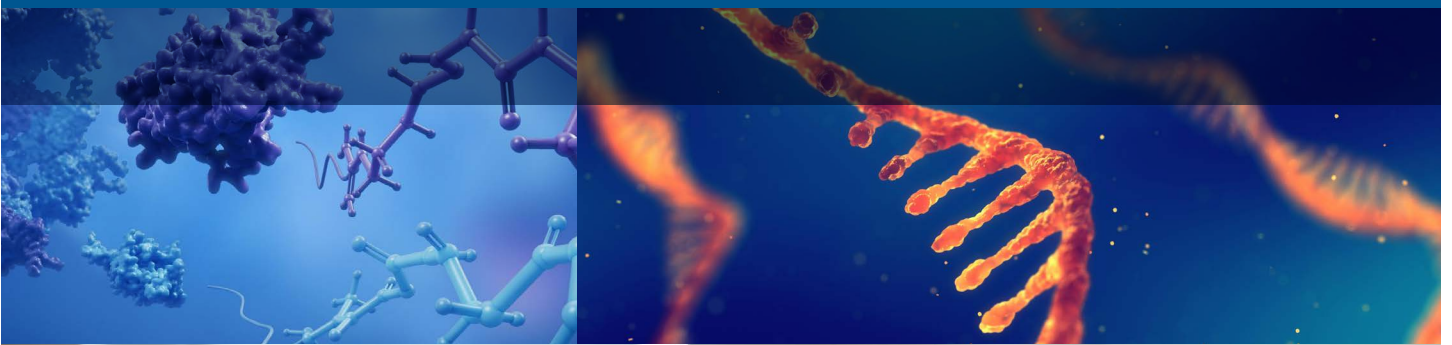
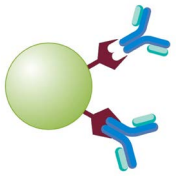


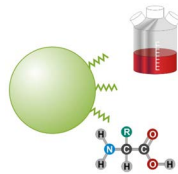
Agilent Solution for Biopharmaceutical Critical Quality Attributes



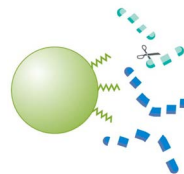
Agilent provides confidence in the monitoring of critical quality attributes of proteins and peptides



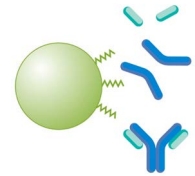
Affinity titer determination



Amino acid analysis



Peptide mapping



Intact protein and subunit analysis



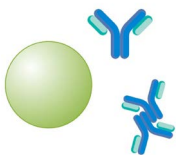
1260 Infinity II bio-inert LC system



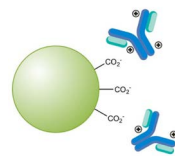
AdvanceBio bio LC columns



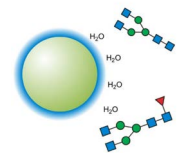
1290 Infinity II bio LC system



Aggregate/fragment analysis



Charge variant analysis



Glycan mapping

Agilent Solution for Monitoring Critical Quality Attributes of Proteins and Peptides

Recommended columns for protein aggregate / fragment analysis

AdvanceBio SEC column, 200Å, 4.6 x 150 mm, 1.9 µm, *part number: [PL1580-3201](#) (for 1290 Infinity II bio LC)*

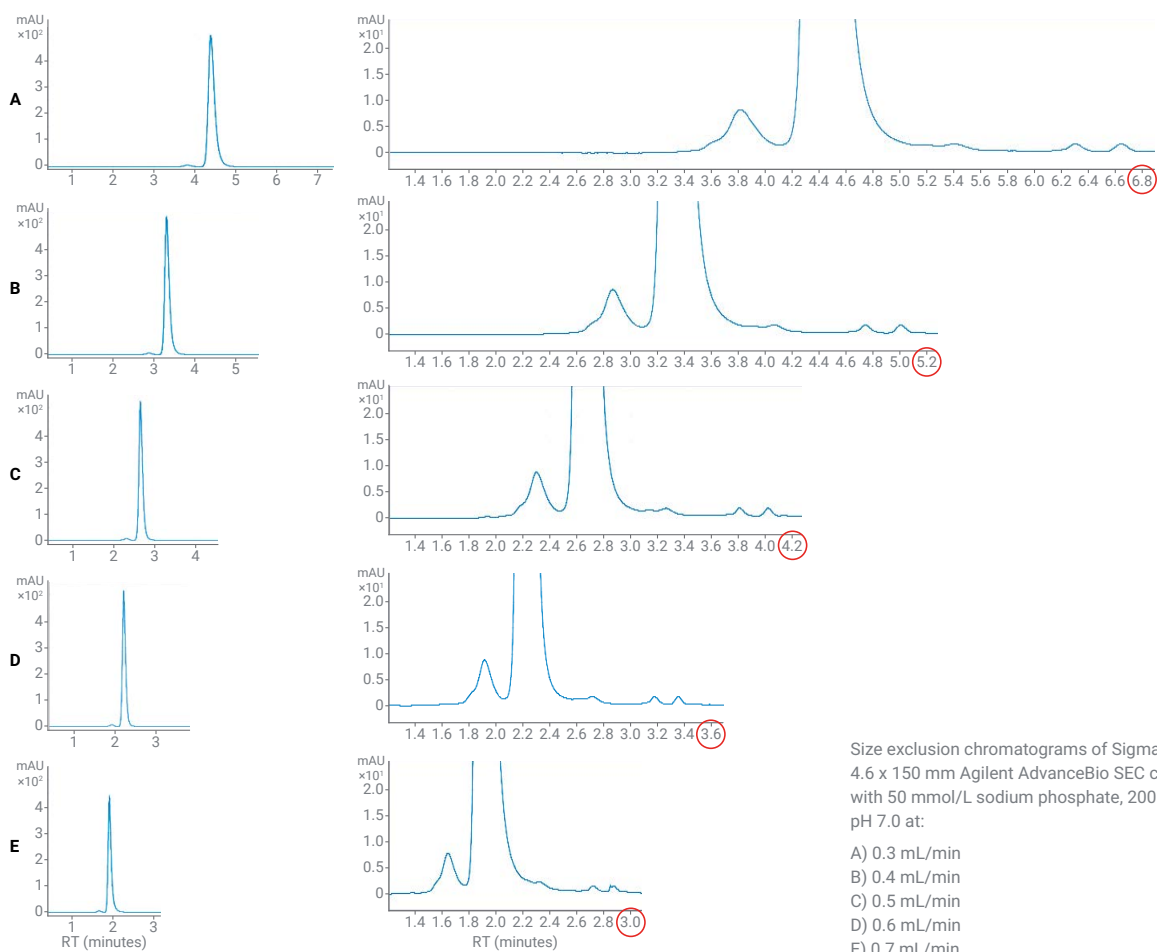
AdvanceBio SEC column, 300Å, 7.8 x 300 mm, 2.7 µm, *part number: [PL1180-5301](#) (for 1260 Infinity II bio-inert LC)*

