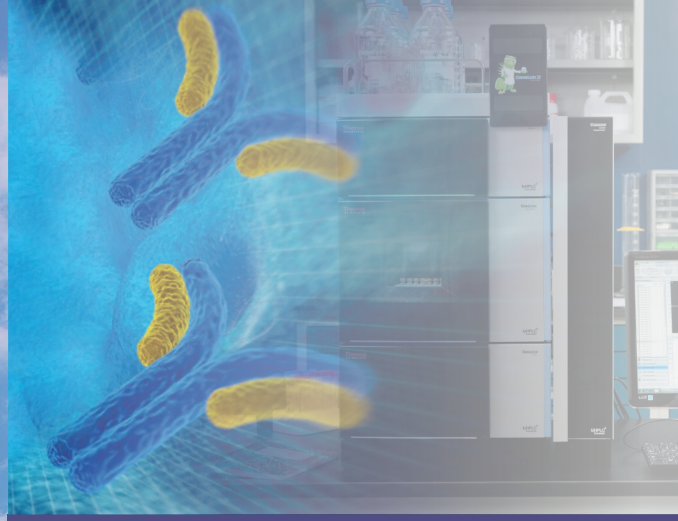

Thermo Scientific
BioLC Columns

Innovative solutions for
mAb analysis and characterization

Thermo
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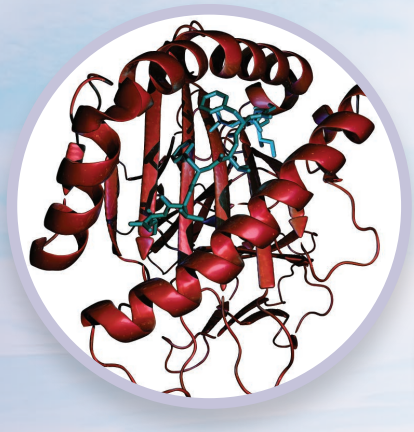
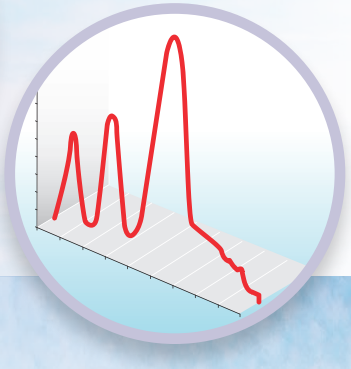
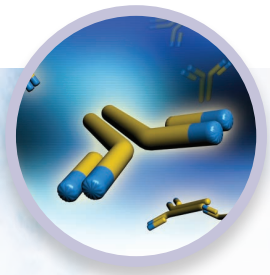
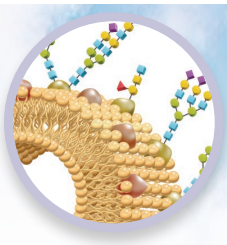




Biopharma Terminology

Term	What does it mean?
Discovery	Process to identify and screen potential drug compounds.
Development	Investigate the most effective compounds from discovery phase; determine efficacy, lifetime, and potential side-effects.
Clinical/pre-clinical	Clinical trials with human volunteers performed in medical clinics and hospitals.
Commercialization	Phase when a new drug achieves legislative success, passes regulatory requirements, and is launched to market.
FDA	Food and Drug Administration, responsible for licensing and control of pharmaceuticals in the US.
EMA	European Medicines Agency.
CFDA	China Food and Drug Administration.
Monoclonal Antibody/mAb	A protein biologic that has been engineered to target certain cells. Many new pharmaceutical products are mAbs.
Biosimilar	A biological product that is demonstrated to be "highly similar" to an FDA-licensed biological product (the reference product).
Isoelectric point (pI)	The pH at which the overall net charge of a protein in zero.
Charge variants	Variations in the mAb amino acid sequence and modifications that lead to alterations in the overall net charge. Acidic charge variants arise from modification such as deamidation and sialylation, (which give increased net negative charge). Basic charge variants result from the presence of C-terminal lysine or glycine amidation, succinimide formation, amino acid oxidation or removal of sialic acid, (which introduce additional positive charge).
Glycan	A carbohydrate/sugar. The quantity and location of glycans on a protein can influence the efficacy of the drug.
Aggregates	Clusters of mAb units that form due to two or more drug compounds attaching to each other.
Peptide	Short chain molecules of multiple amino acids that form the larger protein structure.
Antibody-Drug Conjugate (ADC)	A mAb chemically coupled to a linker and cytotoxic drug. ADCs are a class of therapeutics that harness the antigen-selectivity of mAbs to deliver highly potent cytotoxic drugs to antigen-expressing tumor cells. The use of mAb directed delivery increases both the efficacy and safety of therapy.
DAR (Drug-to-Antibody Ratio)	The ratio of the number of cytotoxic drug linkers per mAb unit present in an ADC.
Titer	Quantitation of therapeutic mAb present in the cell culture.
PTM	Post-translational modification. A change in an amino acid residue side-chain due to an enzymatic or chemical process.



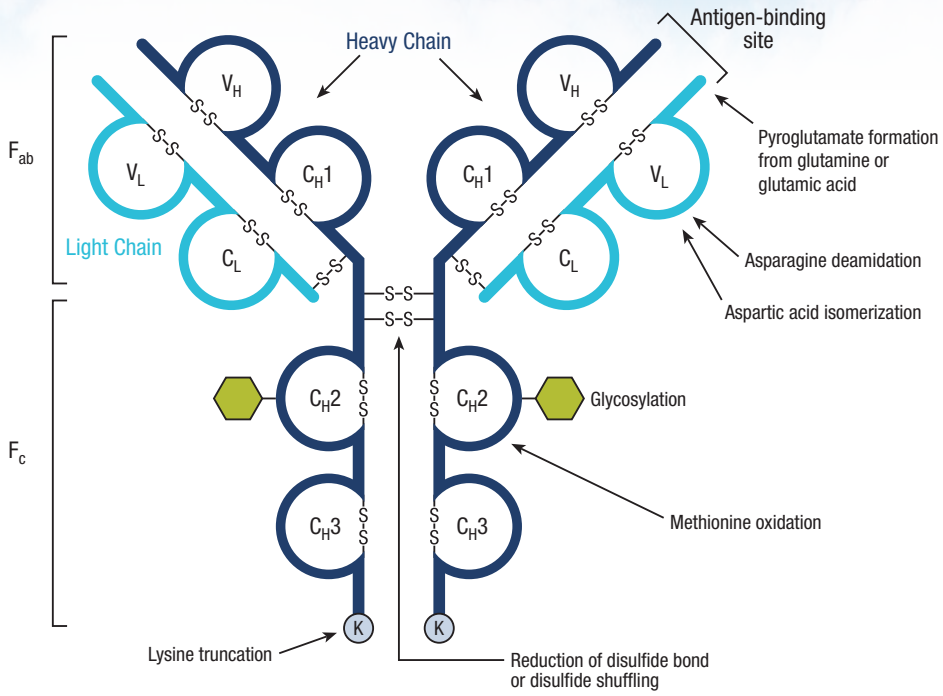


Introduction to Monoclonal Antibody (mAb) Characterization

Protein and monoclonal antibody (mAb) biopharmaceuticals form a major part of the growing biologics drug market, and have been transforming the biotechnology and biopharmaceutical industries in the last decade. mAb treatment is amongst the most effective methods of diagnosis and treatment of a broad range of diseases, including autoimmune disorders, cardiovascular diseases, infectious diseases, cancer and inflammation.

There is an extensive development pipeline for protein and mAb therapeutics, further emphasizing the need for innovative and efficient analytic tools. During development and production of these products it is essential to detect, characterize and quantify structural variants and modifications and to monitor product stability. This is key to demonstrating safety and efficacy and is required by the U.S. FDA, EMA, CFDA and other regulatory agencies. Highlighted in the table below are the various LC and LC-MS protein analytical chemistry methods used in the testing of biological products, as well as the recommended bio-columns and buffers.

Analysis	Description	Columns and Buffers
mAb Capture and Titer Analysis	mAb titer determination (concentration) & screening; mAb capture for analysis workflows	Thermo Scientific™ MAbPac™ Protein A POROS® A, Cartridge
Aggregate Analysis	routine screening for mAb aggregates and fragments	MAbPac SEC-1 MAbPac HIC-10 MAbPac HIC-20 Thermo Scientific™ Acclaim™ SEC-300
Charge Variant Analysis	routine charge variant profiling/screening, including lysine truncation,deamidation & acylation;	MAbPac SCX-10 MAbPac SCX-10RS Thermo Scientific™ ProPac™ WCX-10 Thermo Scientific™ CX-1 pH Gradient Buffer Kit
Methionine & Tryptophan Oxidation	targeted analysis of methionine and tryptophan oxidation	MAbPac HIC-20 MAbPac HIC-10 ProPac HIC-10
Antibody Drug Conjugates (ADCs) Analysis	ADC Drug-to-Antibody Ratio (DAR) DAR analysis	MAbPac HIC-Butyl MAbPac HIC-10 MAbPac HIC-20 MAbPac RP
Intact mAb & Fragments Analysis	light chain (LC) and heavy chain (HC) analysis; Fab and Fc analysis; scFc and $F(ab')_2$ analysis	MAbPac RP Thermo Scientific™ ProSwift™ RP-1S MAbPac SEC-1 MAbPac HIC-20
Sequence & Structural Analysis	primary sequence analysis; peptide mapping; peptide & glycopeptide structural & linkage analysis	Acclaim RSLC 120 C18 Acclaim 300 C18 Thermo Scientific™ Accucore™ 150-C18
Glycan Profiling	profiling of released glycans	Thermo Scientific™ GlycanPac™ AXH-1 GlycanPac AXR-1 Accucore 150-Amide-HILIC



Monoclonal antibodies have very complex structures, with many possible site-specific variations. With such potential for post translational modifications (PTMs), quality control and stability assessment of monoclonal antibodies are very challenging tasks.

High pressure liquid chromatography has established itself as an essential tool in the characterization of mAb and other protein biotherapeutics.

Biopharma Workflows

Routine Screening and Clone Selection Workflow

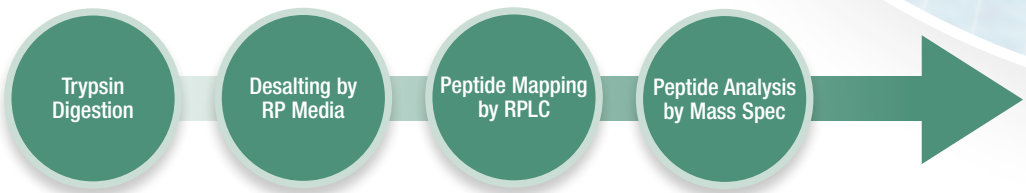
In the early development stage, purification of mAb or IgG from harvested cell culture fluid (HCCF) using Protein A is typically the first step. Often this is followed by size-exclusion chromatography (SEC) for aggregate profiling and ion-exchange chromatography (IEC) for charge variants analysis. Therefore, these separation modes make up a substantial portion of the analysis performed during mAb development.

Intact mAb Profiling and Subunit Analysis Workflow

Intact mAb analysis provides information such as molecular weight, drug-antibody ratio (DAR), and glycan profiles. mAb fragment analysis can quickly pinpoint the location of the modifications without complete digestion of the mAb. MAb light chain (LC) and heavy chains (HC) are generated by the reduction of the four inter disulfide bonds. Papain cleaves above the hinge region containing the disulfide bonds that join the heavy chains, but below the site of the disulfide bond between the light chain and heavy chain. This generates two Fab fragments and an intact Fc fragment. The Immunoglobulin-degrading enzyme from Streptococcus pyogenes (IdeS) is a highly specific protease that cleaves Immunoglobulin G (IgG) at a single site below the hinge region, yielding single chain Fc (scFc) and $F(ab')_2$ fragments. This is also known as the middle-down approach. Optional in-depth analysis of variants and sequence can be performed by MS^n (multiple stages of MS).

