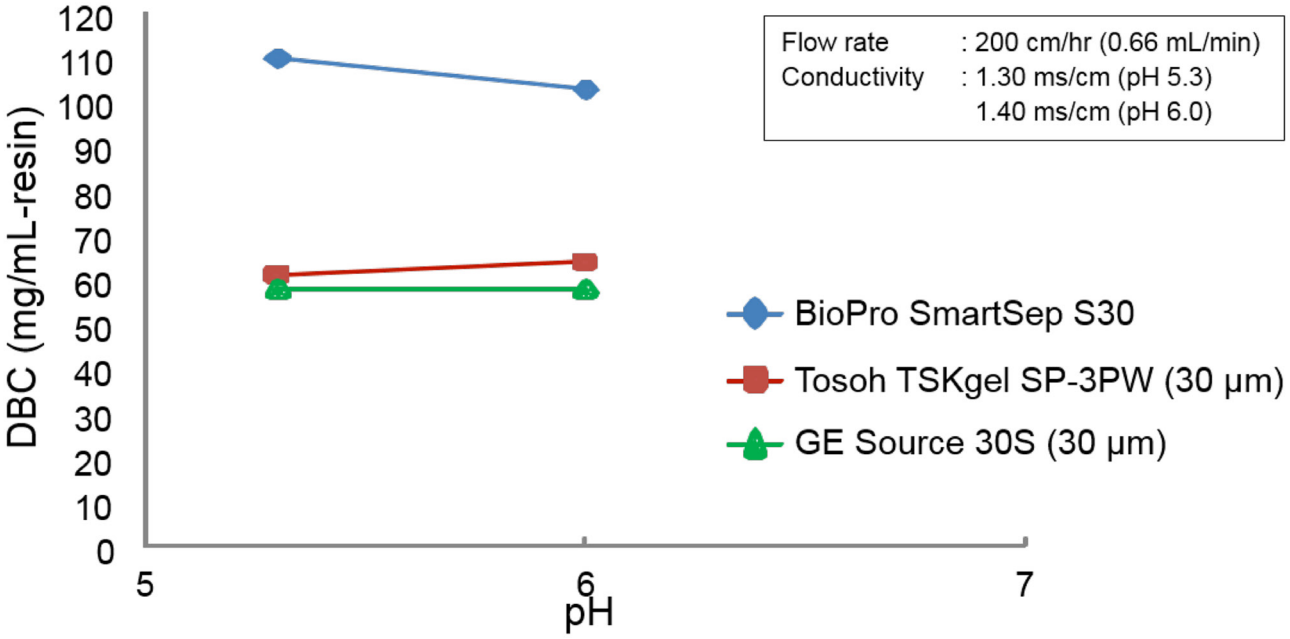
Purification of monoclonal antibodies with BioPro SmartSep

Influence of pH

pH	DBC (mg/mL-resin, 10% breakthrough)	
	pH 5.3	pH 6.0
BioPro SmartSep S30	**110**	**103**
Tosoh TSKgel SP-3PW (30 µm)	61	64
GE Source 30S (30 µm)	58	58



High binding capacities are achieved regardless of elution of pH. Therefore, milder eluting conditions for Adalimumab can be selected.

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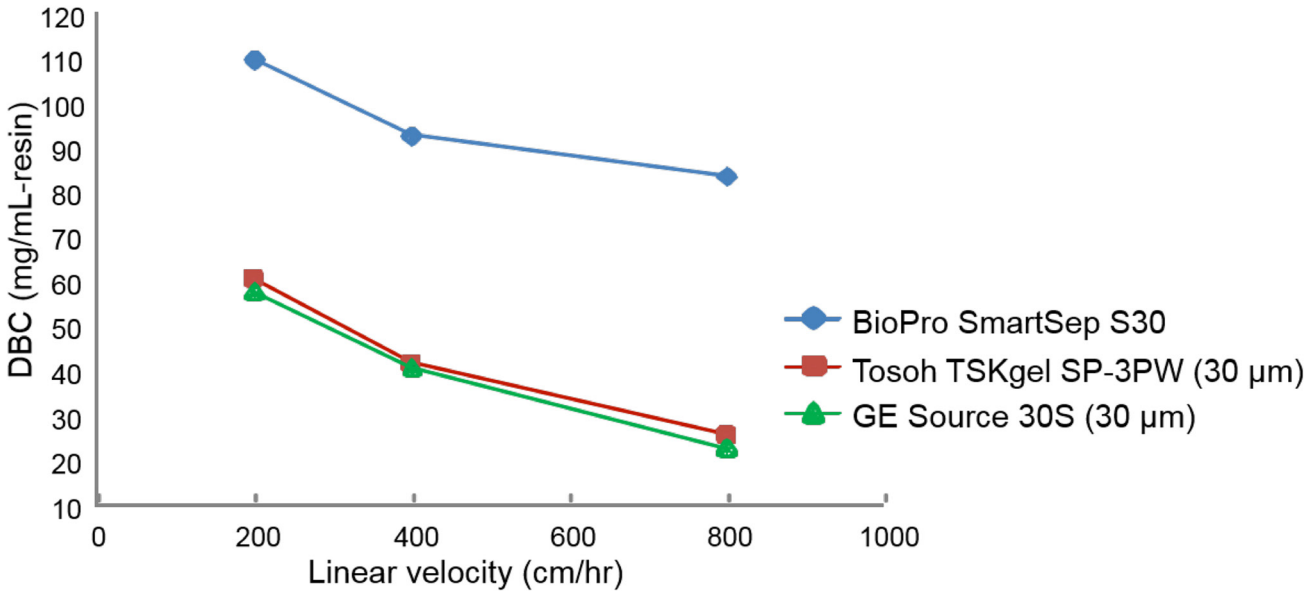
Purification of monoclonal antibodies with BioPro SmartSep

