

LC columns and accessories for biomolecules

Technical resources document

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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

The Thermo Scientific MAbPac RP is a polymer based wide pore column. The 1500Å pore size allows for the separation of large mAbs and their fragments. Compatible with LC-MS analysis, and standard LC-UV, this column offers high throughput separations, with long lifetime. Capable of operating up to 110°C, and pH 1-14, it is a very robust platform for your denaturing protein separations.

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.

Thermo Scientific™ Acclaim™ 300 C18 features 3 µm silica particles for rapid analysis of complex protein digests. Compared to 5 µm 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 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.

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 and MAbPac HIC columns offer high resolution, high capacity columns. Providing excellent resolution and separation, of proteins and variants for analytical and preparative applications. ProPac HIC columns are 300A and offer high capacity, MAbPac HIC columns are 1000A and offer high resolution.

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™ DNAPac™ RP U/HPLC columns deliver high throughput, high resolution ion-pair reversed-phase separations. These LS-MS compatible, long-lasting polymeric columns operate up to pH 14, have temperature stability to 110°C, and easily resolve large dsDNA fragments up to >10,000 bp.

Thermo Scientific™ DNASwift™ is 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 Accucore Amide-HILIC columns are solid core silica columns designed for the separation of glycans. They offer excellent peak capacity, and high resolution separations by fluorescence or LC-MS detection. Excellent resolution for mAb samples that predominantly contain neutral glycans.

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. For neutral glycan analysis, such as those found on mAbs, the Thermo Scientific Accucore 150 Amide-HILIC offers outstanding resolution of glycans and robust lot-to-lot reproducibility.

Columns for proteomics

Nano, capillary and micro columns

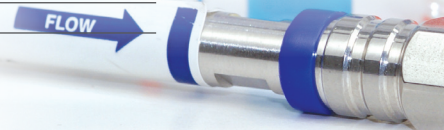
Thermo Scientific™ Acclaim™ PepMap™ and PepMap RSLC columns are specially designed for high-resolution analyses of tryptic, natural, and synthetic peptides. The columns are often applied for LC-MS/MS peptide mapping for protein identification, biomarker discovery, and systems biology. Due to their high 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. 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 Thermo Scientific™ 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 BioBasic AX ProPac Elite	
	Ion exchange	ProPac SCX-10, WCX-10, SAX-10, WAX-10, SCX-20 MAbPac SCX-10, MAbPac SCX-10 RS ProSwift IEX	
		BioBasic 18, 8, 4 MAbPac RP Acclaim 300 C18 Accucore 150-C18, 150-C4 ProSwift RP	
	Reversed-phase	MAbPac HIC-10 MAbPac HIC-20 MAbPac HIC-Butyl ProPac HIC-10 MAbPac Protein A ProPac IMAC-10 ProSwift ConA-1S	
		Proteomics	Acclaim PepMap
	Peptides	Analytical	BioBasic 18, 8, 4 Acclaim 300
		Preparative	BioBasic
		Ion exchange	AminoPac PA10
	Reversed phase		Hypersil GOLD
	Amino acids (derivatized)	Ion exchange	AminoPac PA10
	Amino acids (underivatized)	Reversed phase	Hypercarb
	Oligonucleotides	Ion exchange	BioBasic AX DNAPac PA100, PA200, PA200 RS DNASwift
Reversed-phase		DNAPac RP	
Ligand exchange		HyperREZ XP	
Carbohydrates	Ion exchange	CarboPac	
	Mixed mode	GlycanPac AXH-1 Acclaim HILIC	
	Hilic	Hypersil GOLD HILIC Synchronis HILIC Accucore 150-Amide-HILIC	
		Polar retention	Hypercarb



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 ² /g)	% carbon	Endcapping	USP code	Phase code
Acclaim phase								
300 C18	Spherical, fully porous silica	3	300	100	8	Yes	L1	–
Acclaim PepMap phases								
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	–
Accucore phases								
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
BioBasic phases								
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
BioBasic phases							
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
MABPac SEC-1	Aqueous	Spherical, fully porous silica	5	300	1-500	L33, L59	

Hydrophobic interaction chromatography (HIC) phases

Column	Phase	Target applications	Base matrix material	Particle size (µm)	Pore size (Å)	Nominal surface area (m ² /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	340 mg lysozyme per 7.8 x 75 mm column	Ammonium sulfate/ phosphate salts, organic solvent for cleanup	2.5-7.5