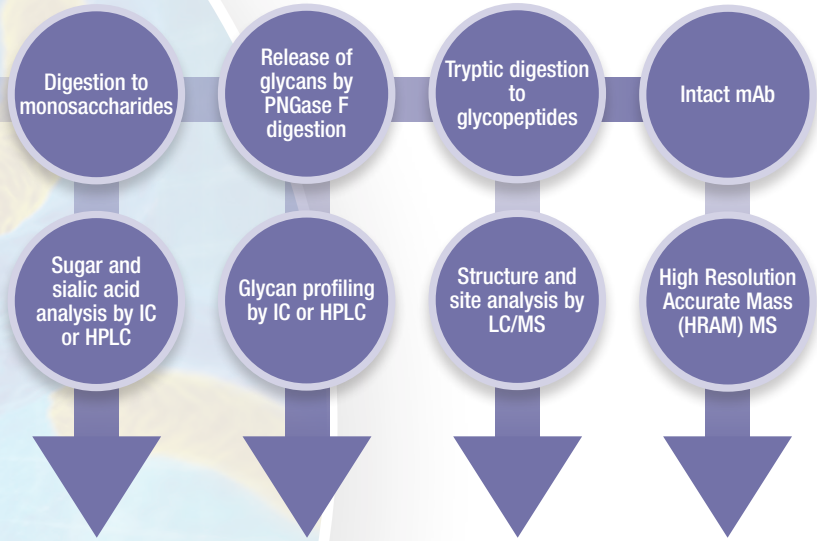
High Resolution Peptide Mapping Workflow

A thorough mAb sequence analysis is achieved by trypsin digestion and analysis of the tryptic peptides by LC-MS/MS using a reversed phase C18 separation. Peptide mapping is commonly used to identify modifications of amino acids, such as deamidation and oxidation. Structural analysis is usually carried out using a high resolution mass spectrometer during the development stage. However, for routine QC batch release, peptide separations are monitored by UV.



Glycan Analysis Workflow

The workflow below shows the most common ways to analyze glycans structures and glycosylation sites. PNGase F is a endoglycosidase cleaving the link between asparagine and N-acetylglucosamines. After PNGase F digestion, the N-linked carbohydrates are released from the mAb and analyzed by either an IC or HPLC methods. For glycosylation site identification, tryptic digestion is often performed and the glycopeptides are analyzed by LC-MS method. For biological product QC testing, intact mAb can be analyzed using a high resolution mass spectrometer. This analysis provides accurate molecular weight and glycan profile information.



The Complete Solution

A combination of these separation workflows allows users to achieve a wide range of analytical tasks, such as:

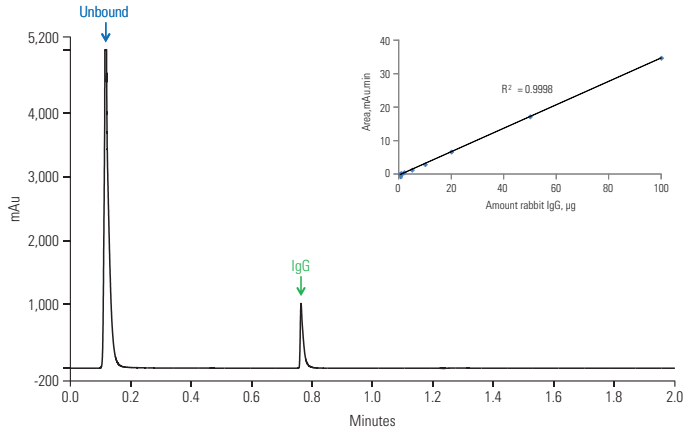
- Purity and titer analysis
- Aggregation analysis
- Charge variant analysis
- Clips and truncations
- Light chain/heavy chain analysis
- Peptide mapping
- Glycan profiling
- Post-translational modification analysis (amino acid substitutions/truncations, deamidation, phosphorylation, etc.)

We offer a complete solution for each of the workflows, enabling scientists to achieve optimum and reliable results.

Affinity Titer Determination

Early in the development of recombinant mAbs, a large number of harvest cell culture (HCC) samples must be screened for IgG titer, i.e. various antibody epitopes must be quantified. The high degree of specificity offered by affinity chromatography provides a powerful alternative to established immunoaffinity methods such as ELISA.

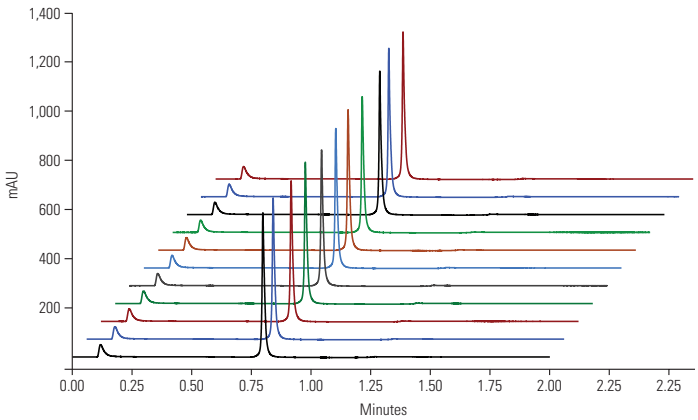
Protein A ligand is often used due to its strong affinity to bind a wide range of antibodies. The MAbPac Protein A HPLC affinity column can be used to determine the MAb concentration in HCC.



Column:	**MAbPac Protein A**
Format:	4.0 × 35 mm
Eluent A:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 7.5
Eluent B:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 2.5
Gradient:	0% B for 0.2 mins, 100% B for 0.60 mins, 0% B for 1.20 mins
Flow Rate:	2 mL/min
Inj. Volume:	10 µL
Temp.:	25 °C
Detection:	280 nm
Sample:	Harvest cell culture (HCC)

The challenge faced by analytical laboratories in the pharmaceutical industry is to the development of a high-throughput and robust titer assay. The MAbPac Protein A column has been engineered specifically for this application from a novel polymeric resin with a hydrophilic surface, covered with a covalently bound recombinant Protein A. The hydrophilic nature of the backbone minimizes nonspecific binding, and therefore enables accurate quantification of the mAb titer. The MAbPac Protein A column format allows rapid automation of loading, binding, elution and collection using the biocompatible HPLC system, such as the Thermo Scientific™ UltiMate™ 3000 BioRS system.

MAbPac Protein A columns feature excellent binding efficiency, high loading capacity, and superior ruggedness. Lower backpressure enables fast analysis. A simple step gradient from near-neutral to acidic pH is all that is required to elute the bound material.

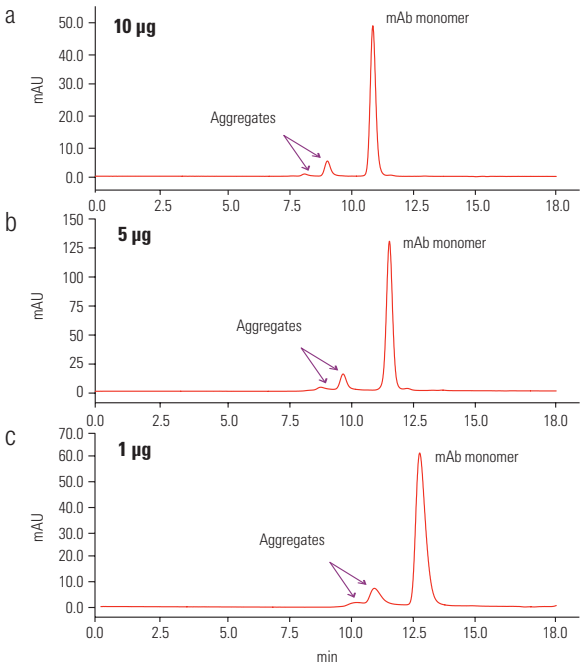


Column:	**MAbPac Protein A**
Format:	4.0 × 35 mm
Eluent A:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 7.5
Eluent B:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 2.5
Gradient:	0% B for 0.2 mins, 100% B for 0.60 mins, 0% B for 1.20 mins
Flow Rate:	2 mL/min
Inj. Volume:	20 µL
Temp.:	25 °C
Detection:	280 nm
Sample:	Rabbit IgG, 1 mg/mL

	t_R (min)	Area (mAu*min)	PWHH (min)
Average	0.80	7.76	0.01
% RSD	1.02	2.20	0.00

Aggregate Separation

Monoclonal antibodies (mAbs) produced from mammalian cell culture may contain significant amounts of dimers and higher-order aggregates. Studies show that these aggregates present in drug products may result in enhanced desired response, but may also cause severe immunogenic and anaphylactic reactions. Thus, biopharmaceutical manufacturers are required to develop analytical methods to monitor size heterogeneity and control levels of dimer and higher-order aggregates.



Column:	**MAbPac SEC-1**, 5 µm,
Format:	a. 7.8 × 300 mm
	b. 4.0 × 300 mm
	a. 2.1 × 300 mm
Mobile Phase:	50mM sodium phosphate pH 6.8, in 300 mM sodium chloride
Flow Rate:	a. 760 µL/min
	b. 200 µL/min
	c. 50 µL/min
Inj. Volume:	a. 10 µL
	b. 5 µL
	a. 1 µL
Temp.:	30 °C
Detection:	280 nm
Sample:	mAb (1 mg/mL)

Size-exclusion chromatography (SEC) is an ideal choice for separation for mAb aggregates. For SEC the separation is based solely on the size of the molecule which does not interact with the stationary phase. Molecules of different sizes penetrate the pores to varying extents. This results in analytes of different sizes travelling through the media at different speeds. Larger molecules do not diffuse deep in the pores and elute first, whilst smaller entities diffuse deeper in the pores and elute later.

The MAbPac SEC-1 column is specially designed for the separation and characterization of monoclonal antibodies (mAbs), their aggregates, and analysis of Fab and Fc fragments. The MAbPac SEC-1 column is based on high-purity, spherical, porous (300 Å), 5 µm silica particles that are covalently modified with a hydrophilic layer, resulting in an extremely low level of undesired interaction sites. The stationary phase is designed to handle different eluent conditions containing both high and low ionic strength mobile phases, as well as mass spectrometry friendly volatile eluents.

The three different column formats meet different analytical needs: the 7.8 mm ID column provides the highest resolution separation and accurate quantitation of mAbs and their aggregates; the 4.0 mm ID column enables baseline separation of mAb monomer and dimer, requiring only ¼ of sample compared to the 7.8 mm ID column; the 2.1 mm ID column uses low flow rate and low sample loading, making it the perfect format for MS detection.

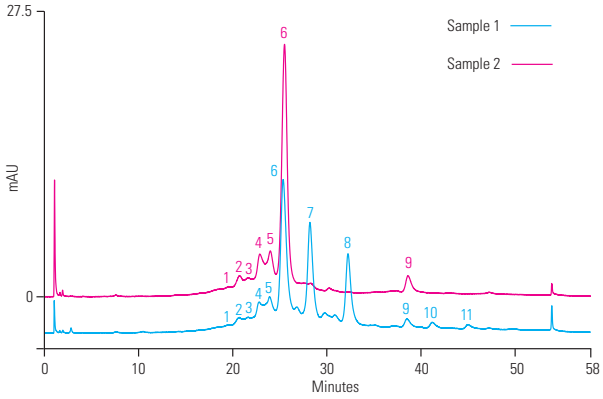
Charge Variant Analysis

Ion Exchange Chromatography (IEC) is based on a stationary phase with a charged ligand on the surface interacting with analytes of the opposite charge. The technique is divided between anion exchange and cation exchange chromatography. In anion exchange, positively charged surface ligands interacts with negatively charged analytes, whilst in cation exchange negatively charged surface ligands interact with positively charged analytes.

Salt Gradient Method

In salt gradient-based IEC, the pH of the buffer is fixed. In addition to choosing the appropriate pH of the starting buffer, the initial ionic strength is kept low since the affinity of proteins for IEC resins decreases as ionic strength increases. The proteins are then eluted by increasing the ionic strength (salt concentration) of the buffer to increase the competition between the buffer ions and proteins for charged groups on the IEC resin. As a result, the interaction between the IEC resin and proteins is reduced, causing the proteins to elute.

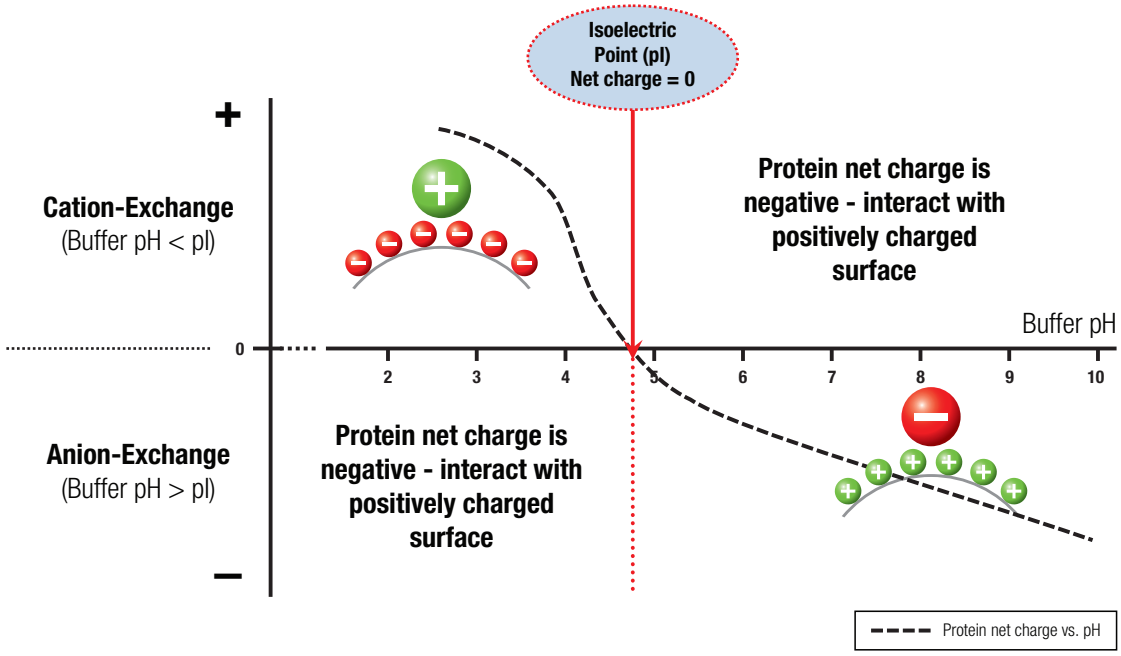
The pI of the majority of antibodies tends to be in the pH 6–10 region, which is the reason why cation exchange is the commonly chosen separation method for mAb charged variants. A range of cation exchange columns, such as the MabPac SCX-10 column family, is available and designed specifically for the separation of mAb variants and other variations affected by charge, such as lysine truncation variants.



