YMC SEPARATION TECHNOLOGY

HPLC DATA SHEET

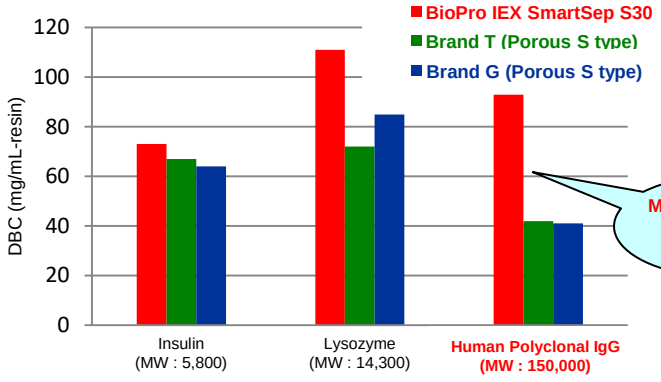
Rapid and effective purification of IgG by using Ion Exchange Media BioPro IEX SmartSep S30

R150521AE

BioPro IEX SmartSep Q/S are ion exchange media dedicated to high-throughput intermediate purification step and polishing step of biopharmaceuticals. BioPro IEX SmartSep media are available in strong ion exchangers of hydrophilic porous polymer beads with low nonspecific adsorption and high binding capacity over a wide range of flow rate. BioPro IEX SmartSep media show both high resolution and recovery even at a high flow rate and high loading condition. DBC is influenced by such as pH, linear velocity and salt concentration. BioPro IEX SmartSep Q/S shows the high DBC in any conditions. BioPro IEX SmartSep Q/S make a big improvement in productivity of biologics, especially, antibody therapeutics.

High Dynamic Binding Capacity (DBC) for IgG

Comparison of DBC of various proteins



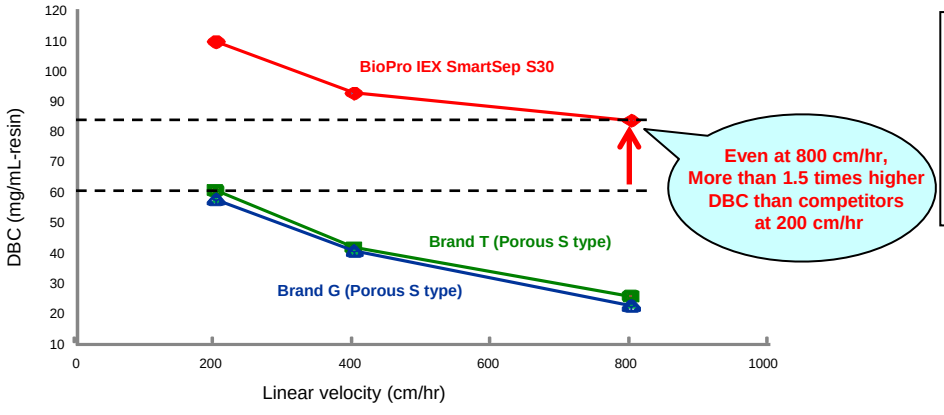
	DBC (mg/mL-resin, 10% breakthrough)		
	Insulin (MW : 5,800)	Lysozyme (MW : 14,300)	Human Polyclonal IgG (MW : 150,000)
BioPro IEX SmartSep S30	73	111	93
Brand T (Porous S type, 30 μm)	67	72	42
Brand G (Porous S type, 30 μm)	64	85	41

Conditions of DBC measurement *

Column size	: 50 X 5.0 mmI.D.
Sample	: 1.5 mg/mL in equilibration buffer
Flow rate	: 400 cm/hr (1.32 mL/min)

*Please inquire us for deails.

