Agilent AdvanceBio Peptide Plus Columns

Charged surface C18 columns with superior performance
for peptide analysis

Agilent

Confidently Analyze Peptides by LC/MS with New Agilent AdvanceBio Peptide Plus Columns

The importance of understanding the critical quality attributes (CQAs) of a biologic drug, and the processes used to create it, cannot be underestimated. Biopharmaceutical labs need to obtain high-quality, accurate characterization of their therapeutic drugs to understand the structure quickly and efficiently, while ensuring seamless transfer to QA/QC and/or release testing.
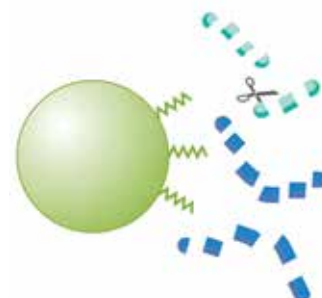
Now, with the new portfolio of Agilent AdvanceBio Peptide Plus columns, you can achieve excellent and reproducible peak shape for target peptides and impurities, while using the common formic acid (FA) mobile phase additive for simplicity and transferability across multiple system platforms.

Unlike traditional C18 columns, this innovative charged surface provides superior performance under the MS-friendly FA mobile phase additive with better peak shape, alternate selectivity, and improved resolution.

Agilent AdvanceBio Peptide Plus columns

Based on Agilent superficially porous Poroshell technology, AdvanceBio Peptide Plus columns feature a hybrid endcapped C-18 stationary phase on a 100 Å pore size, 2.7 µm particle modified to have a charged surface. With UHPLC performance at HPLC pressures, you can use AdvanceBio Peptide Plus columns across all of your LCs.

Agilent AdvanceBio Peptide Plus columns offer the selectivity you need with excellent reproducibility.

– Alternate selectivity that often improves separation of critical components that traditional C18 columns cannot resolve

– Excellent performance with alternate selectivity from the charged surface, improving resolution for peptides with post-translational modifications and degradation products

– FA compatibility and multiple dimensions, so you can choose a single column for early development through QA/QC, and across multiple system platforms

– High loadability, so you can analyze very minor components in samples by injecting a large amount of sample

– Quality assurance, as every column is batch tested with the Agilent peptide mapping standard mix

# Improved resolution of critical post-translational modifications (PTMs)

Compared to traditional C18 columns, AdvanceBio Peptide Plus columns provide better resolution for critical PTMs like deamidation.

Separation of deamidated peptides from the unmodified peptide on an AdvanceBio Peptide Plus column

Greatly improved resolution of deamidated peptides from the unmodified peptide under FA LC/MS conditions.