Column:	**MAbPac SCX-10**, 10 µm
Format:	4.0 × 250 mm
Mobile Phase A:	20 mM MES, 60 mM sodium chloride, pH 5.6
Mobile Phase B:	20 mM MES, 300 mM sodium chloride, pH 5.6
Gradient:	15–36%B in 50 min.
Flow Rate:	1.0 mL/min
Inj. Volume:	5 µL
Temp.:	30 °C
Detection:	UV (280 nm)
Sample:	1: mAb (9 mg/mL) 2: mAb (9 mg/mL) + carboxypeptidase B (CPB)
Peaks:	Sample 1 1–5: Acidic variants 6–8: C-terminal lysine truncation variants of the main peak 9–11: C-terminal Lys truncation variants of a minor variant peak
	Sample 2 6: From peaks 6, 7, 8 after CPB treatment 7: From peaks 9, 10, 11 after CPB treatment

pH Gradient Method

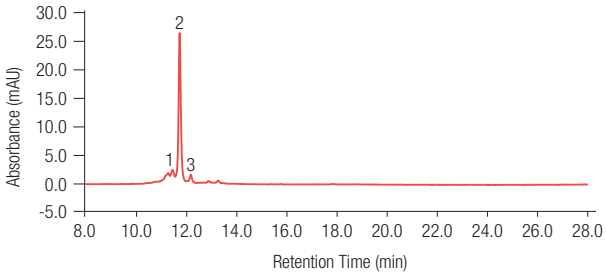
In pH-gradient-based IEC, the starting buffer is held at a constant pH to ensure the proteins have a charge opposite to the stationary phase, thus binding to the column. As the gradient runs, the pH of the buffer is changed so the proteins transition to a net zero charge (the pI), and ultimately the same charge as the resin, thus the protein is released and eluted from the column.



In the fast-paced drug development environment, a platform method to accommodate the majority of MAb analyses is desired. Considerable effort is often required to tailor the salt gradient method for a cation exchange separation of an individual mAb. Ion exchange separations by pH gradient provide the advantage of a generic platform approach, thus saving time on method development.

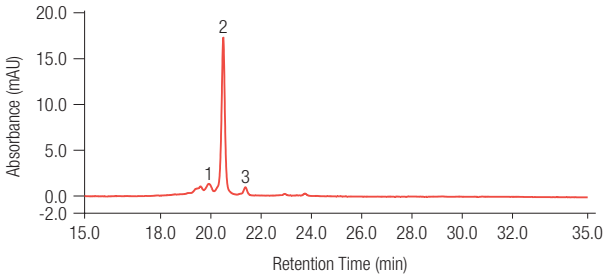
One of the challenges in pH-gradient separations is choosing a buffer system able to cover a wide pH range whilst delivering a linear pH gradient. We offer a novel pH gradient method for cation-exchange chromatography. This method features a multi-component zwitterionic buffer system in which the linear gradient can be run from 100% eluent A (low pH buffer) to 100% eluent B (high pH buffer). The linearity of the pH gradient can be verified using a Thermo Scientific™ Dionex™ UltiMate™ PCM-3000 pH and conductivity online monitor, which is easily incorporated with Thermo Scientific LC instrumentation. Once the approximate pH elution range of the target mAb has been established in the initial run, optimization of the separation is achieved by running a shallower pH gradient over a narrower pH range. Separation of avastin/bevacizumab variants is optimized using a pH gradient. When a broad pH gradient is applied from 0% to 100% B (pH 5.6 to 10.2), all variants are eluted in the pH range 7.0 to 8.0. Adjusting the gradient to 0–50% B (5.6–7.9 pH range, middle), resolution is further improved with minimal optimization steps. For comparison, avastin/bevacizumab variants separation by the salt gradient methods with a steeper (a) and a shallower gradient slope (b).

a Avastin pH Gradient 0–100% B



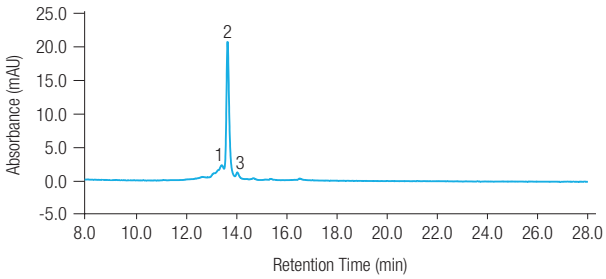
Column:	**MAbPac SCX-10**, 10 µm
Format:	4.0 × 250 mm
Mobile Phase A:	1X CX-1 pH gradient buffer A, pH 5.6
Mobile Phase B:	1X CX-1 pH gradient buffer B, pH 10.2
pH Gradient:	0% B to 100% B from 1 to 31 min
Flow Rate:	1.0 mL/min
Inj. Volume:	5 µL
Temp.:	30 °C
Detection:	UV (280 nm)
Sample:	Avastin/bevacizumab (1 mg/mL)

b Avastin pH Gradient 0–50% B



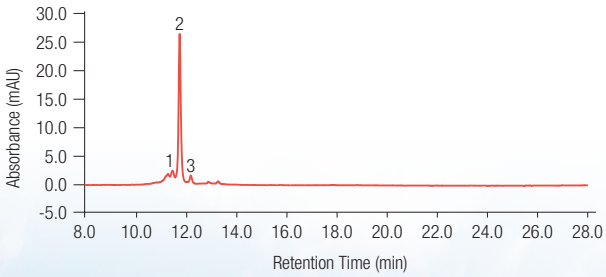
Column:	**MAbPac SCX-10**, 10 µm
Format:	4.0 × 250 mm
Mobile Phase A:	1X CX-1 pH gradient buffer A, pH 5.6
Mobile Phase B:	1X CX-1 pH gradient buffer B, pH 10.2
pH Gradient:	0% B to 50% B from 1 to 31 min
Flow Rate:	1.0 mL/min
Inj. Volume:	5 µL
Temp.:	30 °C
Detection:	UV (280 nm)
Sample:	Avastin/bevacizumab (1 mg/mL)

c Avastin Salt Gradient 0–100% B



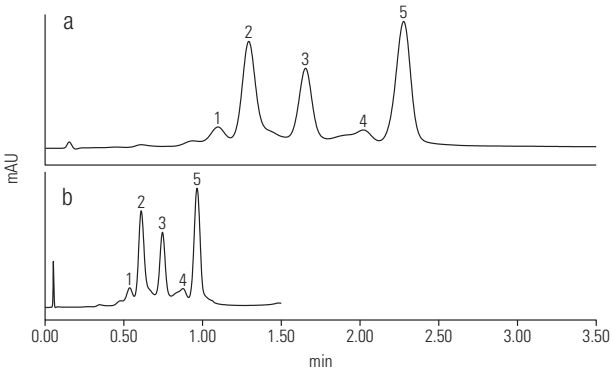
Column:	**MAbPac SCX-10**, 10 µm
Format:	4.0 × 250 mm
Mobile Phase A:	20 mM MES (pH 5.6) + 60 mM sodium chloride
Mobile Phase B:	20 mM MES (pH 5.6) + 300 mM sodium chloride
Salt Gradient:	0% B to 100% B from 1 to 31 min
Flow Rate:	1.0 mL/min
Inj. Volume:	5 µL
Temp.:	30 °C
Detection:	UV (280 nm)
Sample:	Avastin/bevacizumab(1 mg/mL)

d Avastin Salt Gradient 0–50% B



Column:	**MAbPac SCX-10**, 10 µm
Format:	4.0 × 250 mm
Mobile Phase A:	20 mM MES (pH 5.6) + 60 mM sodium chloride
Mobile Phase B:	20 mM MES (pH 5.6) + 300 mM sodium chloride
Salt Gradient:	0% B to 0% B from 1 to 31 min
Flow Rate:	1.0 mL/min
Inj. Volume:	5 µL
Temp.:	30 °C
Detection:	UV (280 nm)
Sample:	Avastin/bevacizumab (1 mg/mL)

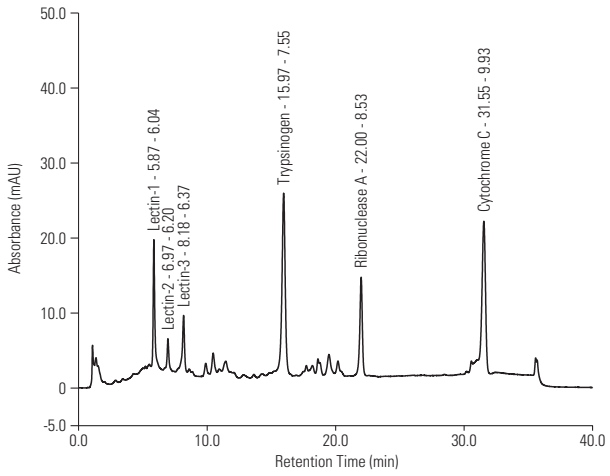
In order to push the throughput of the charge variant assay, fast separation of mAb variants is achieved using a 5 µm MAbPac SCX-10 RS column with a Thermo Scientific™ Vanquish™ UHPLC system. The Vanquish UHPLC system is fully biocompatible, suitable for the analysis of intact proteins. The combination of low gradient delay volume and high precision gradient formation makes it the ideal system for high throughput analysis with gradient elution. An example shown here is an ultra fast separation of infliximab variants separated using pH gradient and a MAbPac SCX-10 RS column (5 µm, 2.1 × 50 mm).



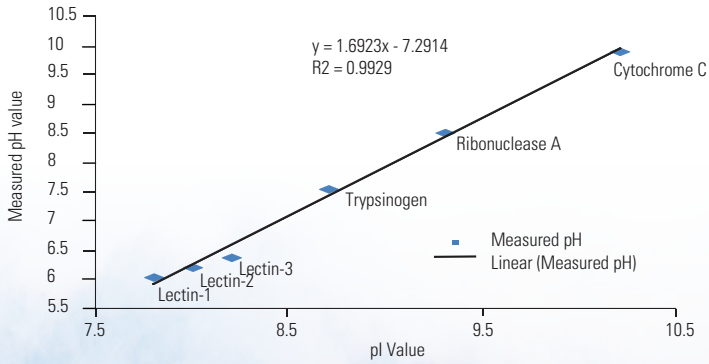
Column:	**MAbPac SCX-10 RS**, 5 µm
Format:	2.1 × 50 mm
Mobile Phase A:	1X CX-1 pH gradient buffer A, pH 5.6
Mobile Phase B:	1X CX-1 pH gradient buffer B, pH 10.2
pH Gradient:	a. 20% B to 40% B in 5 min
	b. 18% B to 27% B in 0.8 min
Flow Rate:	a. 0.5 mL/min
	b. 1.2 mL/min
Inj. Volume:	4 µL
Temp.:	30 °C
Detection:	UV (280 nm)
Sample:	Infliximab (10 mg/mL)

The linearity of the pH gradient can be verified using the UltiMate 3000 pH and conductivity online monitor, which can be added to UltiMate 3000 BioRS system to serve as a platform for pH gradient ion exchange chromatography.

The UltiMate 3000 pH and conductivity monitor is a valuable tool for HPLC method development, particularly for protein and nucleic acid separations. It enables linking the mobile phase pH and/or conductivity with the elution time of the components. The pH and conductivity monitor allows for the monitoring of gradient formation, column equilibration, and understanding column buffering effects in pH gradient ion-exchange chromatography.



