



**Thermo Scientific**  
Technical Resources Document

# LC Columns and Accessories for Biomolecules

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# Thermo Scientific LC Columns for Biomolecules

The diversity of biological samples in terms of compound structure and properties coupled with matrix complexity demands a range of sample separation modes, column chemistries, column configurations and detection techniques for their effective characterization. Thermo Scientific addresses these needs with a range of silica and polymeric columns specifically designed to handle the unique rigors of the analysis of proteins, peptides, oligonucleotides and other biomolecules.

## Columns for proteins

### Reversed Phase

Thermo Scientific™ BioBasic™ reversed-phase columns provide superior chromatography because the extra dense bonding chemistry used for these packings produce a highly stable, reproducible surface for reliable results. BioBasic reversed phase packings are available in C18, C8 and C4, chemistries.

Acclaim 300 C18 features 3µm silica particles for rapid analysis of complex protein digests. Compared to 5µm column packings, the smaller particles support increased flow rates and shallower gradients on shorter columns, for faster separation analysis.

Thermo Scientific™ ProSwift™ RP monolith columns uniquely provide the advantages of high resolution at exceptionally high flow rates for fast protein separation analysis.

### Ion Exchange

Thermo Scientific™ ProPac™ and Thermo Scientific™ MAbPac™ ion exchange columns are based on a pellicular nonporous core particles providing exceptionally high resolution and efficiency for separations of protein variants, resolving isoforms that differ by a single charged residue. A hydrophilic layer prevents unwanted secondary interactions, and a grafted cation exchange surface provides pH-based selectivity control and fast mass transfer for high-efficiency separation and moderate capacity. ProPac WCX and MAbPac SCX columns are specifically developed for monoclonal separation and analytical characterization. Applications include protein variants in a variety of matrices, such as biopharmaceuticals and dairy products. MAbPac columns are specifically designed for the analysis of monoclonal antibody variants.

BioBasic AX and BioBasic SCX ion exchange columns demonstrate superior reproducibility, both column-to-column and batch-to-batch because the 5µm, 300Å silica provides high efficiency. Both phases provide superior performance for proteins, peptides and nucleic acids using protein friendly ion exchange conditions.

ProSwift ion exchange monoliths provide an excellent alternative to porous or non porous ion exchange media. They offer increased loading capacity compared to pellicular phases combined with excellent resolution compared to traditional porous ion exchange media.

### Size Exclusion

BioBasic SEC columns, based on silica with a proprietary polymeric coating, offer the mechanical stability of silica-based size exclusion columns with higher efficiencies than that of polymer-based columns. Four pore sizes (60Å, 120Å, 300Å, 1000Å) are available, making them ideal for molecular weight determination of peptides, proteins and water soluble polymers. They can also be used for sample clean-up prior to other analyses.

MAbPac SEC-1 (300Å 5µm silica) is a size exclusion chromatography (SEC) column specifically designed for separation and characterization of monoclonal antibodies (MAb) and their aggregates, as well as the analysis of Fab and Fc fragments resulting from proteolysis.

### Hydrophobic Interaction

The ProPac HIC-10 column is a high-resolution, high-capacity, 300Å, 5µm silica-based HIC column that provides excellent high resolution separations of proteins and variants for analytical and preparative applications. ProPac HIC columns provide exceptional hydrolytic stability under the highly aqueous conditions used in HIC.

## Affinity

The MAbPac Protein A column is a unique non-porous polymeric column designed for fast, accurate determination of monoclonal antibody titer analysis from harvest cell culture. The ProPac IMAC-10 is a high-resolution analytical and semipreparative column for separation of proteins and peptides by immobilized metal affinity chromatography. It is packed with 10µm, nonporous, polymeric beads coated with a hydrophilic layer, then grafted with poly(IDA) chains.

The ProSwift ConA-1S affinity monolith column is unsurpassed for fast, highly efficient enrichment and purification of Concanavalin A (Con A) binding glycans, glycopeptides, and glycoproteins containing high-mannose regions.

## Columns for oligonucleotides

Thermo Scientific™ DNAPac™ strong anion exchange columns provide industry-leading resolution for analysis and purification of synthetic oligonucleotides. DNAPac columns can resolve full length oligonucleotides from n-1, n+1, and other failure sequences not possible with other columns.