Conditions

Parameter	Value	
Sample:	Tryptic digest of a monoclonal antibody (mAb)	
Flow rate:	0.4 mL/min	
Column temp:	60 °C	
Eluent A:	Water with 0.1% FA	
Eluent B:	Acetonitrile with 0.1% FA	
Gradient:	Time (min)	B %
	0	3
	2	3
	40	40
	50.5	100
	53	3
Stoptime:	60 minutes	
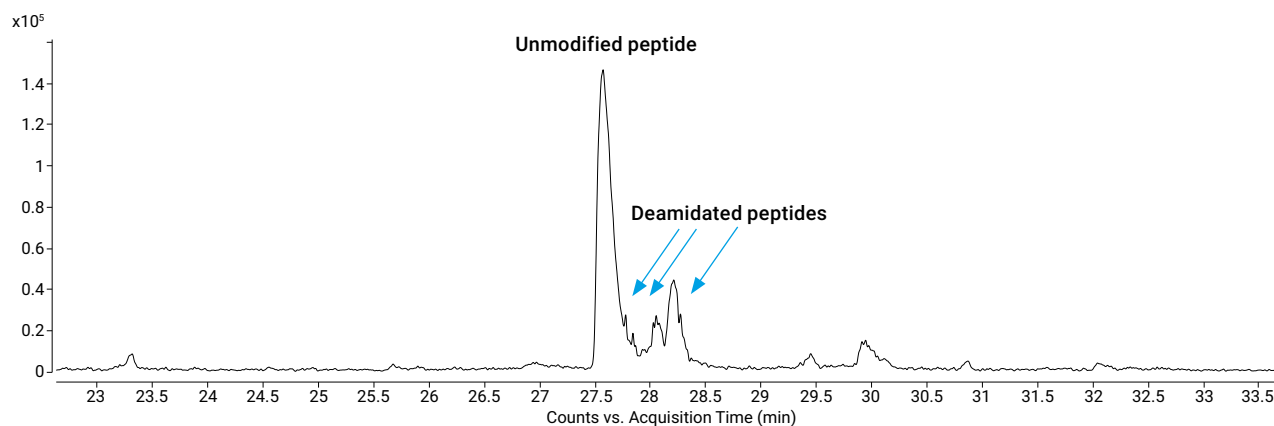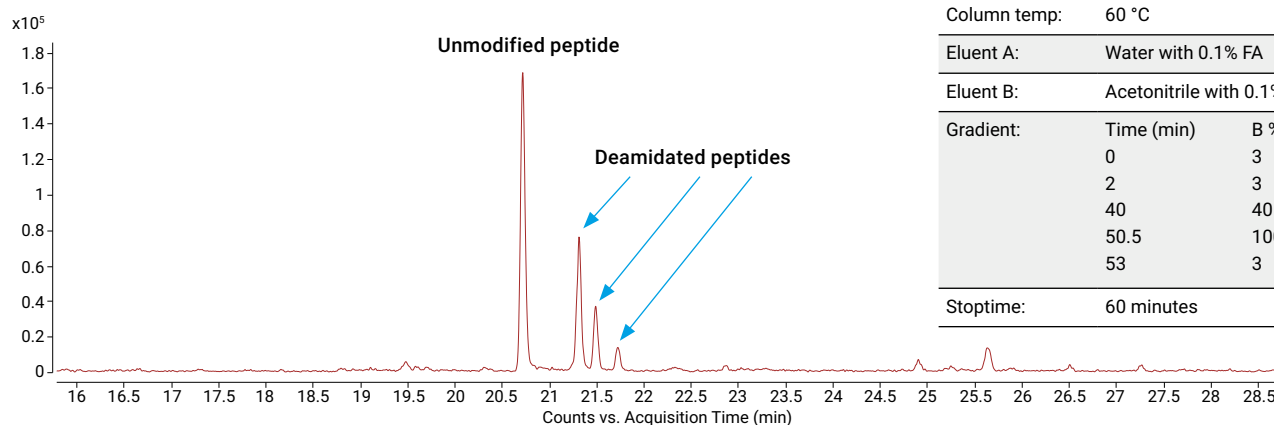
Separation of deamidated peptides from the unmodified peptide on a traditional C18 column
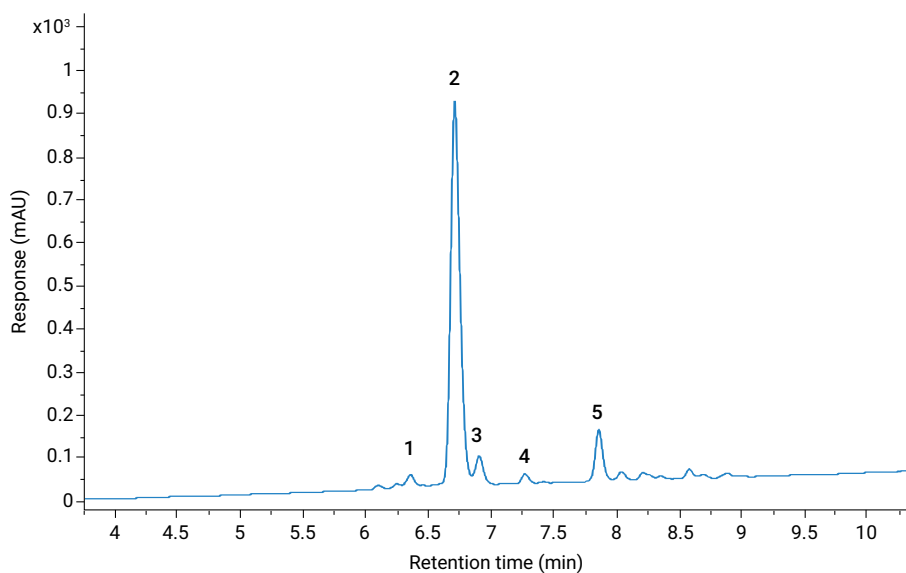
Poor resolution of deamidated peptides from the unmodified peptides under FA LC/MS conditions

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Excellent separation and identification of synthetic peptide impurity

