Cation Exchange Chromatography Workflow

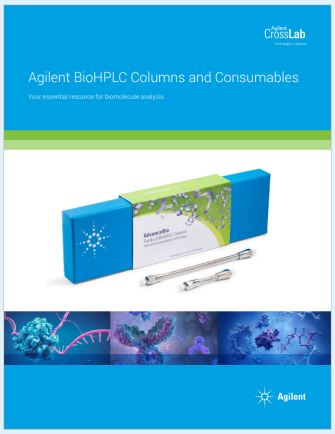
Agilent Bio IEX and Bio MAb HPLC columns

In this document Agilent applications chemists share their recommendations for an optimum LC system and its configuration for characterizing biomolecules. They also offer guidance on a generic method to get you started, and how this method can be further optimized to meet your separation goals.

Additional information is available at www.agilent.com/chem/advancebio

Guidelines

- Basic proteins: strong cation exchange (SCX) or weak cation exchange (WCX).
- Consider the isoelectric point (pI) of your protein when choosing the pH of the mobile phase. If pH<pI, your protein will have a net positive charge.
- The pH of the starting buffer should be 0.5 to 1 pH unit from the pI (below pI for cation exchange).
- If pI is unknown, start with pH 6 for cation exchange.
- Start with SCX columns, which have the widest operating range, WCX can be used to provide a difference in selectivity.
- Buffers for cation exchange (pH 4 to 7) include formate, acetate, MES, phosphate, HEPES.



BioHPLC columns catalog

Find everything you need to measure what matters, including tips and tools to advance your characterization.

Download now

Bonded phase	
SCX	SO_3H
WCX	COOH
Samples	**Column**
Monoclonal antibody	Bio MAb
Peptides and proteins	Bio SCX and WCX
Globular proteins and peptides	PL-SCX 1000Å
Very large biomolecules/high speed	PL-SCX 4000Å
Proteins, antibodies	Bio-Monolith SO3

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Agilent 1260 Infinity II Bio-Inert LC System

Mobile phases

Mobile phase should contain buffer to maintain the desired operating pH, typically 20 mM. Elution salt is typically 400 to 500 mM.

Agilent Buffer Advisor is used to develop the necessary gradient profile by mixing different proportions from the four stock solutions.

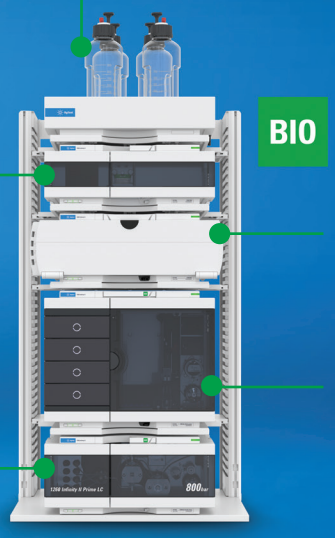
Detection

UV, with a 10 mm bio-inert standard flow cell.

Pump

Typical flow rate for 4.6 mm id columns is 0.5 to 1.0 mL/min.

Typical flow rate for 2.1 mm id columns is 0.2 to 0.4 mL/min.



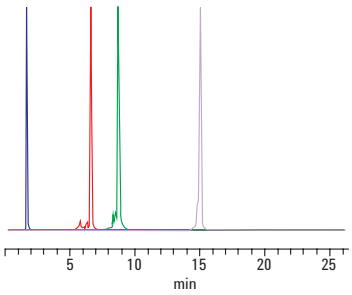
Column compartment

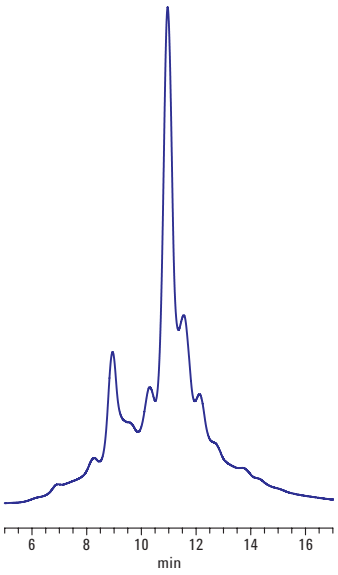
Maximum limit 80 °C. Column lifetime is optimized when used between 10 to 50 °C.