Thermo Scientific™ DNASwift™ a strong anion exchange monolith column that provides exceptionally high oligonucleotide purity. This semipreparative column incorporates the high resolution and selectivity of the DNAPac column, with increased loading capacity.

## Columns for carbohydrates

Thermo Scientific™ GlycanPac™ AXH-1 and AXR-1 columns are HPLC columns designed for the simultaneous separation of glycans by charge, size and polarity. Separating either fluorescently labeled or native glycans.

## Columns for proteomics

### Nano, capillary and micro columns

Thermo Scientific™ Acclaim™ PepMap™ and PepMap RSLC columns are specially designed for high-resolution analyses of tryptic, natural, and synthetic peptides. The columns are often applied for LC-MS/MS peptide mapping for protein identification, biomarker discovery, and systems biology. Due to their high loading capacity, the columns are exceptionally suitable for the analysis of low abundant peptides in complex proteomics samples. Acclaim PepMap Trap columns are typically applied for the desalting and preconcentration of peptides

before LC separation with MS detection. The columns are designed to provide the highest efficiencies for one dimensional peptide mapping experiments and 2D-LC analyses. Thermo Scientific™ PepSwift™ monolithic columns are specially designed for fast separation and identification of proteins and peptides using nano and capillary LC coupled to MS.

Using highly pure chromatographic media and biocompatible, metal-free fused silica capillaries, Thermo Scientific™ EASY-Column™ capillary LC columns are produced with a focus on design simplicity and strict quality control. As a result,

they deliver outstanding chromatographic performance on any nano LC system.

Thermo Scientific™ EASY-Spray™ columns provide a fully integrated and temperature controlled combined column-emitter design with only a single nanoViper connection between the LC and the MS ion source. This dead volume reduction is a critical component in helping to deliver state of the art performance with ease of use.

## Bio Columns Selection Guide

Analyte	Mode of Analysis	Recommended Column
Monoclonal antibodies and Proteins	Size Exclusion	BioBasic SEC
		MABPac SEC-1
	Ion Exchange	BioBasic AX
		ProPac SCX-10, WCX-10, SAX-10, WAX-10, SCX-20
		MABPac SCX-10, MABPac SCX-10 RS
	Reversed Phase	ProSwift IEX
		BioBasic 18, 8, 4
		Acclaim 300 C18
	Hydrophobic Interaction	Accucore 150-C18, 150-C4
		ProSwift RP
Affinity	ProPac HIC-10	
	MABPac Protein A	
	ProPac IMAC-10	
Peptides	Proteomics	ProSwift ConA-1S
		Acclaim PepMap
		PepSwift
	Analytical	EASY-Column
		EASY-Spray Columns
		BioBasic 18, 8, 4
Preparative	Acclaim 300	
	BioBasic	
Amino Acids (derivatized)	Ion Exchange	AminoPac PA10
	Reversed phase	Hypersil GOLD
Amino Acids (underivatized)	Ion Exchange	AminoPac PA10
	Reversed phase	Hypercarb
Nucleotides	Anion Exchange	BioBasic AX
	Polar retention	Hypercarb
Oligonucleotides	Ion Exchange	BioBasic AX
		DNAPac PA100, PA200, PA200 RS
		DNASwift
Carbohydrates	Ligand Exchange	HyperREZ XP
	Ion Exchange	CarboPac
	Mixed Mode	GlycanPac AXH-1
	HILIC	Acclaim HILIC
		Hypersil GOLD HILIC
		Synchronis HILIC
		Accucore 150-Amide-HILIC
	Polar retention	Hypercarb