A single LC method can be run with either UV or MS detection to separate synthetic peptide impurities using FA as a mobile phase additive. This LC/MS method can be used in discovery and early development for impurity identification before using UV for quantitation of critical pairs, eliminating the need for costly, time-consuming method redevelopment.
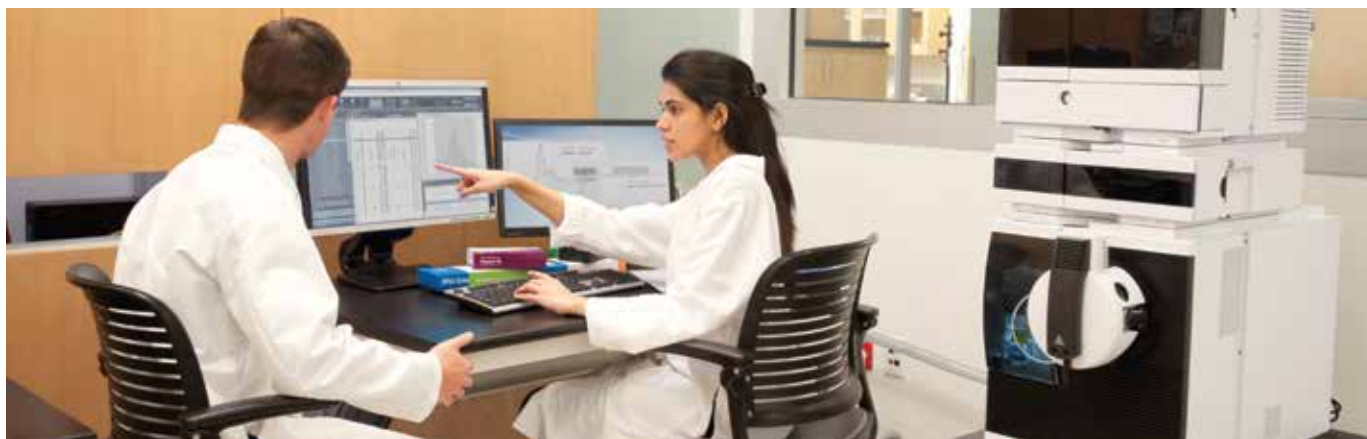
Separation of synthetic peptide and impurities under FA conditions

Excellent separation of bivalirudin peptide and impurities using FA mobile phase.

Conditions

| Parameter	Value	
Column:	AdvanceBio Peptide Plus 2.1 x 150 mm	
Column temp:	60 °C	
Flow rate:	0.4 mL/min	
Eluent A:	Water with 0.1% FA	
Eluent B:	Acetonitrile with 0.1% FA	
Gradient:	Time (min)	B %
	0	17
	2	17
	22	37
	24	95
	26	95
	26.1	17
Post time:	5 minutes	
Peak:	Peak ID:	
1	Deletion of Glu	
2	Product	
3	Deletion of Gly	
4	Loss of H_2O	
5	Deamidation	

Optimize your separations with high column loadability and alternate selectivity

Peak shape comparison in 0.1% FA-modified mobile phase at increasing sample loads

The AdvanceBio Peptide Plus column is designed to maintain narrow, symmetrical peak shapes and stable retention times in FA even as sample load is increased to very high levels. Excellent performance even with high sample loads can facilitate LC/MS determination of minor components such as peptides from host cell proteins in biologics.