Recommended columns for peptide aggregate analysis

AdvanceBio SEC column, 120 Å, 4.6 x 150 mm, 1.9 µm, *part number: [PL1580-3250](#) (for 1290 Infinity II bio LC)*

AdvanceBio SEC column, 130 Å, 7.8 x 300 mm, 2.7 µm, *part number: [PL1180-5350](#) (for 1260 Infinity II bio-inert LC)*

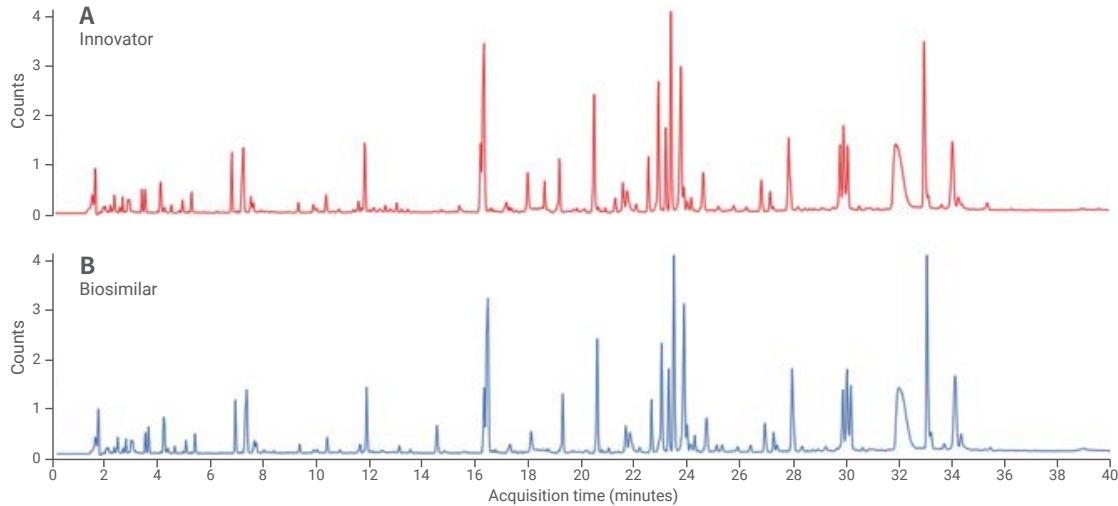
Column	Feature	Benefit
AdvanceBio SEC	Proprietary hydrophilic chemistry ensures surface inertness of packing materials	Minimized secondary interactions of hydrophobic molecules
	Excellent particle strength	Over 400 injections of column lifetime in stop/run tests
	Recommended flow rate of 0.1–2.0 mL/min for 7.8 mm id Recommended flow rate of 0.1–0.7 mL/min for 4.6 mm id Recommended flow rate of 0.05–0.10 mL/min for 2.1 mm id (Long column lengths require reduced flow rates to avoid overpressure)	Improve speed of high-throughput analysis while maintaining relatively high resolution
Tips	InfinityLab Quick Connect assembly recommended for the 1290 Infinity II bio LC for best performance. Fittings part number: 5067-5965; MP35N inert tube (0.12 x 105 mm) part number: 5500-1578	
Applications	Fast Separations for Aggregates and Fragments with Agilent AdvanceBio SEC 200 Å 1.9 µm columns	5994-0873EN
	Analysis of Antibody Fragment-Drug Conjugates Using an Agilent AdvanceBio SEC 120 Å 1.9 µm PEEK-lined Column	5994-3045EN
	Size Exclusion Chromatography of Biosimilar and Innovator Insulin	5991-6872EN



Recommended column for peptide mapping / host cell protein analysis / synthetic peptide analysis

AdvanceBio Peptide Mapping column, 2.1 x 150 mm, 2.7 μm , *part number: 653750-902*

Column	Feature	Benefit
AdvanceBio Peptide Mapping column (2.7 μm Poroshell particle, 120 \AA , C18)	Quality and performance tests	Excellent batch-to-batch reproducibility
	Superficially porous particles (SPPs)	Achieve UHPLC-level resolution using conventional HPLC
Applications	An Integrated Workflow for Peptide Mapping of Monoclonal Antibodies	5991-7811EN
	Automatic Protein Disulfide Bond Mapping of a Monoclonal Antibody Using the Agilent Accurate-Mass Q-TOF LC/MS Platform and MassHunter BioConfirm Software Algorithm	5991-6951EN



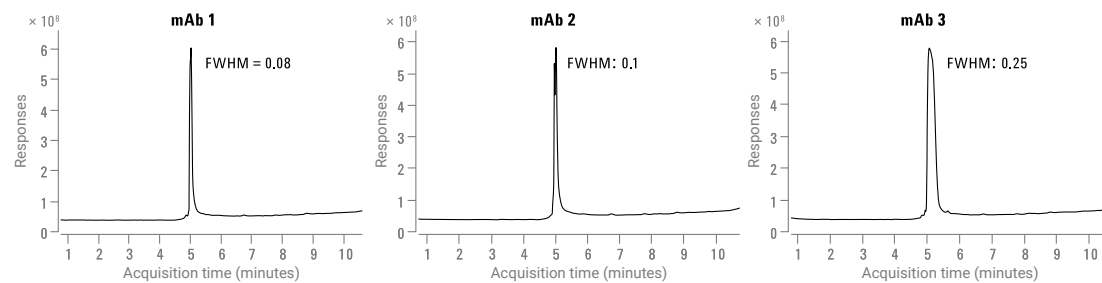
Total ion chromatogram (TIC) of peptide digest from innovator (A) and biosimilar (B).