DBC of human polyclonal IgG at various flow rates

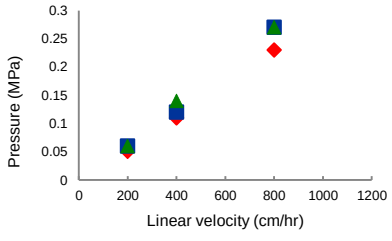


Conditions of DBC measurement

Column	: 50 X 5.0 mmI.D.
Equilibration buffer	: 20 mM citric acid-NaOH (pH 5.3)
Elution buffer	: Equilibration buffer containing 0.5 M NaCl
Flow rate	: 200-800 cm/hr (0.66-2.62 mL/m in)
Temperature	: ambient (25℃)
Detection	: UV at 280 nm
Sample	: **1.5 mg/mL human polyclonal IgG** in equilibration buffer

Linear velocity	DBC (mg/mL-resin, 10% breakthrough)		
	200 cm/hr	400 cm/hr	800 cm/hr
BioPro IEX SmartSep S30	110	93	84
Brand T (Porous S type, 30 μm)	61	42	26
Brand G (Porous S type, 30 μm)	58	41	23

Change of Pressure at various flow rate

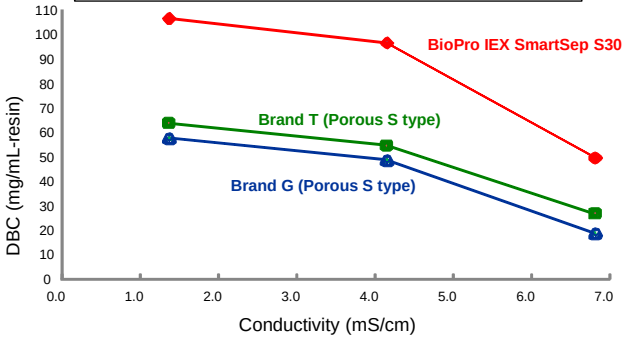


- **BioPro IEX SmartSep S30 provides higher DBC on various proteins.**
  For IgG, BioPro IEX SmartSep has more than twice as high DBC as competitors'
  ⇒Enabling column downsizing (gel cost reduction) during antibody purification
- **Larger difference of DBC at high flow rate**
  →Expect improvement of productivity by increasing flow rate.
  ⇒BioPro IEX SmartSep S30 makes purification productivity per unit time double or more.

High DBC under various conditions

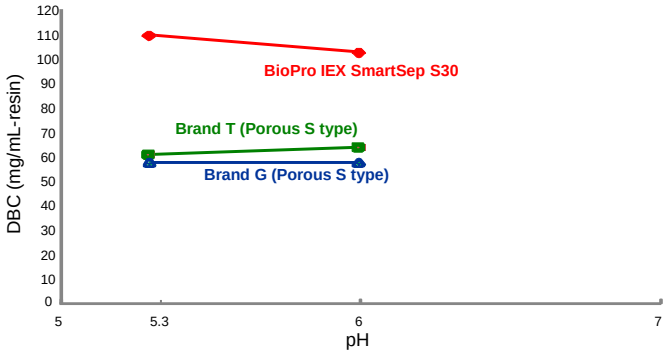
Effect of sample buffers/salts concentration

Sample: 1.5 mg/mL human polyclonal IgG
in equilibration buffer containing 0, 25, 50 mM NaCl
＊Equilibration buffer pH : 5.3



Effect of equilibration buffer pH

Conductivity : 1.30 mS/cm (pH 5.3)
1.40 mS/cm (pH 6.0)



Column : 50 X 5.0 mmI.D.
Equilibration buffer : 20 mM citric acid-NaOH (pH 5.3 or 6.0)
Elution buffer : Equilibration buffer containing 0.5 M NaCl
Flow rate : 200 cm/hr (0.66 mL/min)
Temperature : ambient (25℃)
Detection : UV at 280 nm
Sample : **1.5 mg/mL human polyclonal IgG** in equilibration buffer