## Peak shape of peptide GPSVFPLAPSSK in a mAb digest
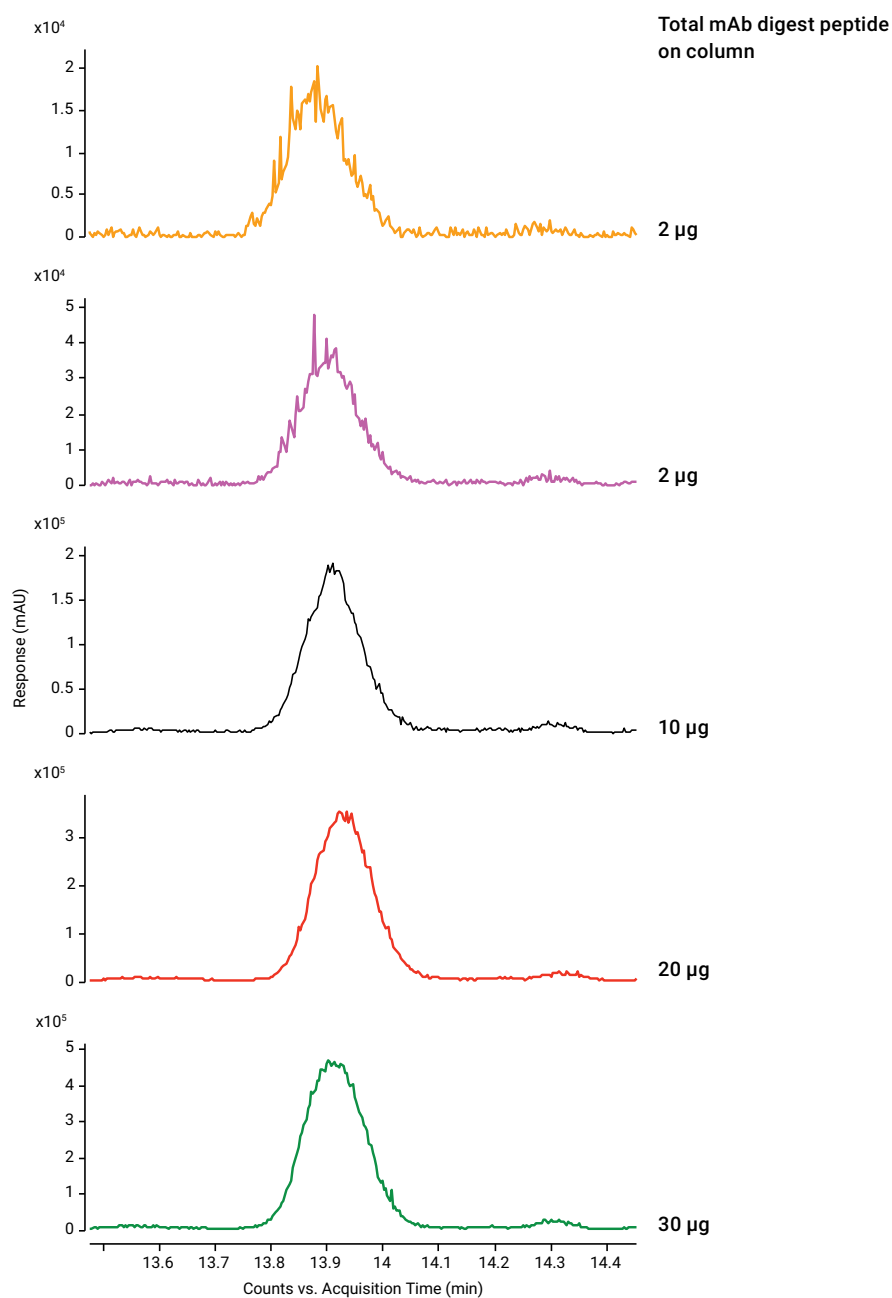
Alternative selectivity for optimizing separations

The AdvanceBio Peptide Plus column has a highly distinct selectivity for peptide structures, offering an excellent chance to improve critical pair resolution when switching from a standard C18 column.
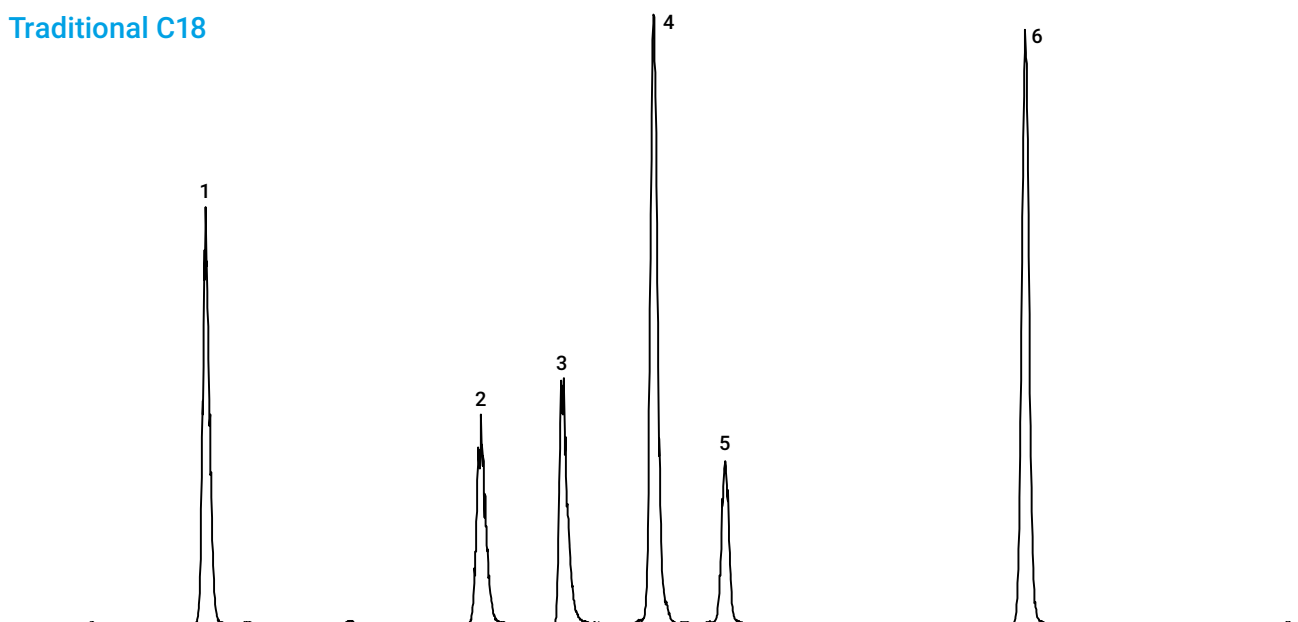
Conditions