Recommended columns for reversed-phase analysis of intact proteins and subunits

Intact protein: PLRP-S column, 1000 \AA , 2.1 x 50 mm, 8 μm , *part number: PL1912-1802*

Subunit analysis: PLRP-S column, 1000 \AA , 2.1 x 150 mm, 8 μm , *part number: PL1912-3802*

Column	Feature	Benefit
PLRP-S column for biomolecules (pH range: 1–14)	Robust polymeric materials	Longer lifetime at extreme pHs and temperatures
	Wide range of pore sizes	Suitable for the separation and analysis of intact proteins and fragments with low carryover
	Good peak shape under formic acid conditions	Compatible with mass spectrometry without loss of sensitivity or resolution
Applications	Monitoring Product-Related mAb Fragments: Intact Protein Analysis with the Agilent 1290 Infinity II Bio LC System Enables UV and MS Detection of Low Molecular Weight Species	5994-3021EN
	PLRP-S Polymeric Reversed-Phase Column for LC/MS Separation of mAbs and ADC	5991-7163EN



TIC of intact mAb/ADC LC/MS analysis on an Agilent PLRP-S column, 2.1 x 50 mm, 5 μm , 1000 \AA .

Recommended tools for the analysis of glycans and polar peptides

AdvanceBio Glycan Mapping column, 2.1 x 150 mm, 1.8 μm , **part number: 859700-913 (for 1290 Infinity II bio LC)**

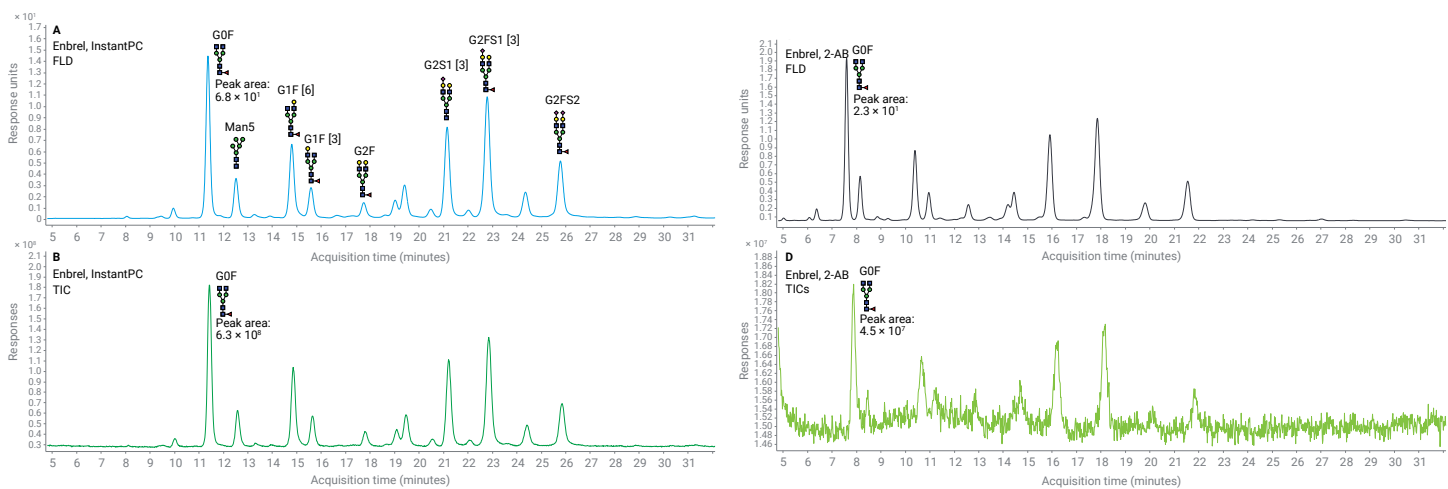
AdvanceBio Glycan Mapping column, 2.1 x 150 mm, 2.7 μm , **part number: 683775-913 (for 1260 Infinity II bio-inert LC)**

Kits and standards (please consult an Agilent consumables sales representative for protocol of complete workflow):

AdvanceBio Gly-X N-glycan prep with InstantPC kit, 96 count, **part number: GX96-IPC**

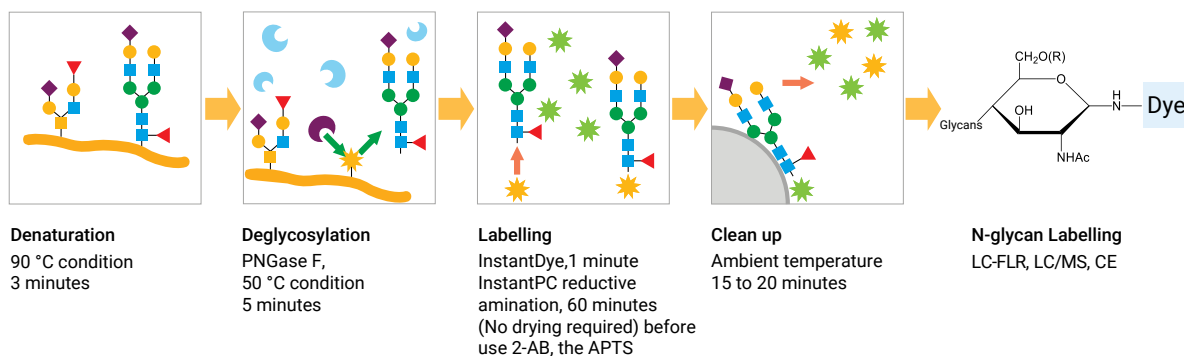
AdvanceBio InstantPC Human IgG N-glycan library, **part number: GKPC-005**