Silica-based hydrophobic interaction chromatography phases (continued)

Column	Phase	Target applications	Base matrix material	Functional groups	Breakthrough capacity	Recommended flow rate	Solvent compatibility	Maximum backpressure	pH range
MABPac HIC-10	Hydrophobic Interaction	Separation of mAbs, bispecifics, ADCs with hydrophobic heterogeneity	Spherical, high purity silica particles	Proprietary polyamide	30 mg/mL	0.5-1 mL/min	0-100%	6000psi (100mm) 8000psi (250mm)	2-8
MABPac HIC-20	Hydrophobic Interaction	Separation of mAbs, bispecifics, ADCs with hydrophobic heterogeneity	Spherical, high purity silica particles	Proprietary amide	24 mg/mL	0.5-1 mL/min	0-100%	6000psi (100mm) 8000psi (250mm)	2-8
MABPac HIC Butyl	Hydrophobic Interaction	Separation of mAbs, bispecifics, ADCs with hydrophobic heterogeneity	Non-porous hydrophilic polymer	Butyl	9 mg/mL	0.5-1 mL/min	0-100%	4000psi	2-12

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 Elite	Weak cation exchange	High resolution separations of proteins and protein variants	Ethylvinylbenzene cross linked with 55% divinylbenzene 5µm nonporous particles	Carboxylate		0.1-1 mL/min	80% ACN, acetone. Incompatible with alcohols and MeOH	4500 psi (31 MPa)	2.0-12
ProPac WCX-10	Weak cation exchange	High resolution separations of proteins and protein variants	Ethylvinylbenzene cross linked with 55% divinylbenzene 10µm nonporous particles	Carboxylate	6 mg/mL lysozyme	0.2-2.0 mL/min	80% ACN, acetone. Incompatible with alcohols and MeOH	3000 psi (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	3 mg/mL lysozyme	0.2-2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2.0-12
ProPac SCX-20	Strong cation exchange	High Resolution separations of proteins and protein variants	Divinylbenzene 10µm nonporous particles	Sulfonic	20 µg/mL Dynamic capacity	0.2-2.0 mL/min	50% acetonitrile	3000 psi (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 10 µm nonporous particles	Tertiary amine	5 mg/mL BSA	0.2-2.0 mL/min	80% ACN, acetone, MeOH,	3000 psi (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 10 µm nonporous particles	Quaternary ammonium	15 mg/mL BSA	0.2-2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (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	5.5 mg/mL Insulin	2.0-4.0 mL/min	Most common organic solvents	2800 psi (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	1.0 mg/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	0.5 mg/mL Lysozyme	1.0-16 mL/min	Most common organic solvents	2800psi (19.3 MPa)	1.0-14

Polymeric ion-exchange, reversed-phase and affinity columns (continued)

Column	Phase	Target applications	Base matrix material	Functional groups	Breakthrough capacity	Recommended flow rate	Solvent compatibility	Maximum backpressure	pH range
MABPac RP	Reversed-phase	High resolution intact and fragment protein analysis	Supermacroporous polymer, 4µm	Phenyl	20 µg	75-150µL/min for 1.0mm ID 300-600µL/min for 2.1mm ID 500-1000µL/min for 3.0mm ID	Up to 100% ACN, IPA, MeOH	4000psi (28Mpa)	0-14 for 2.1 and 3.0mm ID 1-7 for 1mm ID
ProSwift RP-4H	Reversed-phase	Fast protein analysis with high resolution	Monolith; polystyrene-divinylbenzene	Phenyl	2.3 mg/mL Lysozyme	0.1-0.3 mL/min	Most common organic Solvents	1500 psi	1.0-14
ProSwift SAX-1S	Strong anion exchange	Fast protein analysis with high resolution	Monolith; polymethacrylate	Quaternary amine	18 mg/mL BSA	0.5-1.5 (4.6mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2.0-12
ProSwift SCX-1S	Strong cation exchange	Fast protein analysis with high resolution	Monolith; polymethacrylate	Sulfonic acid	30 mg/mL Lysozyme	0.5-1.5 mL/min (4.6 mm)	Most common organic solvents	1000 psi (4.6 mm)	2.0-12
ProSwift WAX-1S	Weak anion exchange	Fast protein analysis with high resolution	Monolith; polymethacrylate	Tertiary amine (DEAE)	18 mg/mL BSA	0.5-1.5 mL/min (4.6 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2.0-12
ProSwift WCX-1S	Weak cation exchange	Fast protein analysis with high resolution	Monolith; polymethacrylate	Carboxylic acid	23 mg/mL Lysozyme	0.5-1.5mL/min (4.6mm), 0.05-0.20	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	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	>6.0 mg lysozyme/mL gel (4 x 250 mm)	1.0 mL/min	EtOH, urea, NaCl, non-ionic detergents, glycerol, acetic acid, guanidine HCl	3000 psi (21M Pa)	2.0-12
ProSwift ConA-1S	Affinity	Concanavalin A binding (high-mannose) glycans, glycopeptides and proteins	Monolith; polymethacrylate	Concanavalin A ligands	12-16 mg of protein	0-1.0 mL/min	Up to 10% methanol	2000 psi	5.0-8