View product information and application notes



## HPLC Phases for Biomolecules

### Silica-based Reversed Phase and Ion Exchange Phases

Phase	Particle Type	Particle Size (µm)	Pore Size (Å)	Nominal Surface Area (m <sup>2</sup> /g)	% Carbon	Endcapping	USP Code	Phase Code
<b>Acclaim Phase</b>								
300 C18	Spherical, fully porous silica	3	300	100	8	Yes	L1	–
<b>Acclaim PepMap Phases</b>								
100 C18	Spherical, fully porous silica	2, 3, 5	100	300	15	Yes	L1	–
300 C18	Spherical, fully porous silica	5	300	100	9	Yes	L1	–
100 C8	Spherical, fully porous silica	3, 5	100	300	9	Yes	L7	–
300 C4	Spherical, fully porous silica	5	300	300	3	Yes	L26	–
<b>Accucore Phases</b>								
150-C18	Spherical, solid core silica	2.6	150	80	7	Yes	L1	161
150-C4	Spherical, solid core silica	2.6	150	80	2	Yes	L26	165
150-Amide-HILIC	Spherical, solid core silica	2.6	150	80	–	–	–	167
<b>BioBasic Phases</b>								
18	Spherical, fully porous silica	5	300	100	9	Yes	L1	721
8	Spherical, fully porous silica	5	300	100	5	Yes	L7	722
4	Spherical, fully porous silica	5	300	100	4	Yes	L26	723
AX	Spherical, fully porous silica	5	300	100	3	No	–	731
SCX	Spherical, fully porous silica	5	300	100	3	No	L52	733

## Columns for Protein Separations

### Silica-based Size Exclusion Chromatography Phases

Phase	SEC Type	Particle Type	Particle Size (µm)	Pore Size (Å)	Exclusion Limit Operating Range (kDa)	USP Code	Phase Code
<b>BioBasic Phases</b>							
SEC 60	Aqueous	Spherical, fully porous silica	5	60	0.1 – 6	–	733
SEC 120	Aqueous	Spherical, fully porous silica	5	120	0.1 – 50	L33	734
SEC 300	Aqueous	Spherical, fully porous silica	5	300	1 – 500	L33, L59	735
SEC 1000	Aqueous	Spherical, fully porous silica	5	1000	20 – 4000	L33	736

### Silica-based Hydrophobic Interaction Chromatography Phases

Column	Phase	Target Applications	Base Matrix Material	Particle Size (µm)	Pore Size (Å)	Nominal Surface Area (m <sup>2</sup> /g)	Breakthrough Capacity	Solvent Compatibility	pH Range
ProPac HIC-10	Hydrophobic Interaction	High resolution separations of proteins and protein variants	Spherical, porous ultrapure silica with amide/ethyl surface chemistry	5	300	100	340mg lysozyme per 7.8 x 75mm column	Ammonium sulfate/phosphate salts, organic solvent for cleanup	2.5-7.5

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**Polymeric Ion Exchange, Reversed Phase and Affinity Columns**