Column	Feature	Benefit
AdvanceBio Glycan Mapping column (2.7 μm , 120 \AA Poroshell particle and 1.8 μm , 300 \AA totally porous particle "TPP")	Successful separation within 10 minutes (1.8 μm TPPs)	Fast analysis
	SPP structure	Achieve UHPLC-level resolution using conventional HPLC
	Quality and performance tests	Excellent batch-to-batch reproducibility
Application	Streamlined Workflows for N-Glycan Analysis of Biotherapeutics Using Agilent AdvanceBio Gly-X InstantPC and 2-AB Express Sample Preparation with LC/FLD/MS	5994-1348EN



FLD and MS of InstantPC- and 2-AB-labeled N-glycans from Enbrel. A) InstantPC FLD; B) InstantPC TIC; C) 2-AB FLD; D) 2-AB TIC.

AdvanceBio Gly-X N-glycan sample preparation workflow

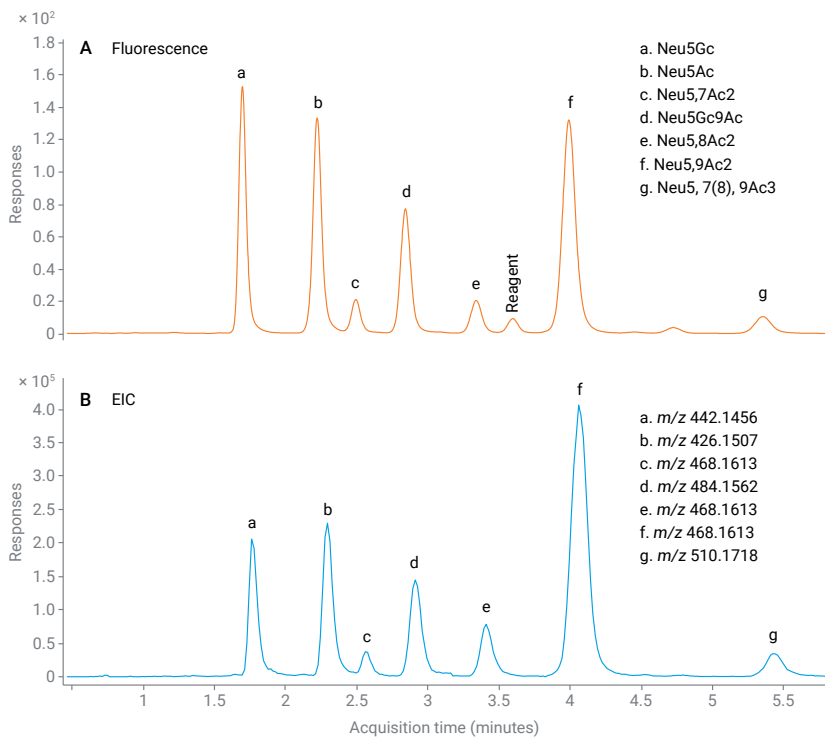


Recommended tools for sialic acid analysis

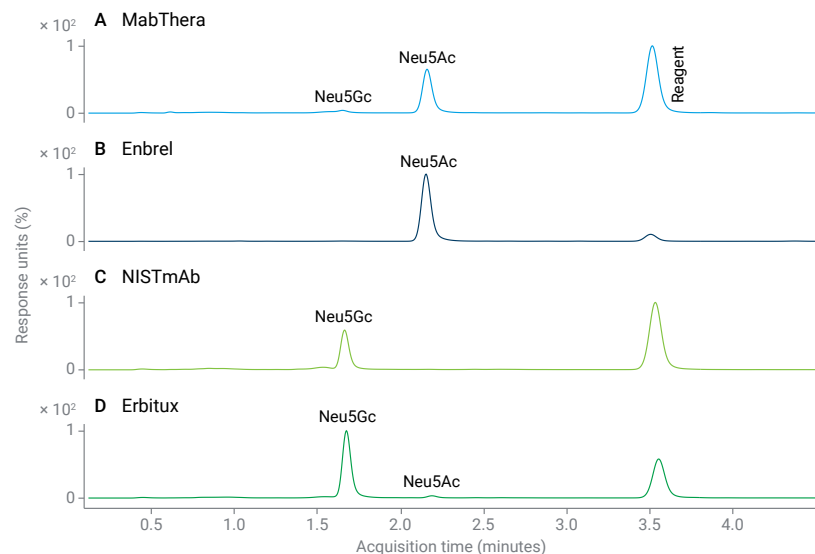
InfinityLab Poroshell 120 EC-C18, 4.6 x 75 mm, 2.7 μm , *part number: 697775-902*

Reagent kit: AdvanceBio Sialic Acid profiling and quantitation kit, *part number: GS24-SAP*

Kit	Feature	Benefit
AdvanceBio Sialic Acid profiling and quantitation kit	No need to dry samples before assays	Less preparation time
	Reduced sample dilution factors	Higher assay sensitivity (1–2000 pmol)
	Contains sialic acid qualitative controls, Neu5Ac and Neu5Gc quantitative standards	All reagents and standards conveniently available from a single supplier
Application	An Improved Workflow for Profiling and Quantitation of Sialic Acids in Biotherapeutics	5994-2352EN



UHPLC chromatogram of DMB-labeled SARP. (A) Fluorescence; (B) Extracted ion chromatogram (EIC) of DMB-labeled sialic acid species, $[M+H]^+$.