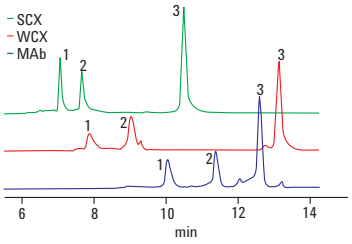
Sample injection

1 to 10 µL injection for maximum resolution. Sample must be soluble in the mobile phase.

	Monoclonal antibodies		Monoclonal antibodies Proteins and peptides
	Salt gradient	pH gradient	Salt gradient
Columns	Bio WCX, 4.6 x 250 mm, 10 µm Bio WCX, 4.6 x 250 mm, 5 µm	Bio MAb, 4.6 x 250 mm, 5 µm	Bio SCX, 4.6 x 50 mm, 3 µm WCX, 4.6 x 50 mm, 3 µm Bio MAb, 4.6 50 mm, 3 µm
Mobile phase	A: Water B: 1.6 M NaCl C: 40.0 mM NaH_2PO_4 D: 40.0 mM Na_2HPO_4 By combining predetermined proportions of C and D, 20 mM buffer solutions at the desired pH range are produced.	A: Water B: 1.6 M NaCl C: 40.0 mM NaH_2PO D: 40.0 mM Na_2HPO_4 By combining predetermined proportions of C and D, buffer solutions at the desired pH range are produced at the selected buffer strengths.	A: 20 mM sodium phosphate, pH 5.0 for WCX, pH 6.0 for SCX B: Buffer A + 1 M NaCl
Gradient	0 to 50% B, 0 to 20 min (constant pH, for example, pH 6.0) 50% B, 20 to 25 min 0% B, 25 to 35 min	pH 6.0 to 8.0, 0 to 20 min 0 to 800 mM NaCl, 20 to 25 min 800 mM NaCl, 25 to 30 min	1 to 100% B in 30 min for 50 mm columns, 60 min for 250 mm columns
Flow rate	1 mL/min	1 mL/min	0.5 mL/min
Temperature	Ambient	Ambient	Ambient
Injection	10 µL	10 µL	10 µL
Sample	2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)	2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)	
Detection	UV, 220 nm	UV, 220 nm	UV, 220 nm
	Separation of protein standards at pH 7.0 using an Agilent Bio WCX, 4.6 x 250 mm, 10 µm column. Ovalbumin (pI 4.5) Ribonuclease (pI 9.4) Cytochrome C (pI 9.8) Lysozyme (pI 11)	Analysis of a IgG monoclonal antibody using a pH gradient of 6.5 to 7.5 (0-20 min), 50 mM, Agilent Bio MAb, 4.6 x 50 mm, 5 µm column.	Separation of protein standards on Agilent 3 µm ion exchange columns by cation exchange chromatography. Ribonuclease (pI 9.4) Cytochrome C (pI 9.8) Lysozyme (pI 11)







Ordering Information

Description	Bio IEX HPLC columns, PEEK		Bio MAb HPLC columns, PEEK
	Bio SCX part number	Bio WCX part number	Part number
4.6 x 250 mm, 10 µm	5190-2435	5190-2455	5190-2415
4.6 x 50 mm, 10 µm	5190-2436	5190-2456	5190-2416
4.6 x 250 mm, 5 µm	5190-2427	5190-2447	5190-2407
4.6 x 50 mm, 5 µm	5190-2428	5190-2448	5190-2408
2.1 x 250 mm, 10 µm	5190-2439	5190-2459	5190-2419
2.1 x 50 mm, 10 µm	5190-2440	5190-2460	5190-2420
2.1 x 250 mm, 5 µm	5190-2431	5190-2451	5190-2411
2.1 x 50 mm, 5 µm	5190-2432	5190-2452	5190-2412

Note: For Bio IEX and Bio MAb stainless steel HPLC columns part numbers, see the BioHPLC columns catalog 5994-6123EN

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