Column	Phase	Target Applications	Base Matrix Material	Functional Groups	Breakthrough Capacity	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
ProPac WCX-10	Weak Cation Exchange	High resolution separations of proteins and protein variants	Ethylvinylbenzene cross linked with 55% divinylbenzene 10µm nonporous particles	Carboxylate	6mg/mL lysozyme	0.2-2.0 mL/min	80% ACN, acetone. Incompatible with alcohols and MeOH	3000psi (21 MPa)	2.0-12
ProPac SCX-10	Strong Cation Exchange	High resolution separations of proteins and protein variants	Ethylvinylbenzene cross linked with 55% divinylbenzene 10µm nonporous particles	Sulfonate	3mg/mL lysozyme	0.2-2.0 mL/min	80% ACN, acetone, MeOH	3000psi (21 MPa)	2.0-12
ProPac SCX-20	Strong Cation Exchange	High Resolution separations of proteins and protein variants	Divinylbenzene 10µm nonporous particles	Sulfonic	20µg/mL Dynamic capacity	0.2-2.0 mL/min	50% acetonitrile	3000psi (21 MPa)	2.0-12
ProPac WAX-10	Weak Anion Exchange	High resolution separations of proteins and protein variants	Ethylvinylbenzene cross linked with 55% divinylbenzene 10µm nonporous particles	Tertiary amine	5mg/mL BSA	0.2-2.0 mL/min	80% ACN, acetone, MeOH,	3000psi (21 MPa)	2.0-12
ProPac SAX-10	Strong Anion Exchange	High resolution separations of proteins and protein variants	Ethylvinylbenzene cross linked with 55% divinylbenzene 10µm nonporous particles	Quaternary ammonium	15mg/mL BSA	0.2-2.0 mL/min	80% ACN, acetone, MeOH	3000psi (21 MPa)	2.0-12
ProSwift RP-1S	Reversed Phase	Fast protein analysis with high resolution of large peptides to medium proteins	Monolith; polystyrene-divinylbenzene	Phenyl	5.5mg/mL Insulin	2.0-4.0 mL/min	Most common organic solvents	2800psi (19.2 MPa)	1.0-14
ProSwift RP-2H	Reversed Phase	Fast protein analysis with high resolution over a wide size range	Monolith; polystyrene-divinylbenzene	Phenyl	1.0mg/mL Lysozyme	1.0-10 mL/min	Most common organic solvents	2800psi (19.3 MPa)	1.0-14
ProSwift RP-3U	Reversed Phase	Fast protein analysis with high resolution of large proteins	Monolith; polystyrene-divinylbenzene	Phenyl	0.5mg/mL Lysozyme	1.0-16 mL/min	Most common organic solvents	2800psi (19.3 MPa)	1.0-14
ProSwift RP-4H	Reversed Phase	Fast protein analysis with high resolution	Monolith; polystyrene-divinylbenzene	Phenyl	2.3mg/mL Lysozyme	0.1-0.3 mL/min	Most common organic Solvents	1500psi	1.0-14
ProSwift SAX-1S	Strong Anion Exchange	Fast protein analysis with high resolution	Monolith; polymethacrylate	Quaternary amine	18mg/mL BSA	0.5-1.5 (4.6mm)	Most common organic solvents	1000psi (4.6mm) 2000psi (1.0mm)	2.0-12
ProSwift SCX-1S	Strong Cation Exchange	Fast protein analysis with high resolution	Monolith; polymethacrylate	Sulfonic acid	30mg/mL Lysozyme	0.5-1.5 mL/min (4.6mm)	Most common organic solvents	1000psi (4.6mm)	2.0-12
ProSwift WAX-1S	Weak Anion Exchange	Fast protein analysis with high resolution	Monolith; polymethacrylate	Tertiary amine (DEAE)	18mg/mL BSA	0.5-1.5 mL/min (4.6mm)	Most common organic solvents	1000psi (4.6mm) 2000psi (1.0mm)	2.0-12
ProSwift WCX-1S	Weak Cation Exchange	Fast protein analysis with high resolution	Monolith; polymethacrylate	Carboxylic acid	23mg/mL Lysozyme	0.5-1.5mL/min (4.6mm), 0.05-0.20	Most common organic solvents	1000psi (4.6mm) 2000psi (1.0mm)	2.0-12
ProPac IMAC-10	Immobilized Metal Affinity	High resolution separation of certain metal-binding proteins and peptides	Polystyrene divinylbenzene 10µm nonporous particles	Poly (IDA) grafts	>60mg lysozyme/mL gel (4x250mm)	1.0mL/min	EtOH, urea, NaCl, non-ionic detergents, glycerol, acetic acid, guanidine HCl	3000psi (21MPa)	2.0-12
ProSwift ConA-1S	Affinity	Concanavalin A binding (high-mannose) glycans, glycopeptides and proteins	Monolith; polymethacrylate	Concanavalin A ligands	12-16mg of protein	0-1.0mL/min	Up to 10% methanol	2000psi	5.0-8

## Columns for Monoclonal Antibody Separations

### Polymeric Ion Exchange Columns

Phase	IEX Type	Particle Type	Particle Size (µm)	Pore Size (Å)	Dynamic Capacity	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
MABPac SCX-10	Strong cation exchange (sulfonic)	Polymeric, Highly crosslinked DVB	3, 5, 10	non-porous	MABPac SCX-10 PEEK 3µm: 60µg/mL 5µm: 40µg/mL 10µm: 20µg/mL MABPac SCX-10 RS 5µm: 40 µg/mL	0.2-2.0mL/min (Depending on the particle size and column pressure limits)	50% Acetonitrile	3000psi (21MPa) RS columns 7000psi	2.0-12
ProPac WCX-10	Weak cation exchange (carboxylate)	Polymeric, Non-porous DVB	10	non-porous	6mg/mL	0.2-2.0mL/min	80% ACN, acetone. Incompatible with alcohols including methanol	3000psi (21MPa)	2.0-12

### Silica-based Size Exclusion Chromatography Columns

Phase	SEC Type	Particle Type	Particle Size (µm)	Pore Size (Å)	Exclusion Limit Operating Range (kDa)	USP Code
MABPac SEC-1	Aqueous	Spherical, fully porous silica	5	300	1-500	L33, L59