Influence of linear velocity

Linear velocity	DBC (mg/mL-resin, 10% breakthrough)		
	200 cm/hr	400 cm/hr	800 cm/hr
BioPro SmartSep S30	**110**	**93**	**84**
Tosoh TSKgel SP-3PW (30 µm)	61	42	26
GE Source 30S (30 µm)	58	41	23



BioPro SmartSep maintains higher binding capacity values over a wider range of linear velocity. This will increase product throughput for the purification work without loss of efficiency.

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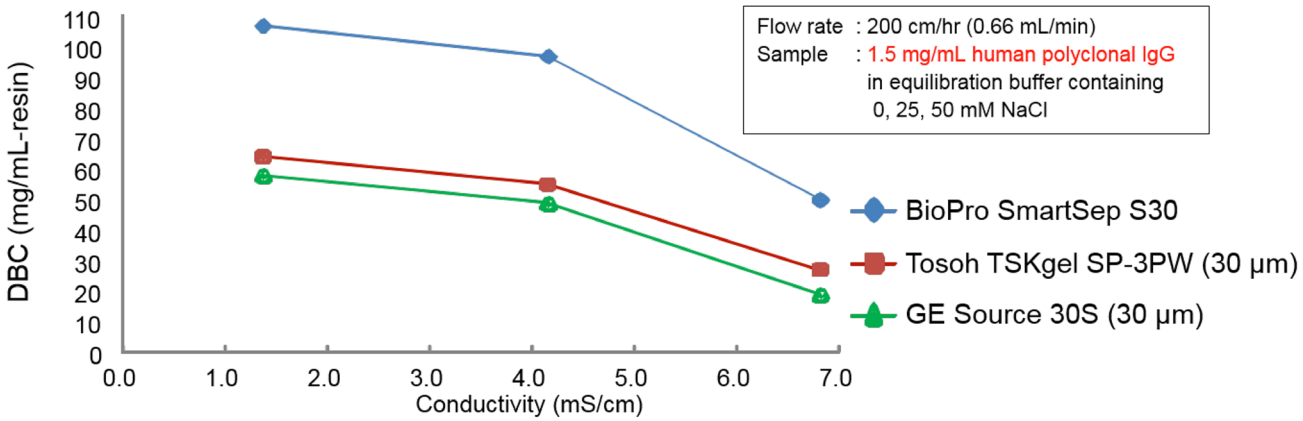
Purification of monoclonal antibodies with BioPro SmartSep

Influence of salt concentration

	DBC (mg/mL-resin, 10% breakthrough)		
pH	5.3		
NaCl concentration	0 mM	25 mM	50 mM
Conductivity	1.36 mS/cm	4.14 mS/cm	6.8 mS/cm
BioPro SmartSep S30	**107**	**97**	**50**
Tosoh TSKgel SP-3PW (30 µm)	64	55	27
GE Source 30S (30 µm)	58	49	19



BioPro SmartSep has higher salt concentration tolerance. This simplifies the desalting process after Protein A chromatography and will help to shorten the production process.

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Conclusions

BioPro SmartSep materials meet the highest demands for the purification of monoclonal antibodies. High binding capacity is achieved regardless of elution of pH, linear velocity or salt concentration. This allows purification processes to be carried out more efficiently.

- **Milder eluting conditions can be selected**
- **Higher throughput at stable efficiencies**
- **Simplification of desalting processes for shorter processes**