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	100 µg IgG/column	< 2.5	30	1000	2.5-7.5

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 3 µm: 60 µg/mL 5 µm: 40 µg/mL 10 µm: 20 µg/mL MABPac SCX-10 RS 5 µm: 40 µg/mL	0.2-2.0 mL/min (Depending on the particle size and column pressure limits)	50% acetonitrile	3000 psi (21 MPa) RS columns 7000 psi	2.0-12

Columns for carbohydrate separations

Polymeric ligand exchange columns

Phase	Particle type	Particle size (µm)	Pore size (Å)	Nominal surface area (m ² /g)	% carbon	Endcapping	USP codec	Phase code
HyperREZ XP phases								
Carbohydrate H ⁺	Spherical, polymer	8	–	–	–	–	L17	690
Carbohydrate Pb ²⁺	Spherical, polymer	8	–	–	–	–	L34	691
Carbohydrate Ca ²⁺	Spherical, polymer	8	–	–	–	–	L19	692
Carbohydrate Na ⁺	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 ² /g)	% carbon	Endcapping	USP code	Phase code
GlycanPac phases								
AXH-1	Spherical, fully porous silica	1.9	175	220	-	Yes	-	-
AXH-1	Spherical, fully porous silica	3	120	300	-	Yes	-	-
Accucore phase								
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 ² /g)	% carbon	Endcapping	USP Code	Phase Code
ProSwift Phases								
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	13 µm diameter nonporous substrate agglomerated with alkyl quaternary ammonium functionalized latex 100 nm MicroBeads	55%	5%	40 µeq	Hydroxide or sodium and lithium salts of Chloride or Perchlorate	1.5 mL/min	0-100%	4000 psi (28 MPa)	2-12.5
DNAPac PA200	Improved high resolution separations of single and double stranded DNA or RNA orthogonal to DNAPac PA100	8 µm diameter nonporous substrate agglomerated with alkyl quaternary ammonium functionalized latex 130 nm MicroBeads	55%	5%	40 µeq	Hydroxide, acetate/ or sodium and lithium salts of Chloride or Perchlorate	1.2 mL/min	0-100%	4000 psi (28 MPa)	2-12.5
DNAPac PA200 RS	UHPLC-resolution separations of single and double stranded DNA or RNA Best available resolution	4 µm diameter nonporous substrate agglomerated with alkyl quaternary ammonium functionalized latex 130 nm MicroBeads	55%	5%	40 µeq	Hydroxide, acetate/ hydroxide or sodium and lithium salts of Chloride or Perchlorate	1.3 mL/min	0-100%	10,000 psi (69 MPa)	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%	8 mg, of a 20 mer oligonucleotide	NaClO ₄ and NaCl	0.5-2.5 mL	Most Common Organic Solvents	1500 psi, 10 MPa	6.0-12.4
DNAPac RP	Ion pair reversed phase separations of oligonucleotides and failure sequences. Polymeric backbone for long life at high pH and temperature.	4µm hydrophobic wide-pore polymer resin			29ug/mL of a 25-mer	TEAA, TEA, HFIP	2.1mm ID: 0.2-0.6 mL/min 3.0mm ID: 0.4-1.0 mL/min	Up to 100% ACN, IPA, MeOH	4000 psi (28MPa)	0-14

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