Peak	Peptide Sequence
1	RPPGFSPFR
2	Glp-LYENKPRRPYIL
3	DRVYIHPFHL
4	GLILVGGYGTR
5	GILFVGSGVSGGEEGAR
6	LTILEELR

AdvanceBio Peptide Plus column
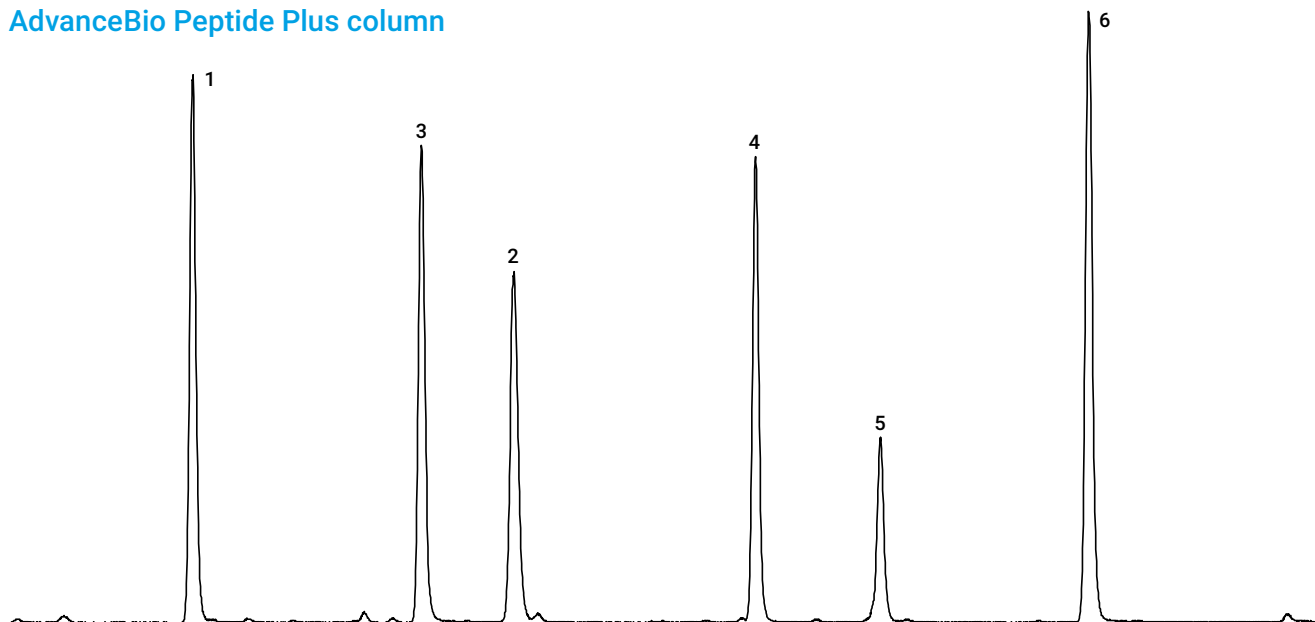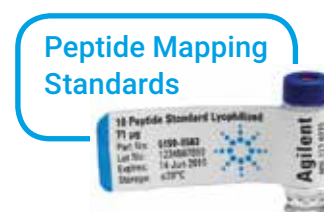

Traditional C18

Quality assurance: Ensure confidence in your results

Agilent research scientists design robustness into each stage of the column manufacturing process—particle design, bonding, and column packing. Testing is performed throughout the production process, and final product QC ensures that each batch of media and each column produced will meet the stringent requirements of your analysis.