	DBC (mg/mL-resin, 10% breakthrough)				
pH	5.3			5.3	6.0
NaCl concentration	0 mM	25 mM	50 mM	-	-
Conductivity	1.36 mS/cm	4.14 mS/cm	6.8 mS/cm	-	-
BioPro IEX SmartSep S30	**107**	**97**	**50**	**110**	**103**
Brand T (Porous S type, 30 μm)	64	55	27	61	64
Brand G (Porous S type, 30 μm)	58	49	19	58	58

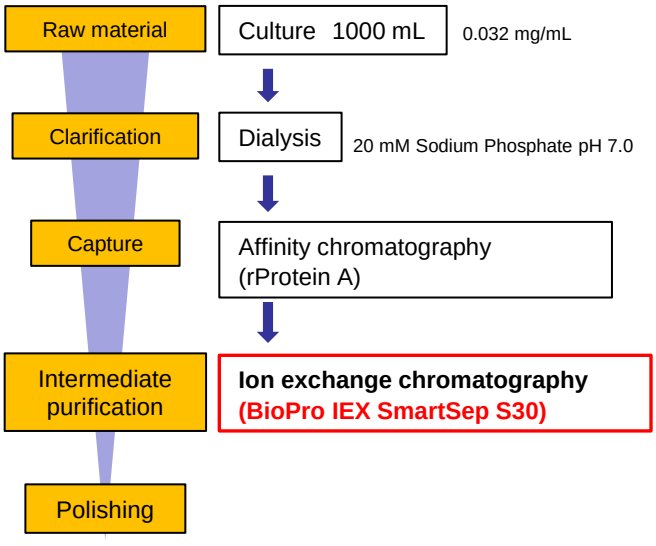
・High DBC than competitors even in the presence of 50 mM NaCl.
⇒ Eluate from Protein A column chromatography could be directly subjected
・High DBC at buffer pH range commonly used in antibody purification by cation exchange chromatography

Purification of IgG1（Anti-hTNFalpha IgG）

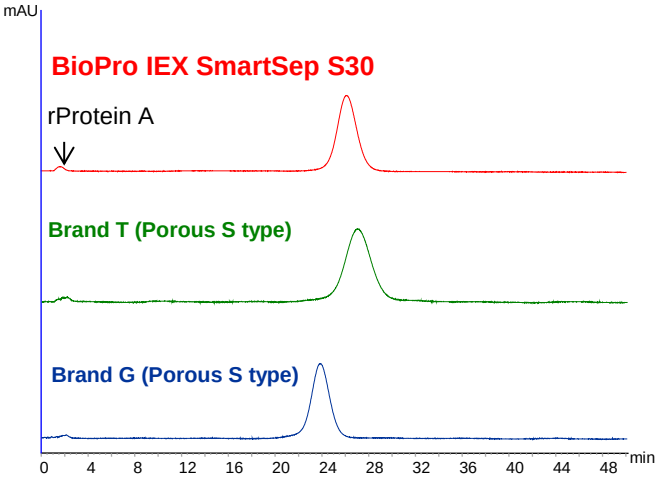
This is an example that an IgG1 monoclonal antibody was purified from cell culture medium. After clarified, it was subjected to initial purification (capture step) by affinity chromatography (rProtein A), followed by ion- exchange chromatography. In the capture step rProtein A derived from affinity media contaminated the eluate, then they were separated and removed by following ion exchange chromatography.

Purification Scheme of IgG1

Sample: Anti-h TNF alpha IgG1



Intermediate purification (cation exchange chromatography)



Column : 50 X 5.0 mmI.D.
Eluent : A) 20 mM citric acid-NaOH (pH 5.3)
B) 20 mM citric acid-NaOH (pH 5.3) containing 0.5 M NaCl
0-100 %B, 30 column volumes
Flow rate : 180 cm/hr (0.59 mL/min)
Temperature : ambient
Detection : UV at 280 nm
Sample : Anti-hTNFalpha IgG1(Purified by Affinity chromatography)
Injection : 0.25 mL (0.1 mg IgG1)

・BioPro IEX SmartSep S30 ion exchange media is effective to remove desorbed rProtein A ligand in the capture step.