Column:	**MAbPac SCX-10**, 10 µm		
Format:	4.0 × 250 mm		
Mobile Phase A:	1X CX-1 pH gradient buffer A, pH 5.6		
Mobile Phase B:	1X CX-1 pH gradient buffer B, pH 10.2		
pH Gradient:	Time (min)	%A	%B
	0.0	100	0
	1.0	100	0
	31.0	0	100
	34.0	0	100
	34.1	100	0
	40.0	100	0
Flow Rate:	1.0 mL/min		
Inj. Volume:	5 µL		
Temp.:	30 °C		
Detection:	UV (280 nm)		
Sample:	Protein Standard		



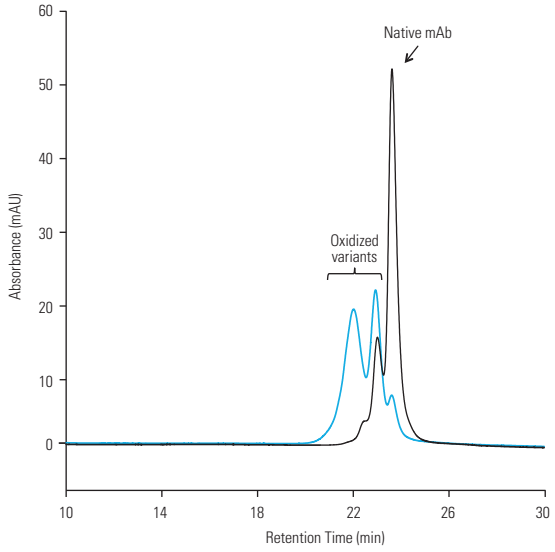
Monitoring the eluent pH during a pH gradient makes charge variant characterization more simple and predictable because proteins will only elute once the eluent pH is above the biomolecules pI. The measured pH values for six protein peaks (lentil lectin-1, lectin-2, lectin-3, trypsinogen, ribonuclease A, and cytochrome C), exhibit a strong linear correlation to the literature based pI values. This shows that the pI of a protein component can be estimated based on the peak retention time and measured pH.

Oxidation Variant Analysis

Hydrophobic Interaction Chromatography (HIC) is an important tool for protein chemists separating proteins under gentle, non-denaturing conditions. Biomolecules are separated by their degree of hydrophobicity. The mobile phase consists of a salting-out agent, which at high concentration retains the protein by increasing the hydrophobic interaction between proteins and the stationary phase. The bound proteins are eluted by decreasing the salt concentration. Since proteins are relatively stable in the salt solutions used as the mobile phase, the biological activity of proteins is usually maintained in HIC. This makes HIC separations ideal for high resolution analyses as well as a purification step or polishing prior to analysis.

Oxidation of exposed amino acid residues such as methionine (Met) and tryptophan (Trp) is a major concern in therapeutic mAb stability studies. Oxidation of amino acid residues on a mAb can alter the hydrophobic nature of the mAb by either an increase in polarity of the oxidized form or a conformational change. This can also have an effect on the activity of the therapeutic. Therefore hydrophobicity-based HPLC methods such as reversed-phase chromatography and hydrophobic interaction chromatography are often used to characterize oxidized mAb products.

The MAbPac HIC-20 column is a high-resolution silica-based HIC column designed for the separation of mAbs and mAb variants. Its unique column chemistry provides high resolution, rugged stability and desired selectivity for mAb and mAb variants. MAbPac HIC-20 provides superior resolution of oxidized mAb variants from unmodified mAb. The MAbPac HIC-20 can resolve oxidized mAb variants without fragmentation or other sample preparation.



Column:	**MAbPac HIC-20**, 5 µm		
Format:	4.6 × 250 mm		
Mobile Phase A:	2 M ammonium sulfate, 100 mM sodium phosphate, pH 7.0		
Mobile Phase B:	100 mM sodium phosphate, pH 7.0		
Gradient:	Time (min)	%A	%B
	-6.0	50	50
	0.0	50	50
	2.0	50	50
	30.0	0	100
	35.0	0	100
Flow Rate:	0.5 mL/min		
Inj. Volume:	Untreated mAb: 20 µL (1.25 mg/mL)		
	Oxidized mAb: 20 µL (1.25 mg/mL)		
Temp.:	30 °C		
Detection:	UV (280 nm)		
Sample:	Untreated mAb H_2O_2 oxidized mAb		

Antibody-Drug Conjugate (ADC) Analysis

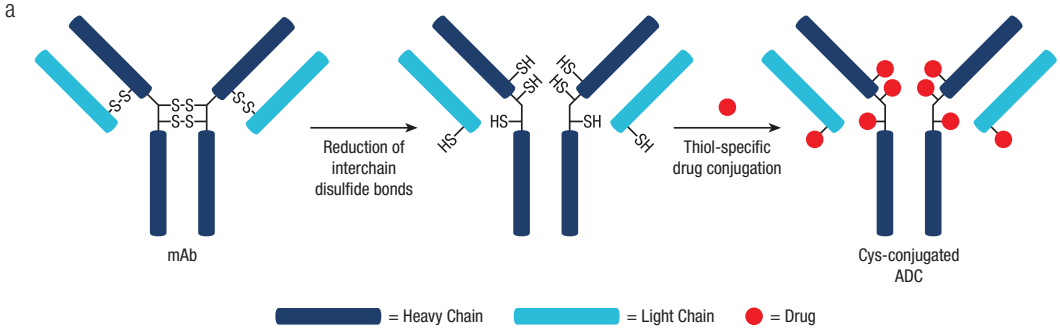
ADCs have gained tremendous interest among pharmaceutical companies due to their significantly improved clinical efficacy over native monoclonal antibodies. The conjugation of drugs often results in an ADC molecule that is heterogeneous with respect to both the distribution and loading of cytotoxic drugs on the mAb. The number of drugs attached to the mAb has been shown to directly affect the safety and the efficacy of the drug. Therefore it is critical to fully characterize and monitor the heterogeneity of ADCs during development and production.

Hydrophobic interaction chromatography (HIC) and reversed-phase chromatography (RPC) are suitable for the separation of ADCs since attachment of toxic drugs alters the hydrophobicity of the antibody. Typically the least hydrophobic unconjugated antibody elutes first and as the number of drugs attached increases the elution time increases.

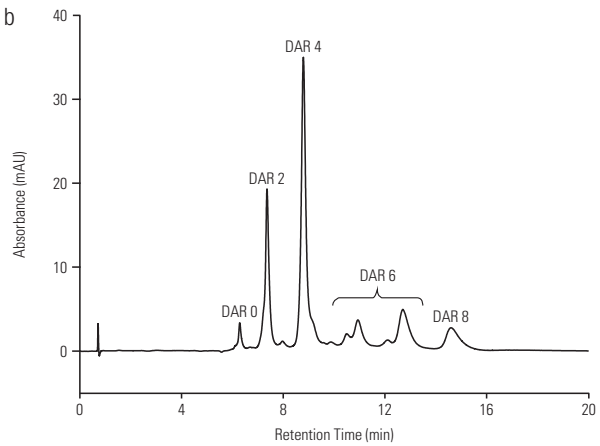
ADC Analysis by Hydrophobic Interaction Chromatography

The MAbPac HIC-Butyl column is based on hydrophilic, non-porous, 5 µm polymer particles functionalized with butyl groups. The hydrophilic resin provides biocompatibility while the optimal density of the butyl groups provides high resolution separation. An ADC mimic sample was analyzed using a MAbPac HIC-Butyl column. The ADC mimics were conjugates between a drug mimic and a mAb via the sulfhydryl group of interchain cysteine residues which results in a mixture of drug-loaded antibody species with 0 to 8 drugs. The unmodified mAb and ADCs with DAR (drug-to-antibody ratio) values ranging from 2 to 8 are well resolved by the MAbPac HIC-Butyl column.

a



b

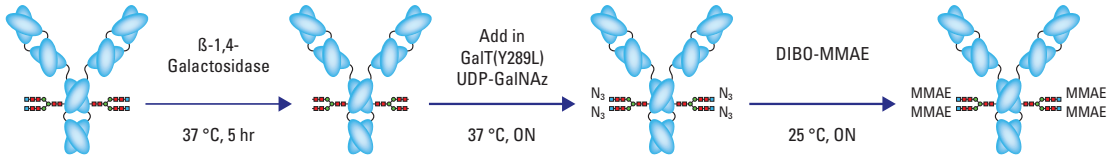


Column:	**MAbPac HIC-Butyl**, 5 µm		
Format:	4.6 × 100 mm		
Mobile Phase A:	1.5 M ammonium sulfate, 50 mM sodium phosphate, pH 7.0/isopropanol (95:5 v/v)		
Mobile Phase B:	50 mM sodium phosphate, pH 7.0/isopropanol (80:20 v/v)		
Gradient:	Time (min)	%A	%B
	-5.0	100	0
	0.0	100	0
	1.0	100	0
	15.0	0	100
	20.0	0	100
Flow Rate:	1.0 mL/min		
Inj. Volume:	5 µL (5 mg/mL)		
Temp.:	25 °C		
Detection:	UV (280 nm)		
Sample:	Cys-conjugated ADC mimic		

ADC Analysis by Reversed-Phase Chromatography

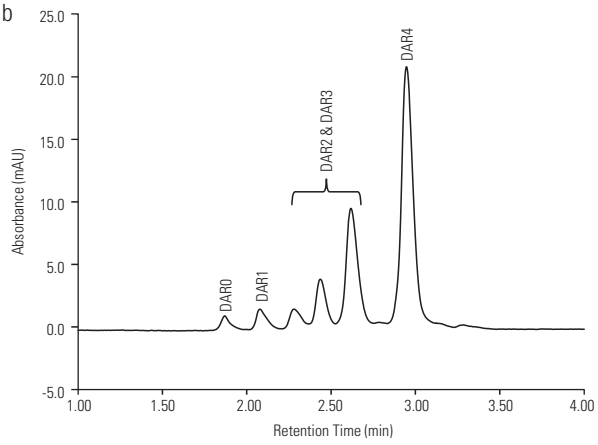
The MAbPac RP column is based on supermacroporous 4 µm polymer particles. The hydrophobic phenyl based resin with its large pore size enables efficient separation of large molecules including mAbs and their conjugates. Shown in the chromatogram below is an ADC created using site-specific antibody labeling technology and analyzed using a MAbPac RP column. ADCs were prepared by enzymatically activating mAb Fc domain glycans with azides using the mutant beta-galactosyltransferase enzyme. The azide-activated antibodies were then conjugated with dibenzocyclooctyne (DIBO) -activated Val-Cit-PAB-Monomethyl Auristatin E (MMAE) toxin resulting in a mixture of drug-loaded antibody species with 0 to 4 MMAE molecules. The unmodified mAb and ADCs with DAR values ranging from 0 to 4 are well resolved by the MAbPac RP column.

a



Unlabeled Ab | **Cleave Terminal Gal** | **Azide-Activated Ab (stable for long-term storage)** | **Antibody Drug Conjugate (ADC)**

b



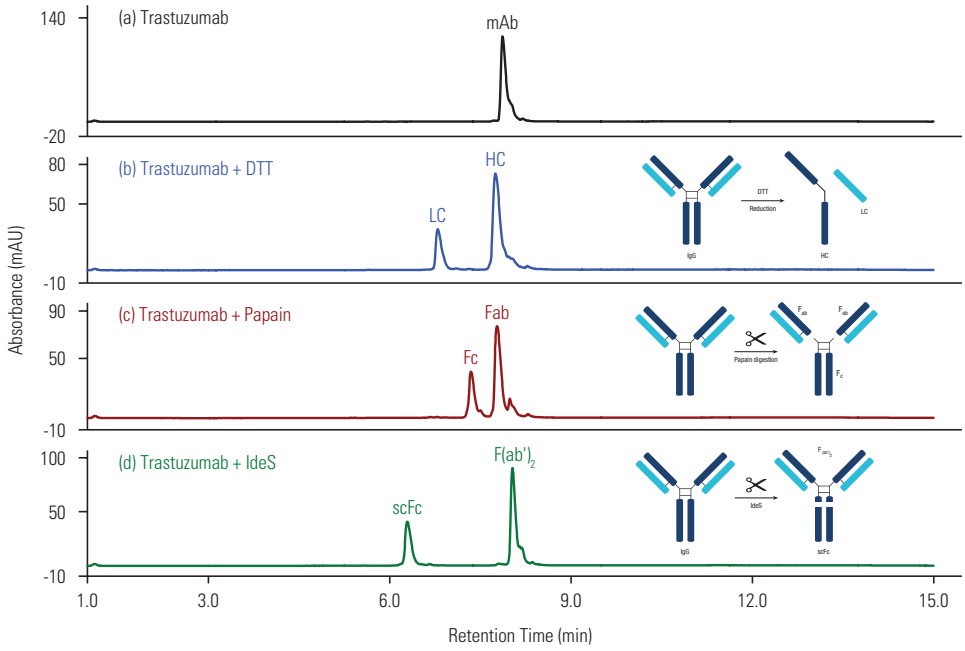
Column:	**MAbPac RP**, 4 µm		
Format:	2.1 × 50 mm		
Mobile Phase A:	Water/trifluoroacetic acid (99.9 : 0.1 v/v)		
Mobile Phase B:	Acetonitrile/water/trifluoroacetic acid (90: 9.9 :0.1 v/v/v)		
Gradient:	Time (min)	%A	%B
	0.0	65	35
	0.5	65	35
	4.5	45	55
	5.0	45	55
	5.5	65	35
	6.0	65	35
Flow Rate:	0.6 mL/min		
Inj. Volume:	2 µL		
Temp,:	80 °C		
Detection:	UV (280 nm)		
Sample:	Trastuzumab-MMAE		

Intact mAb and mAb Fragments Analysis

In order to verify the quality attributes of the overall molecule, and to provide a reproducible, safe and effective biological drug compound, the correct protein sequence as well as the presence and relative abundance of different glycoforms must be confirmed. The simplest way to achieve this is to interface the separation with high resolution MS/MS detection. Typically, mAbs are analyzed in either their native intact form, in reduced light/heavy chain form, or after enzymatic cleavage into antigen binding fragment (Fab) and crystallizable fragment (Fc).