Every batch of AdvanceBio Peptide Plus column is application tested with Agilent peptide standard using LC/MS FA mobile phase. Each column is also tested with a small-molecule probe to ensure column efficiency.
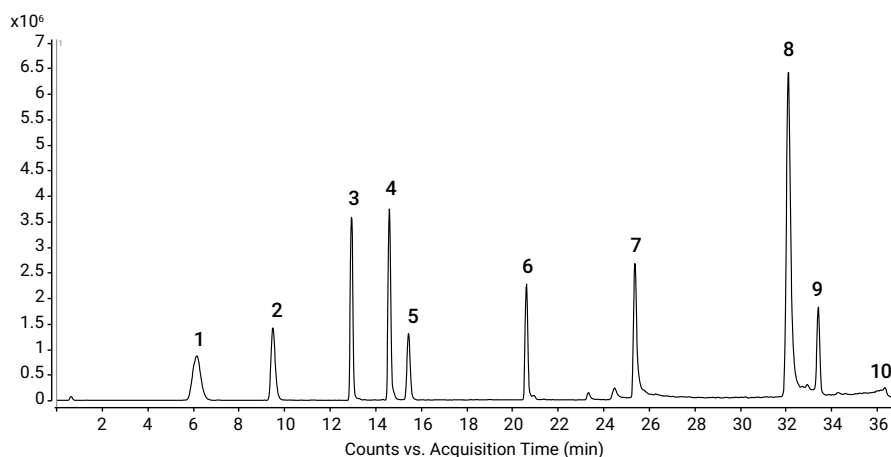
Peptide Mapping Standards

Quality test with peptide mixture standard

Batch to batch consistency ensured with batch test by peptide standard under FA LC/MS conditions.

Conditions

Parameter	Value	
Sample:	Agilent Peptide Mapping Standards Mix made in 100 µL water	
Injection volume:	0.2 µL	
Column:	AdvanceBio Peptide Plus 2.1 x 150 mm	
Column temp:	55 °C	
Flow rate:	0.4 mL/min	
Eluent A:	Water with 0.1% FA	
Eluent B:	Acetonitrile with 0.1% FA	
Gradient:	Time (min)	B %
	0	3
	2	3
	35	30
	40	97
	43	100
	43.5	3
Stoptime:	60 minutes	

Counts vs. Acquisition Time (min)

Conditions

Peak	Peptide Name	Peptide Sequence
1	Bradykinin frag 1-7	RPPGFSP
2	Bradykinin	RPPGFSPFR
3	Angiotensin II (human)	DRVYIHPF
4	Neurotensin	Glp-LYENKPRRPYIL
5	Angiotensin I (human)	DRVYIHPFHL
6	Renin substrate (porcine)	DRVYIHPFHLLVYS
7	[Ace-F-3,-2 H-1] Angiotensinogen (1-14)	Ace-FFHDRVYIHPFHLLVYS
8	Ser/Thr Protein Phosphatase (15-31)	EIFLSQPILLELEAPLK
9	[F14] Ser/Thr Protein Phosphate (15-31)	FEIFLSQPILLELEAPLK
10	Melettin (honey bee venom)	GIGAVLKVLTTGLPALISWIKRKRQQ

Be Agilent sure in your critical quality attribute (CQA) monitoring

Agilent AdvanceBio LC columns are designed and produced to provide results you can be sure of when analyzing highly complex biotherapeutic molecules and monitoring their purity, potency, and other critical quality attributes.