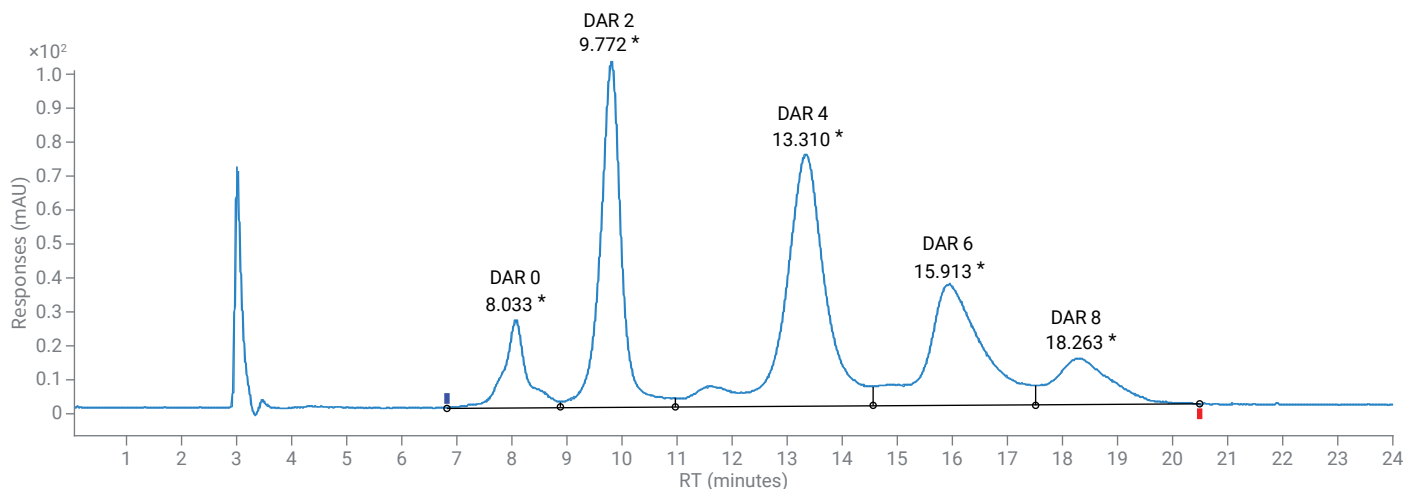


UHPLC fluorescence profiles of DMB-labeled sialic acids from different glycoproteins (A) Rituxan; (B) Enbrel; (C) NISTmAb; and (D) Erbitux

Recommended column for drug-to-antibody ratio (DAR) analysis

AdvanceBio HIC column, 4.6 x 100 mm, *part number: 685975-908*

Column	Feature	Benefit
AdvanceBio HIC column (3.5 µm TPPs)	Proprietary bonded-phase chemistry	Increased selectivity and up to 50% reduction in salt concentration
	Optimized 450 Å pore size	Improved mass-transfer efficiency, better peak shape
	Size 3.5 µm particles	Lower operating pressure, longer column life, reduced costs
Application	An AdvanceBio HIC Column for Drug-to-Antibody Ratio (DAR) Analysis of Antibody Drug Conjugates (ADCs)	
		5994-0149EN



HIC separation of brentuximab vedotin (Adcetris).

Peak area to DAR results

Number	RT (min)	Peak area (mAU*min)	Peak area (%)	DAR
1	8.03	763	6.9	0
2	9.77	2759	25.1	2
3	13.31	3936	35.8	4
4	15.91	2565	23.3	6
5	18.26	978	8.9	8
				DAR
				4.04

Recommended columns for the analysis of amino acids

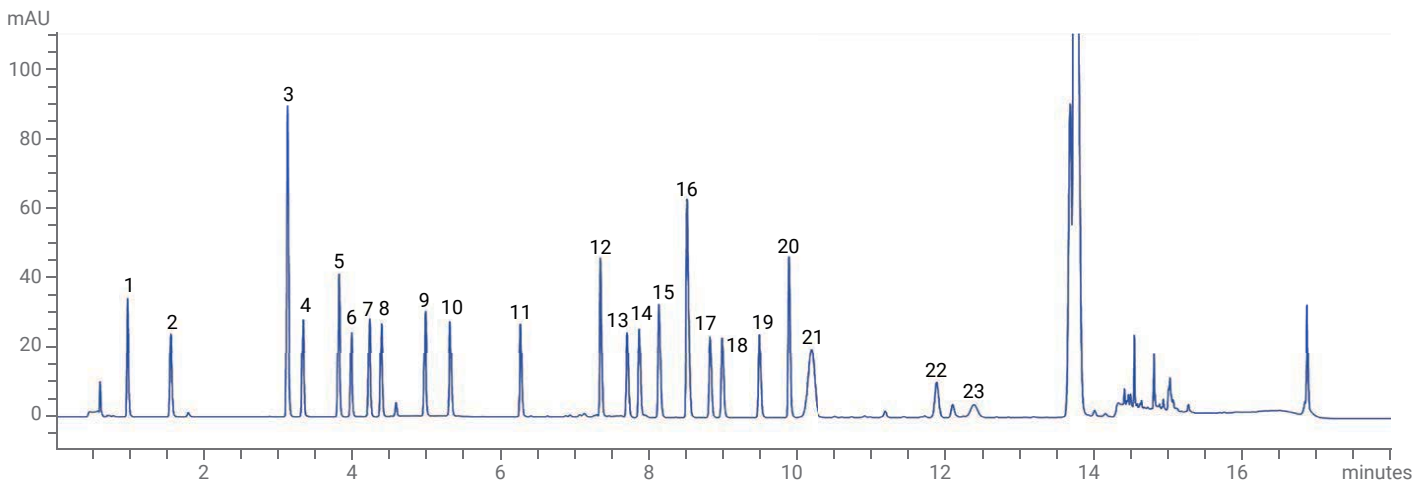
AdvanceBio AAA LC column, 4.6 x 100 mm, **part number: 655950-802** (AdvanceBio amino acid reagents kit, **part number: 5190-9426**)

AdvanceBio AAA guard column, 4.6 x 5 mm, 3 pack, **part number: 820750-931** (optional)