Reversed-phase chromatography has been used for many years as an excellent interface with MS detection. The low concentrations of volatile buffers component combined with the high organic make-up of the mobile phase result in conditions ideal for stable and effective spray. Furthermore, the separation principle offers an alternative selectivity based on hydrophobicity, which provides orthogonal high-resolution separations to ion-exchange method. MAbPac RP uses supermacroporous resin which has large pore size and high loading capacity. This feature has an ideal benefit in antibody analysis, due to the large size of the protein (typical MW of 150 kDa).

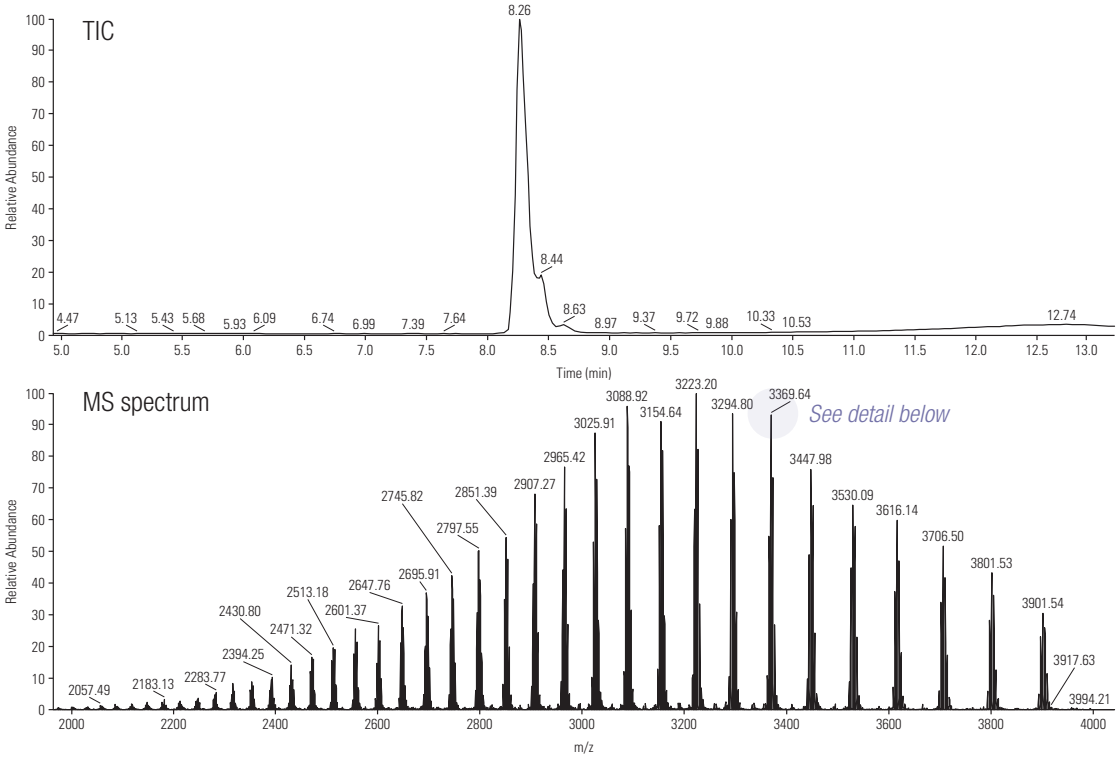
The excellent performance of the MAbPac RP HPLC columns for mAb fragment analysis is shown here for the analysis of trastuzumab fragments: LC and HC, Fc and Fab, scFc and F(ab')$_2$ are baseline separated using a 10-min gradient.

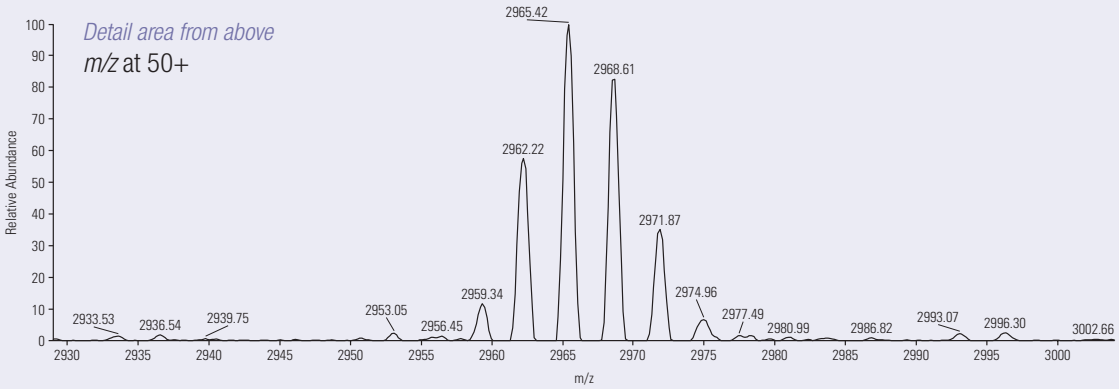


Column:	**MAbPac RP**, 4 µm		
Format:	3.0 × 50 mm		
Mobile Phase A:	Water/formic acid/trifluoroacetic acid (99.88:0.1:0.02 v/v/v)		
Mobile Phase B:	Acetonitrile/water/formic acid/ trifluoroacetic acid (90: 9.88:0.1:0.02 v/v/v/v)		
Gradient:	Time (min)	%A	%B
	0.0	80	20
	1.0	80	20
	11.0	55	45
	12.0	55	45
	14.0	80	20
	15.0	80	20

Flow Rate:	0.5 mL/min
Inj. Volume:	5 µL
Temp.:	80 ºC
Detection:	UV (280 nm)
Sample:	(a) Trastuzumab (5 mg/mL)
	(b) Trastuzumab + DTT (4 mg/mL)
	(c) Trastuzumab + Papain (2 mg/mL)
	(d) Trastuzumab + IdeS (2 mg/mL)

The reversed-phase chromatographic analysis can be coupled to high-resolution mass spectrometry systems to confirm the accurate molecular weight of the target product and identify variants. Here is an example of LC-MS analysis of trastuzumab using a Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap mass spectrometer. The top trace shows the total ion chromatogram and the bottom trace shows the mass spectrum.



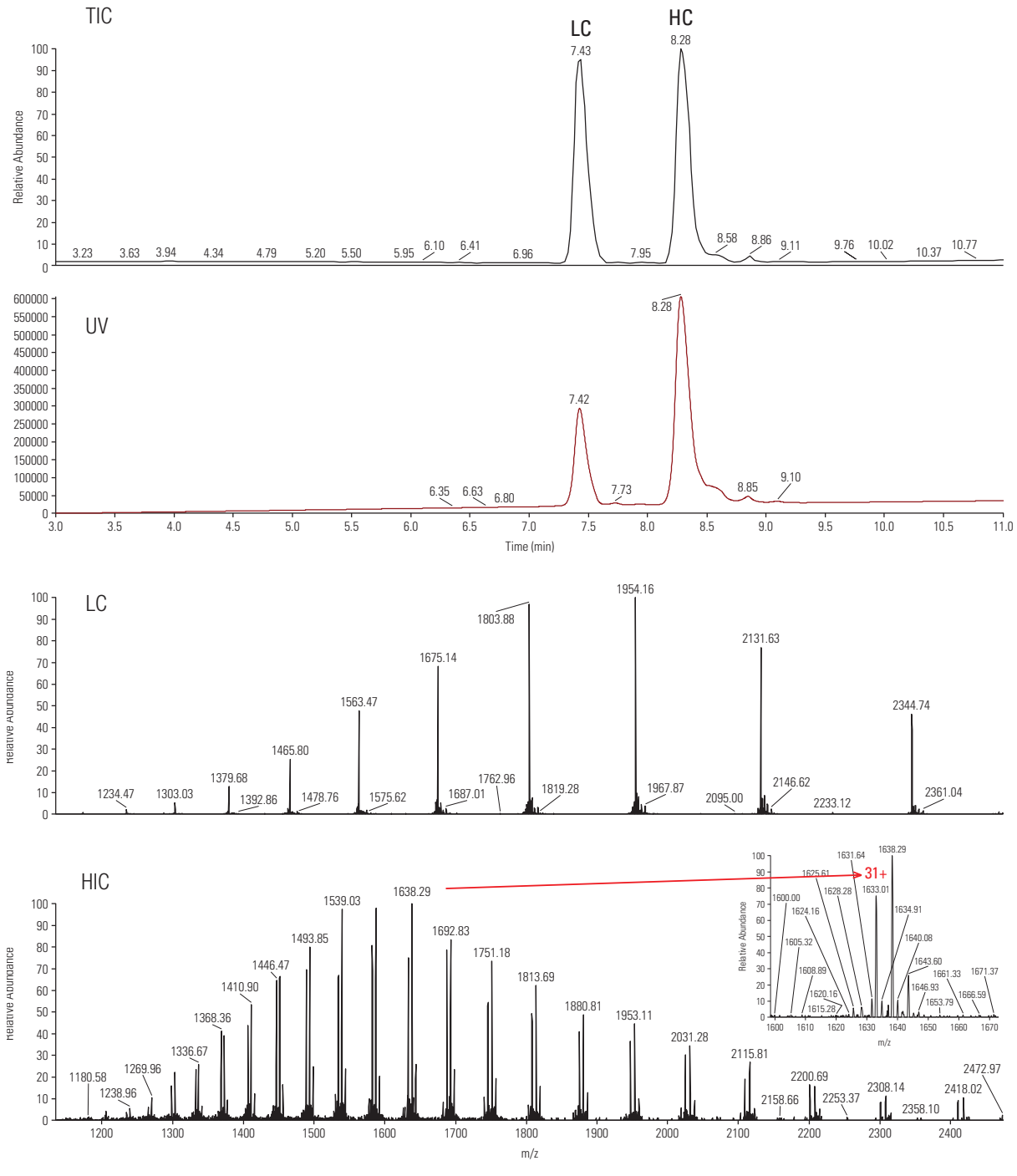


Column:	**MAbPac RP,** 4 µm
Format:	3.0 × 50 mm
Mobile Phase A:	Water/formic acid/trifluoroacetic acid (99.88:0.1:0.02 v/v/v)
Mobile Phase B:	Acetonitrile/water/formic acid/ trifluoroacetic acid (90:9.88:0.1:0.02 v/v/v/v)

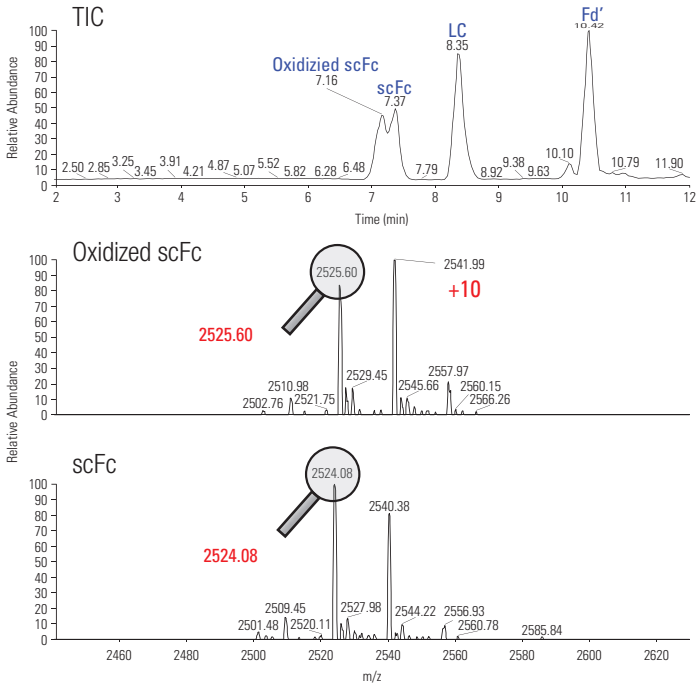
Gradient:	Time (min)	%A	%B
	0.0	80	20
	1.0	80	20
	11.0	55	45
	12.0	55	45
	14.0	80	20
	15.0	80	20

Flow Rate:	0.5 mL/min
Inj. Volume:	1 µL
Temp.:	80 ºC
MS Detection:	positive-ion mode
Mass Spec:	Q Exactive™ Plus
Sample:	Trastuzumab (5 mg/mL)

Further structural elucidation is obtained by reducing the trastuzumab into light chain and heavy chain fragments. Interface with Q Exactive Plus mass spectrometer allows the identification of different glycoforms in the heavy chain as shown below.

