

# CATION EXCHANGE CHROMATOGRAPHY WORKFLOW

## AGILENT BIO IEX HPLC COLUMNS

## AGILENT 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 specific separation goals.

Additional application information is available at [www.agilent.com/chem/advancebio](http://www.agilent.com/chem/advancebio)

### Agilent 1260 Infinity Bio-Inert LC System

#### Guidelines

- Basic proteins: SCX or 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, HEPS

#### 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.

#### Sample injection (G5667A)

1 to 10  $\mu$ L injection for maximum resolution. Sample must be soluble in the mobile phase.

#### Pump (G5611A)

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

#### Column compartment (G1316C)

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

#### Detection (G1315C)

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

**BIO  
inert**



Bonded Phase	
SCX (strong cation-exchange) – SO <sub>3</sub> H	
WCX (weak cation-exchange) – 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 SO <sub>3</sub>

Note: For Bio IEX and Bio MAB stainless steel HPLC columns part number, see Agilent BioHPLC Column Selection Guide, 5990-9384EN.

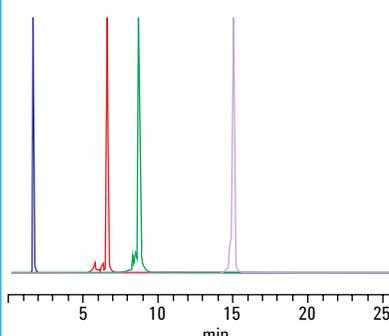
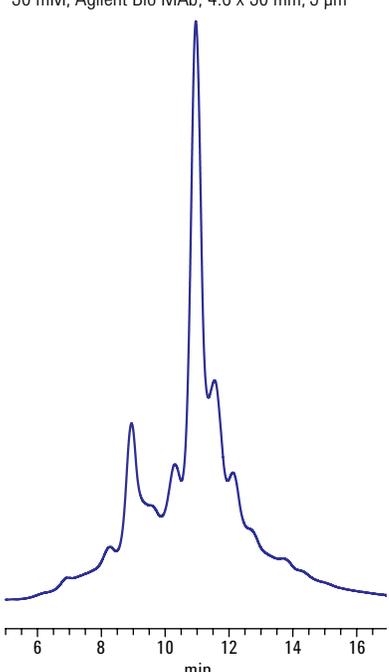
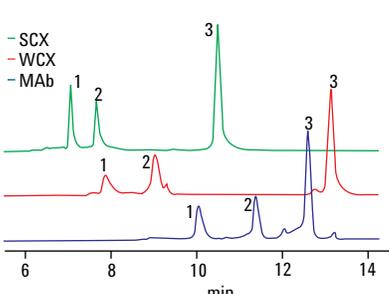
#### Column selection

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 $\mu$ m	5190-2435	5190-2455	5190-2415
4.6 x 50 mm, 10 $\mu$ m	5190-2436	5190-2456	5190-2416
4.6 x 250 mm, 5 $\mu$ m	5190-2427	5190-2447	5190-2407
4.6 x 50 mm, 5 $\mu$ m	5190-2428	5190-2448	5190-2408
2.1 x 250 mm, 10 $\mu$ m	5190-2439	5190-2459	5190-2419
2.1 x 50 mm, 10 $\mu$ m	5190-2440	5190-2460	5190-2420
2.1 x 250 mm, 5 $\mu$ m	5190-2431	5190-2451	5190-2411
2.1 x 50 mm, 5 $\mu$ m	5190-2432	5190-2452	5190-2412



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## Recommended initial conditions

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 x 50 mm, 3 µm
Mobile Phase	A: Water B: 1.6 M NaCl C: 40.0 mM NaH <sub>2</sub> PO <sub>4</sub> D: 40.0 mM Na <sub>2</sub> HPO <sub>4</sub>  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 <sub>2</sub> PO <sub>4</sub> D: 40.0 mM Na <sub>2</sub> HPO <sub>4</sub>  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 or pH 6.0 for SCX B: Buffer A + 1 mM 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
	<p>Separation of protein standards at pH 7.0 using an Agilent Bio WCX, 4.6 x 250 mm, 10 µm column.</p> <p>Ovalbumin (pI 4.5) Ribonuclease (pI 9.4) Cytochrome C (pI 9.8) Lysozyme (pI 11)</p> 	<p>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</p> 	<p>Separation of protein standards on Agilent 3 µm ion-exchange columns by cation-exchange chromatography</p> <p>Ribonuclease (pI 9.4) Cytochrome C (pI 9.8) Lysozyme (pI 11)</p> 

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