Column	Feature	Benefit
Precolumn derivatization, reversed-phase separation: AdvanceBio AAA LC column (2.7 µm Poroshell particle)	Fully automated precolumn derivatization	Easy operation and excellent reproducibility
	Stable up to pH 11	Long column life reduces operating costs
	Quality and performance tests	Excellent batch-to-batch reproducibility
	SPP structure	Achieve UHPLC-level resolution using conventional HPLC
Application	Analysis of Amino Acids Derived Online Using an Agilent AdvanceBio AAA Column	5991-6572EN

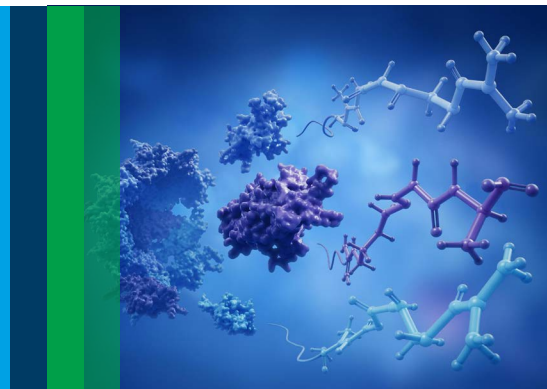
Peak Index	Amino Acid
1.	Aspartate
2.	Glutamate
3.	Asparagine
4.	Serine
5.	Glutamine
6.	Histidine
7.	Glycine
8.	Threonine
9.	Arginine
10.	Alanine
11.	Tyrosine
12.	Cystine

Peak Index	Amino Acid
13.	Valine
14.	Met Enkephalin
15.	Norvaline
16.	Tryptophan
17.	Phenylalanine
18.	Isoleucine
19.	Leucine
20.	Lysine
21.	Hydroxyproline
22.	Sarcosine
23.	Proline



Separation of 23 amino acid standards.

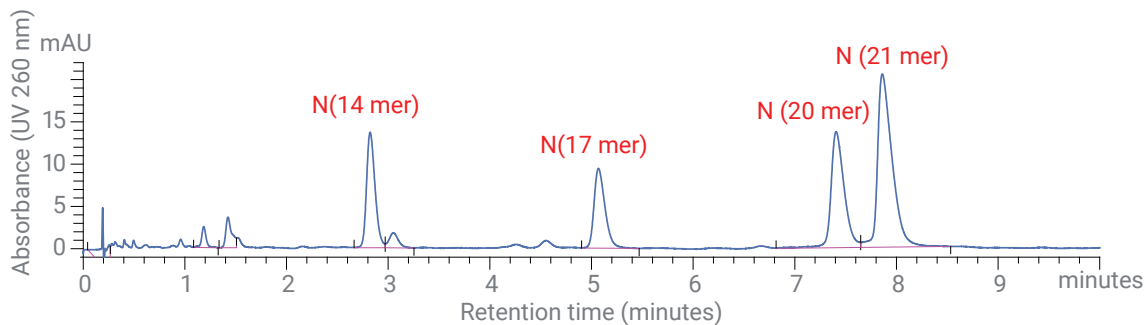
Agilent provides confidence for the monitoring of critical quality attributes in gene-therapy-related molecules



Recommended column for ion-pair reversed-phase (IP-RP) analysis of synthetic oligonucleotides

Reversed-phase chromatography analysis (< 150 Å) of oligo/primer transitions, *part number: 653750-702*

Column	Feature	Benefit
AdvanceBio Oligonucleotide (2.7 µm SPPs), 100 Å	SPPs	Achieve UHPLC-level resolution using conventional HPLC
	Stable up to pH 11	Longer column life, lower costs
Preferred: AdvanceBio Oligonucleotide column, 2.1 × 150 mm		
Applications	Fast and High-Resolution Reversed-Phase Separation of Synthetic Oligonucleotides	5991-6006EN
	Evaluation of Different Ion-Pairing Reagents for LC/UV and LC/MS Analysis of Oligonucleotides	5994-2957EN



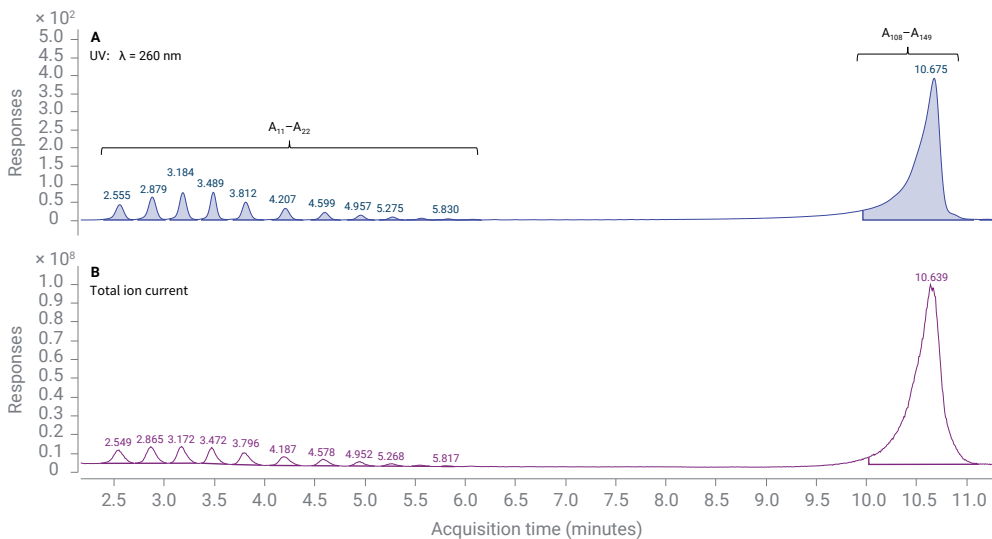
Gradient: 10 to 14% B in 10 minutes
 Stop time: 11 min
 Sample: Agilent RNA resolution standard, oligos at 14, 17, 20, and 21 mer (part number 5190-9028)
 Injection: 10 µL

Separation of Agilent RNA resolution standard using an Agilent AdvanceBio Oligonucleotide column.