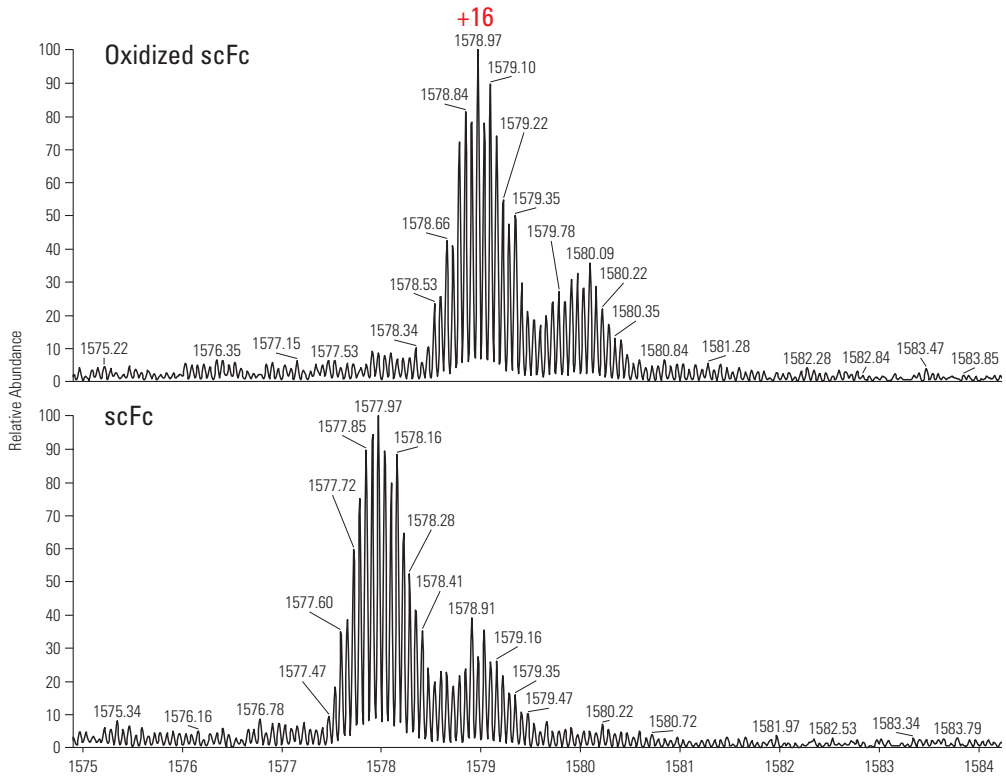


Methionine (Met) oxidation is one of the critical quality attributes required to be closely monitored. The two Met residues in the CH2-CH3 domain interface of recombinant humanized and fully human IgG1 antibodies were found susceptible to oxidation. It is desirable to monitor the progress of the Met oxidation without complete digestion of mAb. A workflow was designed to first reduce mAb and then further digest it with IdeS resulting in smaller (25 kDa) fragments. Further digestion of the HC by IdeS resulted in two smaller fragments: scFc and Fd'. The MAbPac RP baseline separates scFc, LC, and Fd' fragments as shown below. The +10 charge state of the oxidized scFc and non-oxidized scFc are at *m/z* 2525.60 and at *m/z* 2524.08.



Column:	**MAbPac RP**, 4 µm		
Format:	3.0 × 50 mm		
Mobile Phase A:	Water/formic acid/trifluoroacetic acid (99.88 : 0.1:0.02 v/v/v)		
Mobile Phase B:	Acetonitrile/water/formic acid/ trifluoroacetic acid (90: 9.88 :0.1:0.02 v/v/v/v)		
Gradient:	Time (min)	%A	%B
	0.0	75	25
	1.0	75	25
	11.0	63	37
	12.0	63	37
	14.0	75	25
	15.0	75	25
Flow Rate:	0.5 mL/min		
Inj. Volume:	2 µL		
Temp.:	80 ºC		
MS Detection:	positive-ion mode		
Mass spec:	Q Exactive™ Plus		
Sample:	Oxidized trastuzumab, reduced by DTT and digested by IdeS (1 mg/mL)		

Data collected using 280K resolution shows isotopically resolved oxidized scFc and non-oxidized scFc at +16 charge state, see below.

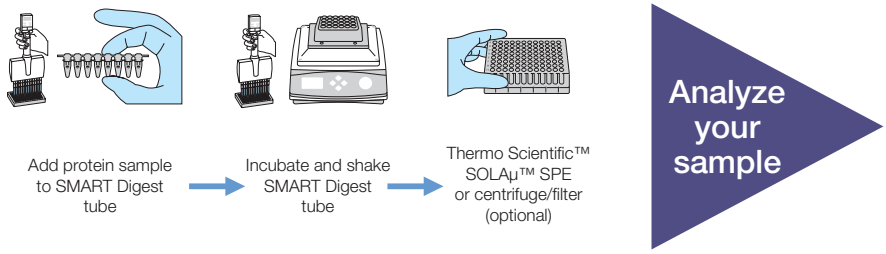


Peptide Mapping

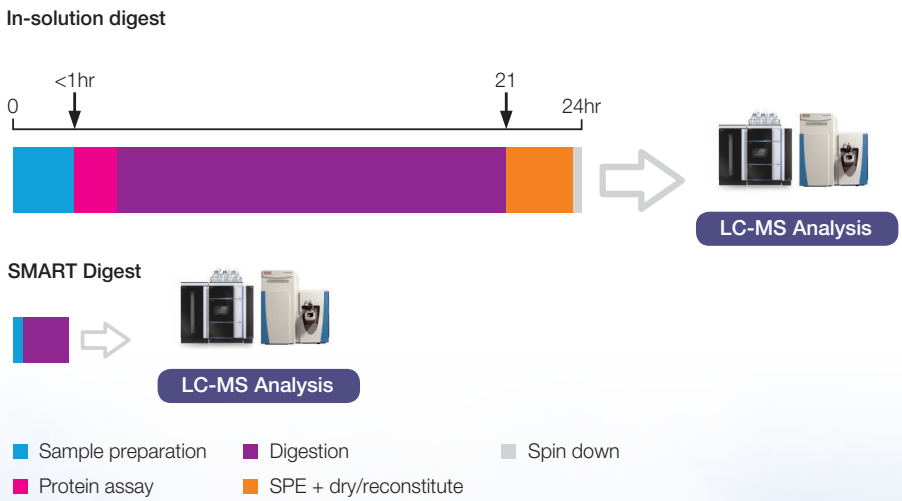
Reversed-phase liquid chromatography of peptides is used in the biopharmaceutical industry to provide information on the nature and quality of protein therapeutics. Reversed-phase chromatography in combination with UV detection is very common in stability studies, process control, quality control, and other cases where all the important attributes of the peptide sequence are extrapolated directly from the chromatogram. In this case the assignment of a peptide to a peak is based on retention time comparison between the investigated sample and the reference one, provided that the identity of the peaks in the reference chromatogram has been determined by mass spectrometry beforehand. Since peak assignment is solely based on retention time, the highest run-to-run retention time precision is required in order to avoid incorrect peptide identification. This is complicated by the employment of in-solution digestion protocols which are time consuming (often taking in excess of 24 hours to complete) and highly irreproducible. In-solution digestion is multifaceted and laborious, with a high potential for chemically induced PTMs and autolysis, all of which can result in variation in the chromatographic profile.

The Thermo Scientific™ SMART™ Digest Kits, which are based on an immobilized trypsin design, help remove these issues by providing a simple to implement process. This delivers highly reproducible digestions quickly and with increased sensitivity compared to in-solution digests.

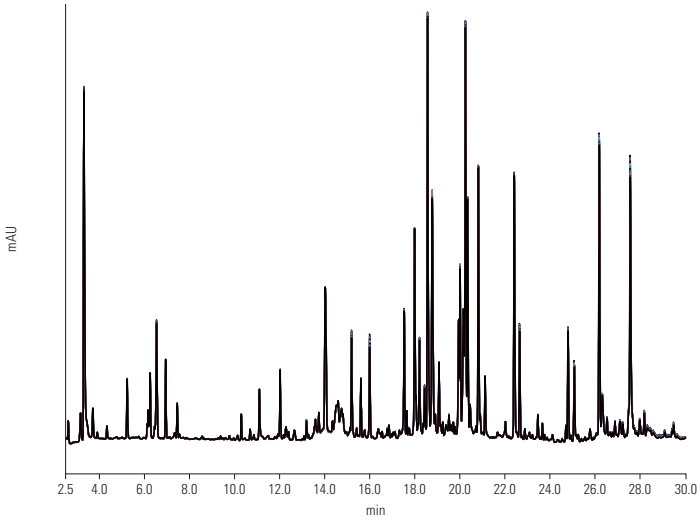
SMART Digest



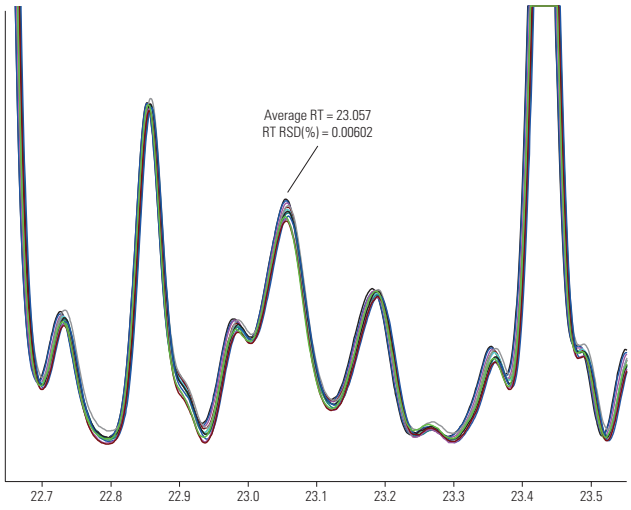
SMART Digest vs conventional in-solution digest protocol



This is complimented by the Vanquish UHPLC system featuring a binary pump with extremely low pulsation ripple, which allows highly stable flow delivery and highly precise gradient formation. The Vanquish UHPLC system is capable of providing unmatched retention time precision for gradient separations. The Acclaim RSLC 120, C18, 2.2 µm Analytical column (2.1 × 250 mm) provides the high resolution separation required by the method.



Column:	**Acclaim RSLC 120**, C18 2.2 µm
Format:	2.1 × 250 mm
Mobile Phase A:	0.05% trifluoroacetic acid in water
Mobile Phase B:	0.04% trifluoroacetic acid in 8/2 acetonitrile/water (v/v)
Gradient:	4% B to 50% B in 30 minutes
Flow Rate:	0.4 mL/min
Inj. Volume:	5 µL
Temp.:	80 °C
Detection:	UV (214 nm)
Sample:	mAb tryptic digest



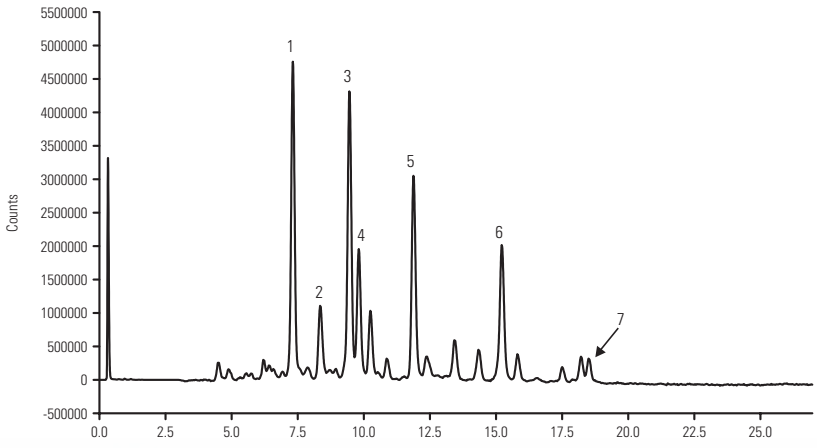
Glycan Analysis

Glycosylation is one of the most common post translational protein modifications (PTMs), that impacts therapeutic protein development. Most of the protein drug candidates in preclinical and clinical development, such as recombinant proteins and monoclonal antibodies (MAbs), are glycosylated. Their biological activites are often dependent on the structure and types of glycans attached to these proteins. Due to processing after en-bloc oligosaccharide transfer to the antibody, their structures can be extremely heterogeneous. Elucidation of the role(s) of antibody glycan structures is necessary for control of development and manufacturing processes for clinically important antibodies and related pharmaceuticals. Because charge, size and isomer variants may be present, structural characterization of glycan charge, size and isomers (linkage and branching) is essential for bio-therapeutic and bio-pharmaceutical projects.