Titer Determination	Aggregate Analysis	Intact Purity & PTM Analysis		Peptide Mapping & PTM Analysis	Charge Variant Analysis	Glycan Analysis	Amino Acid / Cell Culture Media Analysis	
Affinity	Size Exclusion	Reversed Phase >150 Å	Hydrophobic Interaction	Reversed Phase < 150 Å	Ion Exchange	Hydrophilic Interaction	Reversed Phase < 150 Å	Hydrophilic Interaction
	AdvanceBio SEC 1.9 µm PEEK	PLRP-S 1000 Å 5 µm PEEK		AdvanceBio EC-C18 PEEK	Bio mAb / Bio IEX NP5 PEEK			AdvanceBio MS Spent Media PEEK
Bio-Monolith Protein A	AdvanceBio SEC 1.9 µm	PLRP-S	AdvanceBio HIC	AdvanceBio Peptide Mapping	Bio mAb	AdvanceBio Glycan Mapping	AdvanceBio Amino Acid Analysis (HpH)	
Bio-Monolith Protein G	AdvanceBio SEC 2.7 µm	AdvanceBio RP mAb 450Å		AdvanceBio Peptide Plus	Bio IEX (SAX, WAX, SCX, WCX)		ZORBAX AAA	
	Bio SEC-3	ZORBAX RRHD 300 Å, 1.8 µm		ZORBAX RRHD 300 Å, 1.8 µm	PL SCX, SAX			
	Bio SEC-5	ZORBAX 300SB 3.5, 5 & 7 µm			Bio-Monolith (QA, DEAE, SO3)			
	ProSEC 300S	Poroshell 300 5 µm						
	ZORBAX GF250 & GF450							

Key

Stainless steel (SS) column hardware

Solid PEEK or PEEK-lined bioinert column hardware

AdvanceBio

Agilent AdvanceBio columns are designed and produced to provide results you can be sure of when analyzing highly complex biotherapeutic molecules and monitoring their purity, potency, and other critical quality attributes.

For application examples spanning the biocolumn portfolio, please see the Critical Quality Attributes Application Compendium at

www.agilent.com/chem/cqa-applications

Agilent AdvanceBio Peptide Plus column ordering information

Agilent AdvanceBio 2.7 μm columns

Size (mm)	Peptide Plus
2.1 x 50	699775-949
2.1 x 150	695775-949
2.1 x 250	693775-949
3.0 x 150	693975-349
4.6 x 150	693975-949

**Agilent AdvanceBio 2.7 μm Fast Guards: extend column lifetime even further**

Size	Peptide Plus
2.1 x 5	821725-954
3.0 x 5	823750-952
4.6 x 5	820750-940

Agilent AdvanceBio peptide standards

Description	Standard
Ten-peptide standard, 71 μg, lyophilized, in a 2 mL vial	5190-0583