Recommended column for mRNA poly-A tail analysis

PLRP-S column, 1000 Å, 2.1 x 50 mm, 5 µm, **part number: [PL1912-1502](#)**

Column	Feature	Benefit
PLRP-S column for biomolecules (pH range: 1 to 14)	Durable and resilient polymeric materials	Provides highly reproducible results over a longer lifetime
	Wide range of pore sizes	Suitable for separation of small molecules to large complexes and polynucleotides
Preferred: PLRP-S column, 1000 Å, 2.1 x 50 mm, 5 µm, part number: PL1912-1502		
Application	Analysis of mRNA Poly-A Sequence Variants by High-Resolution LC/MS	5994-3005EN

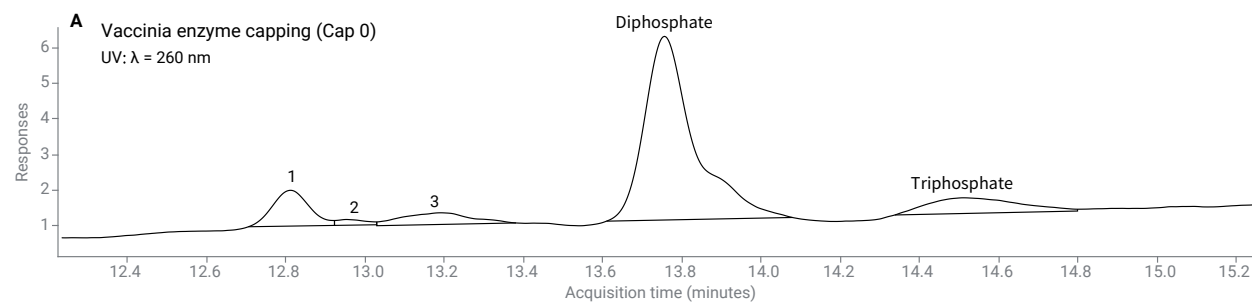


UV absorbance at 260 nm (A: reference = 360 nm) and TIC (B) of RNA primers extended with PAP in the presence of ATP only. Separation was carried out on an Agilent PLRP-S column.

Recommended column for mRNA 5' capping analysis

AdvanceBio Oligonucleotide column, 2.1 x 50 mm, 2.7 µm, **part number: [659750-702](#)**

Column	Feature	Benefit
AdvanceBio Oligonucleotide column (2.7 µm SPPs), 100 Å	SPPs	Achieve UHPLC-level resolution using conventional HPLC
	Tolerates pH up to 11	Longer column life, lower costs
Preferred: AdvanceBio Oligonucleotide column, 2.1 x 50 mm, part number: 659750-702		
Application	Rapid Analysis of mRNA 5' Capping with High-Resolution LC/MS	5994-3984EN

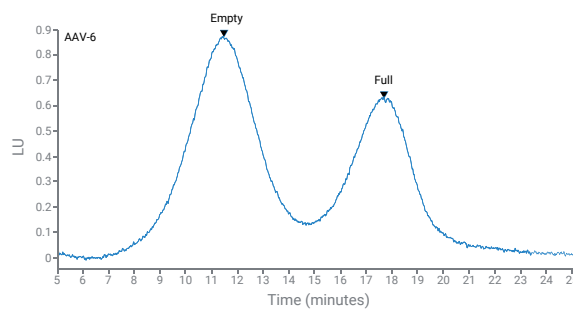
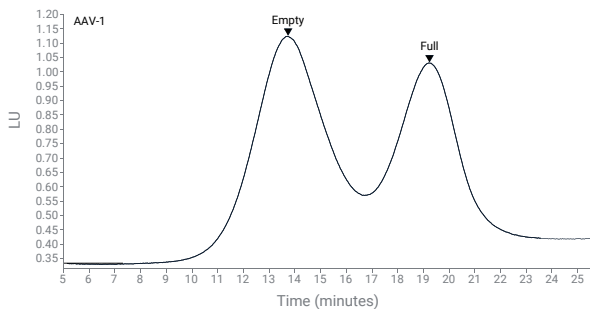


LC/MS of vaccinia virus enzyme-capped oligonucleotides and capping intermediates.

Recommended column for AAV empty/full capsid ratio analysis

Bio SAX column, NP5, 2.1 x 50 mm, PEEK, *part number: 5190-2472*

Column	Feature	Benefit
Bio SAX column	Rigid particles and hydrophilic coating	Inhibition of nonspecific binding
	Nonporous particles	Rapid mass transfer
	PEEK column tube material	Ensure flow path inertness
Application	Analysis of Full/Empty Capsid Ratios in Adeno-Associated Virus 1 and 6 Serotypes Using Biocompatible Liquid Chromatography	5994-4589EN



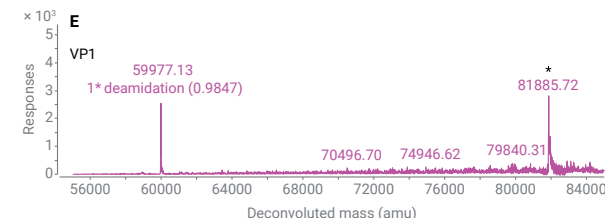
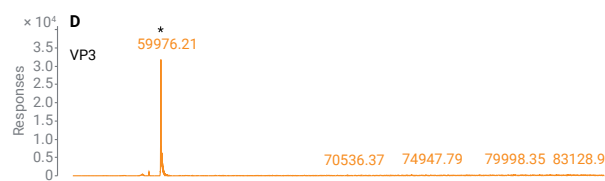
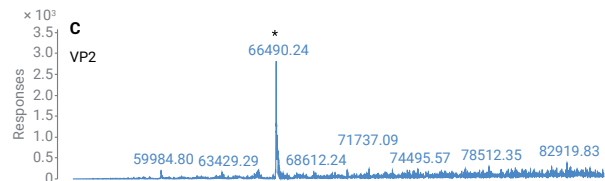
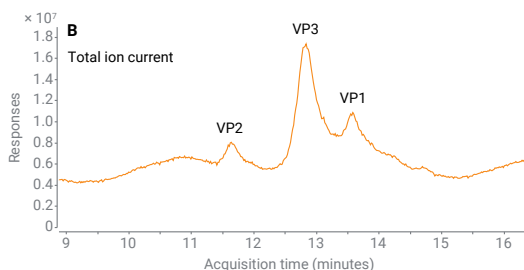
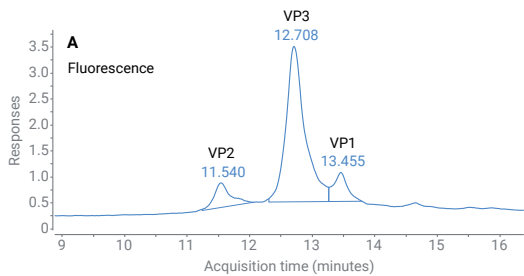
Separation of mixtures of full and empty capsids using the Agilent 1290 Infinity II flexible pump.