In order to meet the challenges of glycan analysis, we offer three different column chemistries with different selectivity.

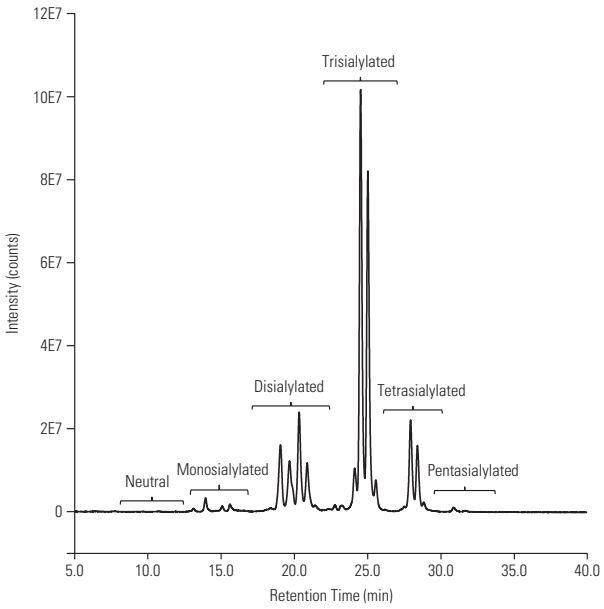
	GlycanPac AXR-1	GlycanPac AXH-1	Accucore 150-Amide-HILIC
Column Chemistry	Alkyl chain with tertiary amine	Polar groups with amine	Polyamide
Retention mechanism	WAX/RP mixed-mode	WAX/HILIC mixed-mode	HILIC
Silica substrate	High-purity totally porous spherical silica (1.9 and 3.0 µm)	High-purity totally porous spherical silica (1.9 and 3.0 µm)	High-purity superficially porous spherical silica (2.6 µm)
Feature	Ultra-high resolution (size, charge and isomerism based separation)	High-resolution with added structural information (size and charge based separation)	High-resolution (size-based separation)
Applications	Charged glycan species (native or labeled)	Neutral or charged glycans (native or labeled)	Neutral glycans with labeling

HILIC columns commonly used for glycan analysis are based on amide, amine, or zwitterionic packing materials. These columns separate glycans mainly by hydrogen bonding, resulting in separations based on size and composition. Accucore 150-Amide-HILIC HPLC phase is designed for the separation of hydrophilic, neutral glycans. Here is an example of human IgG glycans analyzed on an Accucore 150-Amide-HILIC HPLC columns.



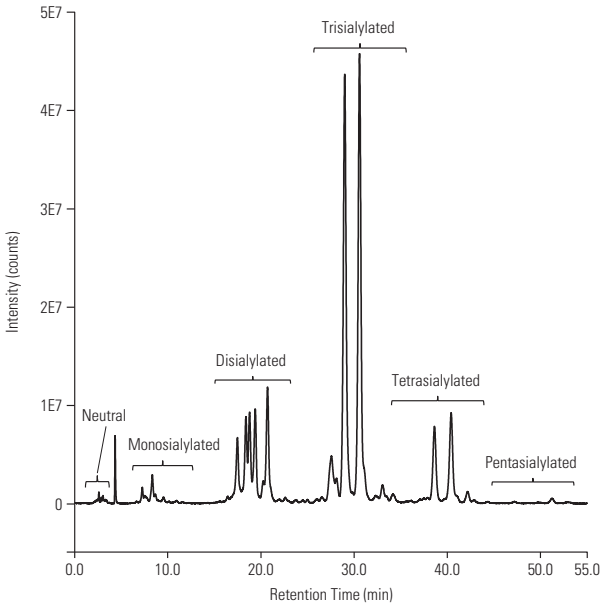
Column:	**Accucore 150-Amide-HILIC,** 2.6 µm
Format:	100 × 2.1 mm
Mobile Phase A:	Acetonitrile
Mobile Phase B:	50 mM ammonium formate pH 4.4 (prepared from LS-N-BUFFX40, Ludger Ltd)
Gradient:	Time (min) %A
	0 20
	26 40
	27 50
Flow Rate:	1 mL/min
Inj. Volume:	5 µL in water, 50 µL loop
Temp.:	60 ºC
Backpressure:	300 bar
Injection Wash Solvent:	Acetonitrile/water (78:22 v/v)
Excitation Wavelength:	330 nm
Emission Wavelength:	420 nm

GlycanPac AXH-1 column combines both weak anion exchange (WAX) and HILIC retention mechanisms for optimal selectivity and high resolving power. The WAX functionality provides retention and selectivity for negatively charged glycans, while the HILIC mode facilitates the separation of glycans of the same charge according to their polarity and size. GlycanPac AXH-1 is designed for neutral and charged glycans analysis, in their native or labeled form. Neutral and acidic 2AB labeled *N*-glycan from bovine fetuin are separated using a GlycanPac AXH-1 (1.9 µm, 2.1 × 150 mm) column. In total, 35 glycan peaks are resolved and detected.



Column:	**GlycanPac AXH-1**, 1.9 µm				
Format:	2.1 × 150 mm				
Mobile Phase A:	Acetonitrile				
Mobile Phase B:	D.I. water				
Mobile Phase C:	Ammonium formate (100 mM, pH4.4)				
Gradient:	Time (min)	A%	B%	C%	Curve
	-10	78	20	2	5
	0	78	20	2	5
	1	78	20	2	5
	35	60	20	20	5
	40	50	20	30	5
	45	50	20	30	5
Flow Rate:	0.4 mL/min				
Inj. Volume:	100 pmoles				
Temp.:	30 ºC				
Detection:	Fluorescence at 320/420 nm				
Sample:	2AB labeled *N*-glycan from bovine fetuin				

The GlycanPac AXR-1 column is based on novel mixed-mode column chemistry, combining the retention mechanisms of both WAX and reversed phase (RP) properties for optimal selectivity and resolution. The WAX functionality provides retention and selectivity for negatively charged glycans, while the reversed phase mode facilitates the separation of glycans of the same charge according to their isomeric structure, and size. As a result the GlycanPac AXR-1 column provides the highest resolution of charged 2AB-labeled *N*-linked glycans. 2AB-labeled *N*-linked glycans from bovine fetuin are separated using a GlycanPac AXR-1 (1.9 µm; 150 × 2.1 mm) column. In total, 59 glycan peaks are resolved and detected.



Column:	**GlycanPac AXR-1**, 1.9 µm				
Format:	2.1 × 150 mm				
Mobile Phase A:	Acetonitrile				
Mobile Phase B:	D.I. water				
Mobile Phase C:	Ammonium formate (100 mM, pH4.4)				
Gradient:	Time (min)	A%	B%	C%	Curve
	-10	0	93	7	5
	0	0	93	7	5
	1	0	93	7	5
	55	0	30	70	5
	56	70	0	30	5
	70	70	0	30	5
Flow Rate:	0.4 mL/min				
Inj. Volume:	100 pmoles				
Temp.:	30 ºC				
Detection:	Fluorescence at 320/420 nm				
Sample:	2AB labeled *N*-glycan from bovine fetuin				

Application Notes

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Column	Application Note	Literature Code
MAbPac Protein A	A Novel Affinity Protein A Column for Monoclonal Antibody (MAb) Titer Analysis	**AN20813**
MAbPac SEC-1	Analysis of monoclonal antibodies and their fragments by Size Exclusion Chromatography coupled with an Orbitrap Mass Spectrometer	**AN20940**
MAbPac SCX-10	High throughput, high resolution monoclonal antibody analysis with small particle size HPLC columns	**AN21008**
CX-1 pH Gradient Buffer	A Novel pH Gradient Separation Platform for Monoclonal Antibody (MAb) Charge Variant Analysis	**AN20784**
	A Fast and Robust Linear pH Gradient Separation Platform for Monoclonal Antibody (mAb) Charge Variant Analysis	**AN20946**
	Separation of Intact Monoclonal Antibody Sialylation Isoforms by pH Gradient Ion-Exchange Chromatography	**AN71062**
	High Resolution Charge Variant Analysis for Top-Selling Monoclonal Antibody Therapeutics Using Linear pH Gradient Separation Platform	**AN21092**
	Development of Ultra-fast pH-Gradient Ion Exchange Chromatography for the Separation of Monoclonal Antibody Charge Variants	**TN160**
MAbPac RP	Fast Analysis of Therapeutic Monoclonal Antibody Fragments Using a Supermacroporous, Reversed-Phase Chromatography Column	**AN21200**
MAbPac HIC-10	High Resolution Separation of a Fusion Protein on MAbPac HIC-10 Column	**AN71205**
	HIC as a Complementary, Confirmatory Tool to SEC for the Analysis of mAb Aggregates	**AN21206**
MAbPac HIC-20	High Resolution Separation of mAb Fragments on MAbPac HIC-20 Column	**AN21047**
	High Resolution Separation of Monoclonal Antibody (mAb) Oxidation Variants on the MAbPac HIC-20 Column	**AN21069**
MAbPac HIC-butyl	High Resolution Separation of Cysteine-Conjugated Antibody Drug Mimics using Hydrophobic Interaction Chromatography	**AN21079**
GlycanPac AXH-1	Separation of 2AB Labeled *N*-Glycans from Bovine Fetuin on a Novel Mixed-Mode Stationary Phase	**AN20754**
	Structural Analysis of Native *N*-Glycans Released from Proteins Using a Novel Mixed-Mode Column and a Hybrid Quadrupole-Orbitrap Mass Spectrometer	**AN20827**
GlycanPac AXR-1	Separation of 2AB-Labeled *N*-Linked Glycans from Bovine Fetuin on a Novel Ultra High Resolution Mixed-Mode Column	**AN20908**
	Separation of 2AA-Labeled *N*-Linked Glycans from Human IgG on a HighResolution Mixed-Mode Column	**AN20909**
	Separation of 2AA-Labeled *N*-Linked Glycans from Glycoproteins on a High Resolution Mixed-Mode Column	**AN20910**
Accucore 150-Amide-HILIC	Analysis of Human IgG Glycans on a Solid Core Amide HILIC Stationary Phase	**AN20703**

Selected Publications

Column	Title
ProPac WCX-10	Vlasak J. and Ionescu R. *Heterogeneity of Monoclonal Antibodies Revealed by Charge-Sensitive Methods.* Current Pharmaceutical Biotechnology **2008**; 9:468-481
ProPac HIC-10	Haverick M., Mengisen S., Shameem M., and Ambrogelly A. *Separation of mAbs molecular variants by analytical hydrophobic interaction chromatography HPLC: Overview and applications.* mAbs **2014**; 6:852–858

Ordering Information

Affinity Columns

MAbPAC Protein A

Particle Size (µm)	Format	Length (mm)	4.0 mm ID
12	HPLC Column	35	**063655**

Size Exclusion Columns

MAbPac SEC-1

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	4.0 mm ID	7.8 mm ID
5	HPLC Column	50		**074697**	
		150	**088790**	**075592**	
		300	**088789**	**074696**	**088460**

Ion Exchange Columns

MAbPac SCX-10

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	4.0 mm ID	4.6 mm ID	9.0 mm ID
3	HPLC Column	50	–	**077907**	–	–
5	HPLC Column	50	**082675**	**078656**	**082674**	–
		150	**088242**	**085198**	**085209**	–
		250	**082515**	**078655**	**082673**	–
10	HPLC Column	50	–	**075603**	–	–
		150	–	**075602**	–	–
		250	**075604**	**074625**	–	**088784**
	HPLC Column Lot Select 3 column	250 (1 lot of Resin)	–	**088782**	–	–
	HPLC Column Lot Select 3 column	250 (3 lots of Resin)	–	**088783**	–	–

