ANION EXCHANGE CHROMATOGRAPHY WORKFLOW
AGILENT BIO IEX HPLC COLUMNS
AGILENT PL-SAX STRONG ANION-EXCHANGE COLUMNS
AGILENT BIO-MONOLITH 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**

Agilent 1260 Infinity Bio-Inert LC System

Guidelines

- Acidic proteins: SAX or WAX
- Consider the isoelectric point (pI) of the protein when choosing the pH of the mobile phase. If pH>pI, your protein will have a net negative charge.
- The pH of the starting buffer should be 0.5 to 1 pH unit from the pI (above pI for anion-exchange)
- If your pI is unknown, start with pH 8.0 for anion-exchange
- Start with SAX columns, which have the widest operating range. WAX can be used to provide a difference in selectivity.
- Buffers for anion-exchange (pH 7 to 10) include bis-tris, tris, diethanoamine, piperazine

Mobile phases

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

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

Sample injection

1 to 10 µL injection for maximum resolution. Sample must be soluble in the mobile phase at lower ionic strength than the starting conditions.

Flow rate

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

Column temperature

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

Detection

UV, G1315D with a 10 mm bio-inert standard flow cell.

Column selection

Bio IEX HPLC Columns, PEEK		
Description	Bio SAX Part Number	Bio WAX Part Number
4.6 x 250 mm, 10 µm	5190-2475	5190-2495
4.6 x 50 mm, 10 µm	5190-2476	5190-2496
4.6 x 250 mm, 5 µm	5190-2467	5190-2487
4.6 x 50 mm, 5 µm	5190-2468	5190-2488
2.1 x 250 mm, 10 µm	5190-2479	5190-2499
2.1 x 50 mm, 10 µm	5190-2480	5190-2400
2.1 x 250 mm, 5 µm	5190-2471	5190-2491
2.1 x 50 mm, 5 µm	5190-2472	5190-2492

Bio IEX HPLC Columns, Stainless Steel		
Description	Bio SAX Part Number	Bio WAX Part Number
21.2 x 250 mm, 5 µm	5190-6883	5190-6877
10 x 250 mm, 5 µm	5190-6882	5190-6876
4.6 x 250 mm, 10 µm	5190-2473	5190-2493
4.6 x 150 mm, 3 µm		5190-6875
4.6 x 250 mm, 5 µm	5190-2465	5190-2485
4.6 x 50 mm, 3 µm	5190-2463	5190-2483
4.6 x 50 mm, 1.7 µm	5190-2461	5190-2481

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Column selection

Bonded Phase	
SAX (strong anion-exchange) – $N(CH_3)_3$	
WAX (weak cation-exchange) – $N(C_2H_5)_2$	
Samples	**Column**
Peptides and proteins	Bio SAX and WAX
Globular proteins and peptides	PL-SAX 1000Å
Very large biomolecules/ high speed	PL-SAX 4000Å
Viruses, DNA, large proteins, plasmid	Bio-Monolith QA
DNS, bacteriophages	Bio-Monolith DEAE

Fast separation protocols

Column: Bio WAX, 4.6 × 250 mm, 5 µm

Buffer A: 20 mM Tris-HCl, pH 8.5

Buffer B: A + 500 mM NaCl

Gradient: 1 to 100% B in 30 min for 50 mm columns, 60 min for 250 mm columns

Flow rate: 0.5 mL/min

Temperature: Ambient

Injection: 10 µL

Sample: 1 mg/mL (in mobile phase)

Detection: UV, 220/280 nm

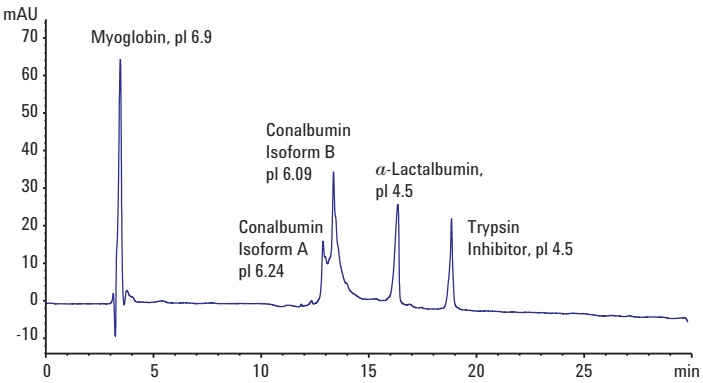
Note: Alternatively, a pH gradient (high to low pH) can be used for elution.