Agilent AdvanceBio 2.7 μm method validation kit

Size	Peptide Plus Method Validation Kit
Three columns packed with three different lots of stationary phase 2.1 x 150	695775-949K
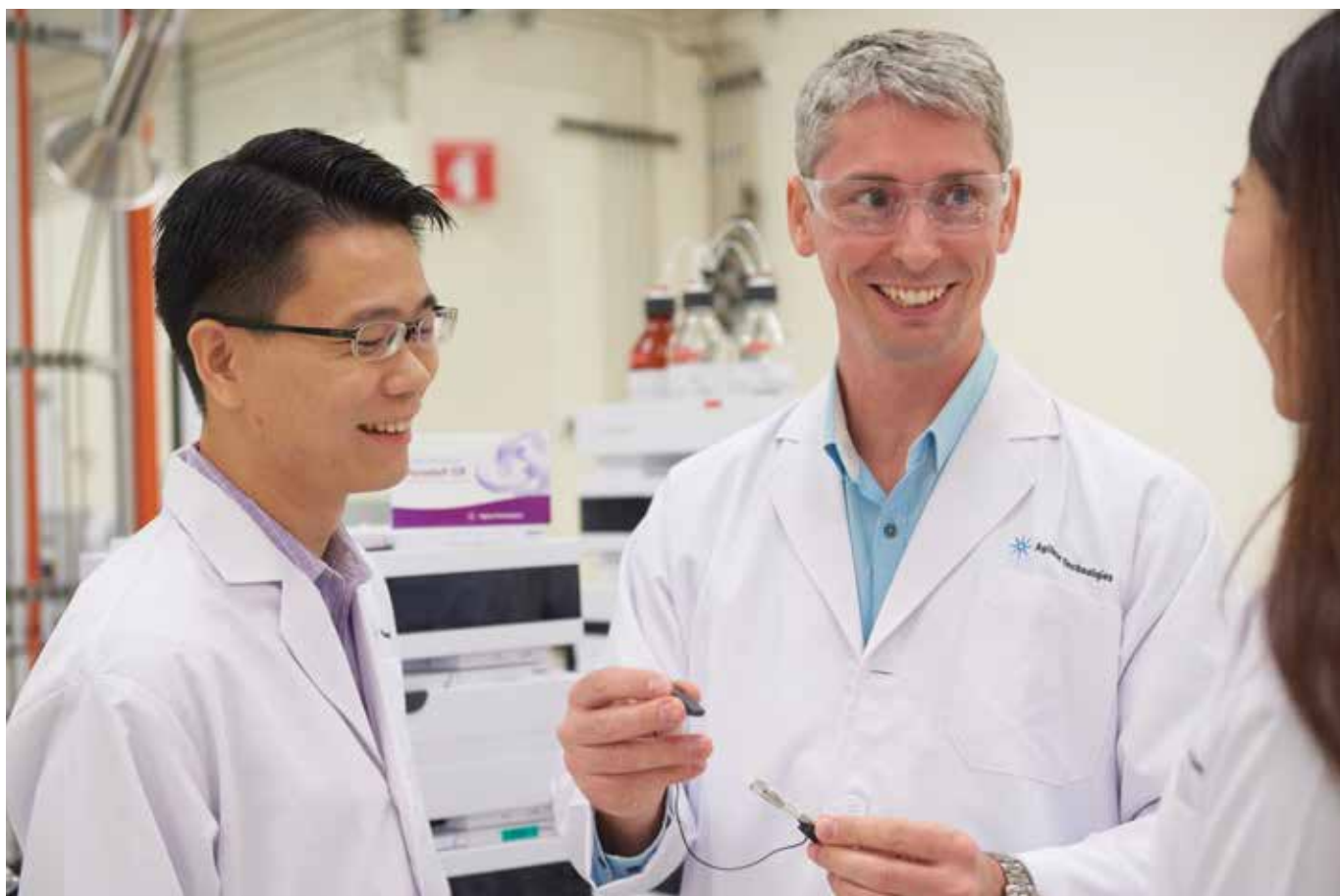
AdvanceBio Peptide Plus columns:
An integral part of your characterization workflow

| **Sample preparation** | **Separation** | **Identification** | **Data analysis** |
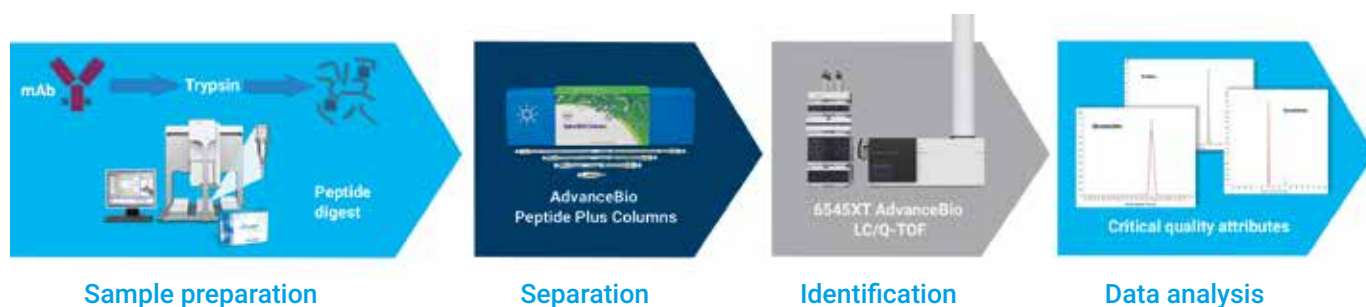
With the highly efficient Agilent peptide mapping workflow, you can get more done, faster, without sacrificing precision.

The secret?

Easy-to-implement automation that takes the tedium (and human error) out of preparing samples… software that's easy to master… instruments that produce precise, repeatable results… and reversed-phase columns that deliver lot-to-lot reproducibility.

Sample preparation

The Agilent AssayMAP Bravo protein and peptide sample prep platform combines the benefits of automation with an optimized workflow interface to enable efficiency and reproducibility.

Separation

Enhance the accuracy and speed of your biomolecule characterization with Agilent AdvanceBio Peptide Plus columns.

Identification

The Agilent 6545XT AdvanceBio LC/Q-TOF system is designed to handle multiple workflows in biomolecule characterization. Agilent MassHunter software efficiently extracts all available information from your samples.

Data analysis

Make data review more objective and less complex with Agilent OpenLab chromatography data system and MatchCompare software.

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