ProPac WCX-10

Particle Size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22.0 mm ID
10	Guard Column	50	**063480**	**054994**	–	–
	HPLC Column	50	–	**074600**	–	–
		100	–	**088778**	–	–
		150	–	**088779**	–	–
		250	**063472**	**054993**	**063474**	**088766**

ProPac SCX-10

Particle Size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22.0 mm ID
10	Guard Column	50	**063462**	**079930**	–	–
	HPLC Column	250	**063456**	**054995**	**063700**	**088769**

ProPac SCX-20

Particle Size (µm)	Format	Length (mm)	4.0 mm ID
10	Guard Column	50	**074643**
	HPLC Column	250	**074628**

ProPac WAX-10

Particle Size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22.0 mm ID
10	Guard Column	50	**063470**	**055150**	–	–
	HPLC Column	250	**063464**	**054999**	**063707**	**088771**

ProPac SAX-10

Particle Size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22.0 mm ID
10	Guard Column	50	**063454**	**054998**	–	–
	HPLC Column	50	–	**078990**	–	–
		250	**063448**	**054997**	**063703**	**088770**

Gradient Buffers

Description	MAbPac SCX-10 Column Size	Buffer Bottle Size 125 mL	Buffer Bottle Size 250 mL
CX-1 pH Gradient Buffer A (pH 5.6)	–	**083273**	**085346**
CX-1 pH Gradient Buffer B (pH 10.2)	–	**083275**	**085348**
CX-1 pH Gradient Buffer Kit: Includes both Buffer A & Buffer B	–	**083274**	**085349**
CX-1 pH Gradient Starter Kit: Includes both Buffer A & Buffer B + MAbPac SCX-10	10 µm, 4 × 250 mm column	**083381**	–
CX-1 pH Gradient High Throughput Kit; Includes both Buffer A & Buffer B + MAbPac SCX-10	5 µm, 4 × 50 mm column	**083378**	–
CX-1 pH Gradient High Resolution Kit: Includes both Buffer A & Buffer B + MAbPac SCX-10	5 µm, 4 × 250 mm column	**083272**	–

Hydrophobic Interaction Columns

MAbPac HIC Family

Description	Particle Size (µm)	Format	Length (mm)	4.6 mm ID
MAbPac HIC-10	5	HPLC Column	100	**088480**
			250	**088481**
		Guard Cartridges (2/pk)*	10	**088482**
MAbPac HIC-20	5	HPLC Column	100	**088553**
			250	**088554**
		Guard Cartridges (2/pk)*	10	**088555**
MAbPac HIC-Butyl	5	HPLC Column	100	**088558**
		Guard Cartridges (2/pk)*	10	**088559**

*Guard Cartridge Holder (P/N 069580)

ProPac HIC-10

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	4.6 mm ID	7.8 mm ID
5	HPLC Column	75	–	–	**063665**
		100	**063653**	**063655**	–
		250	–	**074197**	–

Reversed-Phase Protein Columns

MAbPac RP

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID
4	HPLC Column	50	**088648**	**088645**
		100	**088647**	**088644**
	Guard Cartridges (2/pk)*	10	**088649**	**088646**

*Guard Cartridge Holder (P/N 069580)

ProSwift RP

Packing	Format	Functional Group	Length (mm)	1.0 mm ID	4.6 mm ID
Monolith	HPLC Column	RP-1S	50	–	**064297**
		RP-2H	50	–	**064296**
		RP-3U	50	–	**064298**
		RP-4H	50	**069477**	–
			250	**066640**	–
		RP-10R	5	**164586**	–

Reversed-Phase Peptide Columns

Acclaim RSLC 120 C18, Acclaim RSLC PolarAdvantage II (PA2)

Particle Size (µm)	Format	Length (mm)	ID (mm)	120 C18	Polar Advantage II
2.2	RSLC Column	30	2.1	**071400**	**071402**
			3.0	**071606**	**071609**
		50	2.1	**068981**	**068989**
			3.0	**071605**	**071608**
		100	2.1	**068982**	**068990**
			3.0	**071604**	**071607**
		150	2.1	**071399**	**071401**
		250	2.1	**074812**	**074814**

Acclaim 300 C18

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
3	HPLC Column	50	**060263**	–	**060265**
		150	**060264**	**063684**	**060266**
5	Guard Cartridges (2/pk)*	10	**069690**	**075721**	**069697**

*Guard Cartridge Holder (P/N 069580)

Accucore 150-C18 Columns

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID	0.075 mm ID
2.6	HPLC Column	30	**16126-032130**	**16126-033030**	**16126-034630**	–
		50	**16126-052130**	**16126-053030**	**16126-054630**	–
		100	**16126-102130**	**16126-053030**	**16126-054630**	–
		150	**16126-152130**	**16126-153030**	**16126-154630**	**16126-157569**
		500	–	–	–	**16126-507569**
	Guard Cartridges (2/pk)*	10	**16126-012105**	**16126-013005**	**16126-14005**	–

*Guard Cartridge Holder (P/N 852-00)

SMART Digest Kits

Description	Part Number
SMART Digest Kit (Includes resin, buffer, and collection plate)	**60109-101**
SMART Digest Kit with Thermo Scientific™ SOLAµ™ HRP SPE plate (Includes resin, buffer, collection plate, and SOLAµ SPE plate)	**60109-103**
SMART Digest Kit with 96 well filter plate (Includes resin, buffer, collection plate, and filter plate)	**60109-102**

Glycan Analysis Columns

GlycanPac AXH-1 Columns

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
1.9	HPLC Column	250	**082521**	–	–
		150	**082472**	–	–
		100	**082473**	–	–
3		150	**082470**	**082469**	**082468**
3	Guard Cartridges (2/pk)*	10	**082476**	**082475**	**082474**

*Guard Cartridge Holder (P/N 069580)

GlycanPac AXR-1 Columns

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
1.9	HPLC Column	150	**088136**	–	–
		250	**088135**	–	–
3		150	**088251**	**088252**	**088255**
	Guard Cartridges (2/pk)*	10	**088258**	**088259**	**088260**

*Guard Cartridge Holder (P/N 069580)

Accucore 150-Amide-HILIC Columns

Particle Size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
2.6	HPLC Column	50	**16726-052130**	**16726-053030**	**16726-054630**
		100	**16726-102130**	**16726-103030**	**16726-104630**
		150	**16726-152130**	**16726-153030**	**16726-154630**
		250	**16726-252130**	–	–
	Guard Cartridges (2/pk)*	10	**16726-012105**	**16726-013005**	**16726-014005**

*Guard Cartridge Holder (P/N 852-00)

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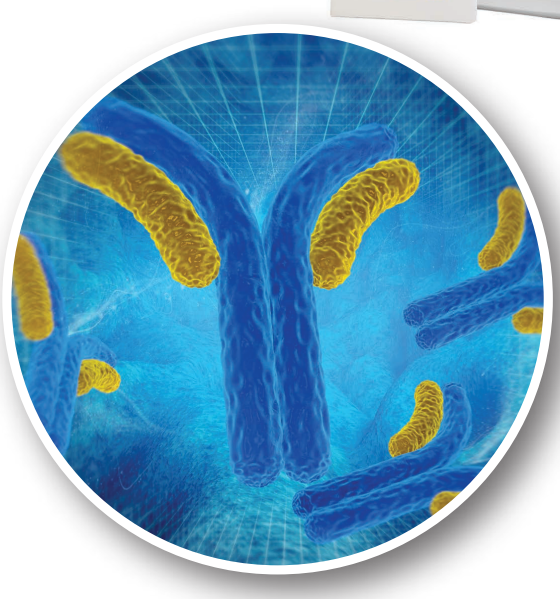

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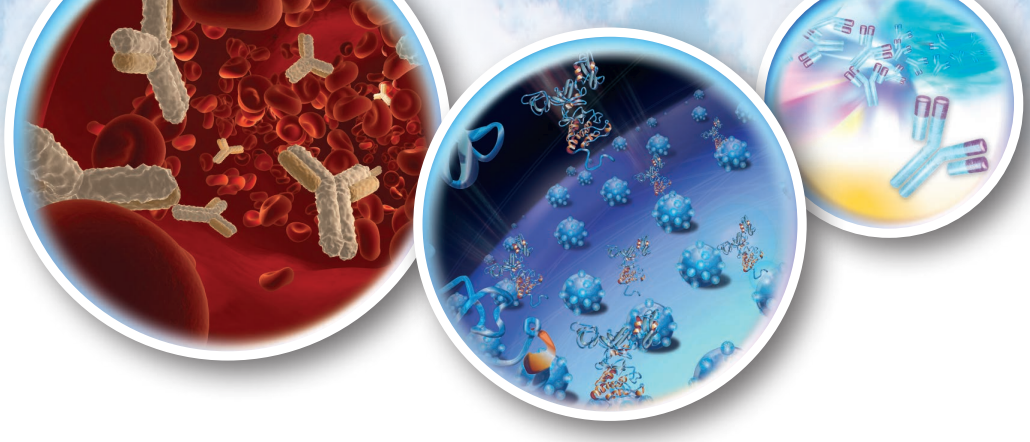

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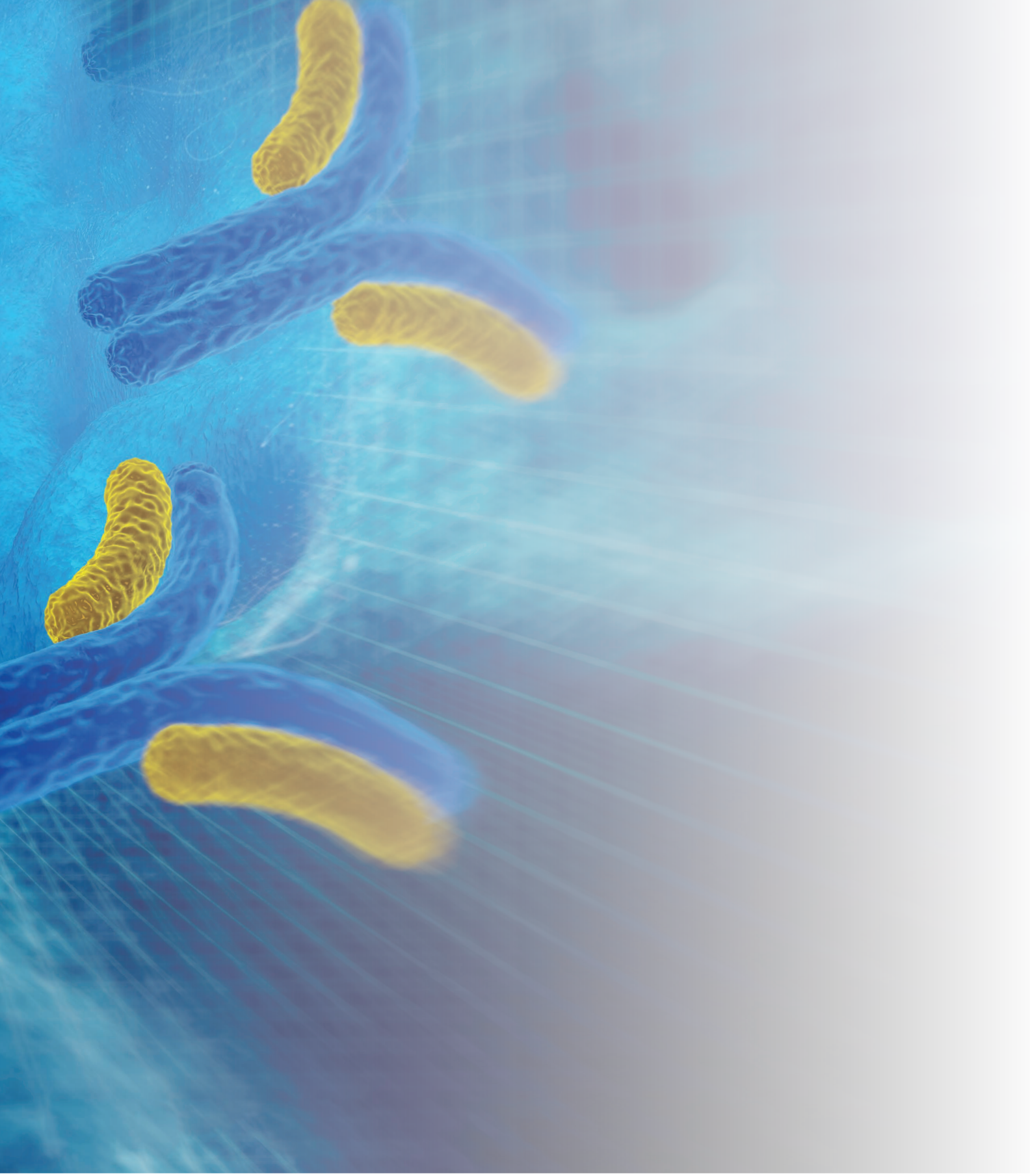

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