PL-SAX Strong Anion-exchange Columns

Description	1000Å Part Number	4000Å Part Number
100 x 300 mm, 10 µm	PL1851-2102	PL1851-2103
50 x 150 mm, 30 µm	PL1751-3702	PL1751-3703
50 x 150 mm, 10 µm	PL1751-3102	PL1751-3103
25 x 150 mm, 30 µm	PL1251-3702	PL1251-3703
25 x 150 mm, 10 µm	PL1251-3102	PL1251-3103
25 x 50 mm, 10 µm	PL1251-1102	PL1251-1103
4.6 x 250 mm, 30 µm	PL1551-5702	PL1551-5703
4.6 x 150 mm, 30 µm	PL1551-3702	PL1551-3703
4.6 x 250 mm, 10 µm	PL1551-5102	PL1551-5103
4.6 x 150 mm, 10 µm	PL1551-3102	PL1551-3103
4.6 x 150 mm, 8 µm	PL1551-3802	PL1551-3803
4.6 x 50 mm, 8 µm	PL1551-1802	PL1551-1803
4.6 x 50 mm, 5 µm	PL1551-1502	PL1551-1503
2.1 x 150 mm, 8 µm	PL1951-3802	PL1951-3803
2.1 x 50 mm, 8 µm	PL1951-1802	PL1951-1803
2.1 x 50 mm, 5 µm	PL1951-1502	PL1951-1503
1 x 50 mm, 5 µm	PL1351-1502	PL1351-1503

Bio-Monolith HPLC Columns

Description	Part Number
Bio-Monolith QA	5069-3635
Bio-Monolith DEAE	5069-3636



Protein separation by AEX by a linear gradient using 2 M NaCl as eluting salt.

Column: Bio WAX, 4.6 × 250 mm, 5 µm

Buffer A: 20 mM Tris, pH 7.6

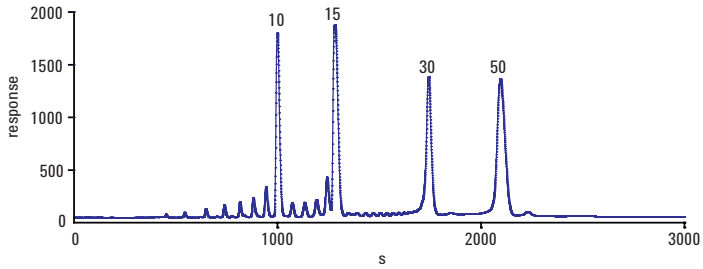
Buffer B: 20 mM Tris, pH 7.6 + 2 M NaCl

Gradient: 5 min – 100% A, 20 min – 70% B, 25 min – 100% B

Temperature: 25 °C

Flow rate: 0.5 mL/min

Injection volume: 5 µL



High resolution separation of a poly-T-oligonucleotide size standard spiked with 10, 15, 30 and 50 mer (main peaks).

Column: PL-SAX 1000Å, 4.6 x 50 mm, 8 µm

Buffer A: 7:93 v/v ACN: 100 mM TEAA, pH 8.5

Buffer B: 7:93 v/v ACN: 100 mM TEAA, 1 M NH_4Cl, pH 8.5

Gradient: 0 to 40% B in 10 min, followed by 40 to 70% B in 14 min and 70 to 100% B in 25 min

Temperature: 60 °C

Flow rate: 1.5 mL/min

Detector: 220 nm

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