### Affinity Columns

Phase	Affinity ligand	Particle Type	Particle Size (µm)	Pore Size (Å)	Capacity	Recommended Flow Rate	Maximum temperature	Maximum Backpressure	pH Range
MABPac Protein A	Protein A	Polymeric	12	non-porous	100µg IgG/column	< 2.5	30	1000	2.5-7.5

## Columns for Carbohydrate Separations

### Polymeric Ligand Exchange Columns

Phase	Particle Type	Particle Size (µm)	Pore Size (Å)	Nominal Surface Area (m <sup>2</sup> /g)	% Carbon	Endcapping	USP Code	Phase Code
<b>HyperREZ XP Phases</b>								
Carbohydrate H <sup>+</sup>	Spherical, polymer	8	—	—	—	—	L17	690
Carbohydrate Pb <sup>2+</sup>	Spherical, polymer	8	—	—	—	—	L34	691
Carbohydrate Ca <sup>2+</sup>	Spherical, polymer	8	—	—	—	—	L19	692
Carbohydrate Na <sup>+</sup>	Spherical, polymer	10	—	—	—	—	—	693
Organic Acid	Spherical, polymer	8	—	—	—	—	L17	696
Sugar Alcohol	Spherical, polymer	8	—	—	—	—	L19	697

### Silica-based HILIC and Mixed-Mode Columns

Phase	Particle Type	Particle Size (µm)	Pore Size (Å)	Nominal Surface Area (m <sup>2</sup> /g)	% Carbon	Endcapping	USP Code	Phase Code
<b>GlycanPac Phases</b>								
AXH-1	Spherical, fully porous silica	1.9	175	220	-	Yes	-	-
AXH-1	Spherical, fully porous silica	3	120	300	-	Yes	-	-
<b>Accucore Phase</b>								
150-Amide-HILIC	Spherical, solid core silica	2.6	150	80	-	-	-	167

## Monolithic Affinity Columns

Phase	Particle Type	Particle Size (µm)	Pore Size (Å)	Nominal Surface Area (m <sup>2</sup> /g)	% Carbon	Endcapping	USP Code	Phase Code
<b>ProSwift Phases</b>								
ConA-1S	Concanavalin A binding (high mannose) glycans, glycopeptides and proteins	"Monolith;	175	220	-	Yes	-	-

## Columns for Oligonucleotide Separations

Column	Target Applications	Base Matrix Material	Substrate Crosslinking	Latex Crosslinking	Capacity	Recommended Eluents	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
DNAPac PA100	High resolution separations of single and double stranded DNA or RNA oligonucleotides	13µm diameter nonporous substrate agglomerated with alkyl quaternary ammonium functionalized latex 100nm MicroBeads	55%	5%	40µeq	Hydroxide or sodium and lithium salts of Chloride or Perchlorate	1.5mL/min	0-100%	4000psi (28MPa)	2-12.5
DNAPac PA200	Improved high resolution separations of single and double stranded DNA or RNA orthogonal to DNAPac PA100	8µm diameter nonporous substrate agglomerated with alkyl quaternary ammonium functionalized latex 130nm MicroBeads	55%	5%	40µeq	Hydroxide, acetate/ or sodium and lithium salts of Chloride or Perchlorate	1.2mL/min	0-100%	4000psi (28MPa)	2-12.5
DNAPac PA200 RS	UHPLC-resolution separations of single and double stranded DNA or RNA Best available resolution	4µm diameter nonporous substrate agglomerated with alkyl quaternary ammonium functionalized latex 130nm MicroBeads	55%	5%	40µeq	Hydroxide, acetate/ hydroxide or sodium and lithium salts of Chloride or Perchlorate	1.3mL/min	0-100%	10,000 psi (69MPa)	2-12.5
DNASwift	High resolution separations for purification of oligonucleotides Highest latex-based capacity	Monolith; polymethacrylate substrate agglomerated with quaternary amine functionalized latex	70%	3%	8mg, of a 20 mer oligonucleotide	NaClO <sub>4</sub> and NaCl	0.5-2.5mL	Most Common Organic Solvents	1500psi, 10MPa	6.0-12.4

# Resources

for Chromatographers



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