Recommended columns for primary structure validation of AAV capsid protein

ZORBAX RRHD StableBond C18 column, 300 Å, 2.1 x 100 mm, 1.8 µm, *part number: 858750-902*

ZORBAX RRHD StableBond Diphenyl column, 300 Å, 2.1 x 100 mm, 1.8 µm, *part number 858750-944*

Column	Feature	Benefit
ZORBAX RRHD StableBond C18 column, 300 Å, 1.8 µm	Maximum pressure of 1200 bar	UHPLC compatible
	Size 1.8 µm particles	Maximized resolution
	Various chemistries	Flexible method development options
Application	LC/MS of Intact Adeno-Associated Virus Capsid Proteins for Rapid Confirmation of Product Identity	5994-2434EN



LC/MS of denatured AAV2 capsid proteins using the optimized method on an Agilent ZORBAX RRHD StableBond C18, 300 Å AAV2 column. (A) Fluorescence chromatogram depicting the three capsid proteins. (B) Total ion current. (C) to (E) Deconvoluted mass spectra of the three capsid proteins with the relevant mass peaks marked by asterisks.

Bio LC recommended supplies

InfinityLab LC fitting portfolio

InfinityLab Quick Connect fittings

- InfinityLab Quick Connect fittings (**part number: 5067-5965**), requiring the following MP35N capillary tubes separately
- InfinityLab Quick Connect Capillary MP35N 0.12 x 150 mm (**part number: 5500-1579**)
- InfinityLab Quick Connect Capillary MP35N 0.17 x 150 mm (**part number: 5500-1585**)



InfinityLab solvent filtration assembly

- InfinityLab solvent filtration assembly (**part number: 5191-6776**), including glass funnel (250 mL), membrane holder glass base, glass flask (1 L), and aluminum clamp
- InfinityLab solvent filtration flask, glass, 2 L (**part number: 5191-6781**)
- Filter membrane, regenerated cellulose, 47 mm, pore size 0.2 µm, 100 pack (**part number: 5191-4340**)



Other recommendations for LC

- InfinityLab Stay Safe purging bottle (**part number: 5043-1339**)
- InfinityLab Stay Safe solvent cap starter kit, including four caps (**part number: 5043-1222**)



Bio LC recommended biocolumns

Biopharmaceutical Applications	Recommended Products	Part Number
Recommended columns for protein aggregate/fragment analysis	AdvanceBio SEC column, 200 Å, 4.6 x 150 mm, 1.9 µm (for 1290 Infinity II bio LC)	PL1580-3201
	AdvanceBio SEC column, 300 Å, 7.8 x 300 mm, 2.7 µm (for 1260 Infinity II bio-inert LC)	PL1180-5301
Recommended column for peptide aggregate analysis	AdvanceBio SEC column, 120 Å, 4.6 x 150 mm, 1.9 µm (for 1290 Infinity II bio LC)	PL1580-3250
	AdvanceBio SEC column, 130 Å, 7.8 x 300 mm, 2.7 µm (for 1260 Infinity II bio-inert LC)	PL1180-5350
Recommended column for peptide mapping	AdvanceBio Peptide Mapping column, 2.1 x 150 mm, 2.7 µm	653750-902
Recommended columns for intact and subunit analysis, reversed phase (> 150 Å)	Intact protein: PLRP-S column, 1000 Å, 2.1 x 50 mm, 8 µm	PL1912-1802
	Subunit analysis: PLRP-S column, 1000 Å, 2.1 x 150 mm, 8 µm	PL1912-3802
Recommended columns for glycan mapping / hydrophilic peptide mapping	AdvanceBio Glycan Mapping column, 2.1 x 150 mm, 1.8 µm (for 1290 Infinity II bio LC)	859700-913
	AdvanceBio Glycan Mapping column, 2.1 x 150 mm, 2.7 µm (for 1260 Infinity II bio-inert LC)	683775-913
Recommended column for sialic acid analysis	InfinityLab Poroshell 120 EC-C18 column, 2.1 x 75 mm, 2.7 µm	697775-902
Recommended column for antibody ratio (DAR) analysis of cysteine-based drug conjugates	AdvanceBio HIC column, 4.6 x 100 mm	685975-908
Recommended column for reversed-phase chromatography analysis (< 150 Å) of oligo/primer transitions	AdvanceBio Oligonucleotide column, 2.1 x 150 mm, 2.7 µm	653750-702
Recommended column for mRNA poly-A tail analysis	PLRP-S column, 1000 Å, 2.1 x 50 mm, 5 µm	PL1912-1502
Recommended column for mRNA 5' capping analysis	AdvanceBio Oligonucleotide column, 2.1 x 50 mm, 2.7 µm	659750-702
Recommended column for AAV empty capsid ratio analysis	Bio SAX column, NP5, 2.1 x 50 mm, PEEK	5190-2472
Recommended column for primary structure validation of AAV capsid protein	ZORBAX RRHD StableBond C18 column, 300 Å, 2.1 x 100 mm, 1.8 µm	858750-902
Recommended column for amino acid analysis (LC online derivatization)	AdvanceBio Amino Acid Analysis (AAA) LC column, 4.6 x 100 mm, 2.7 µm	655950-802

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