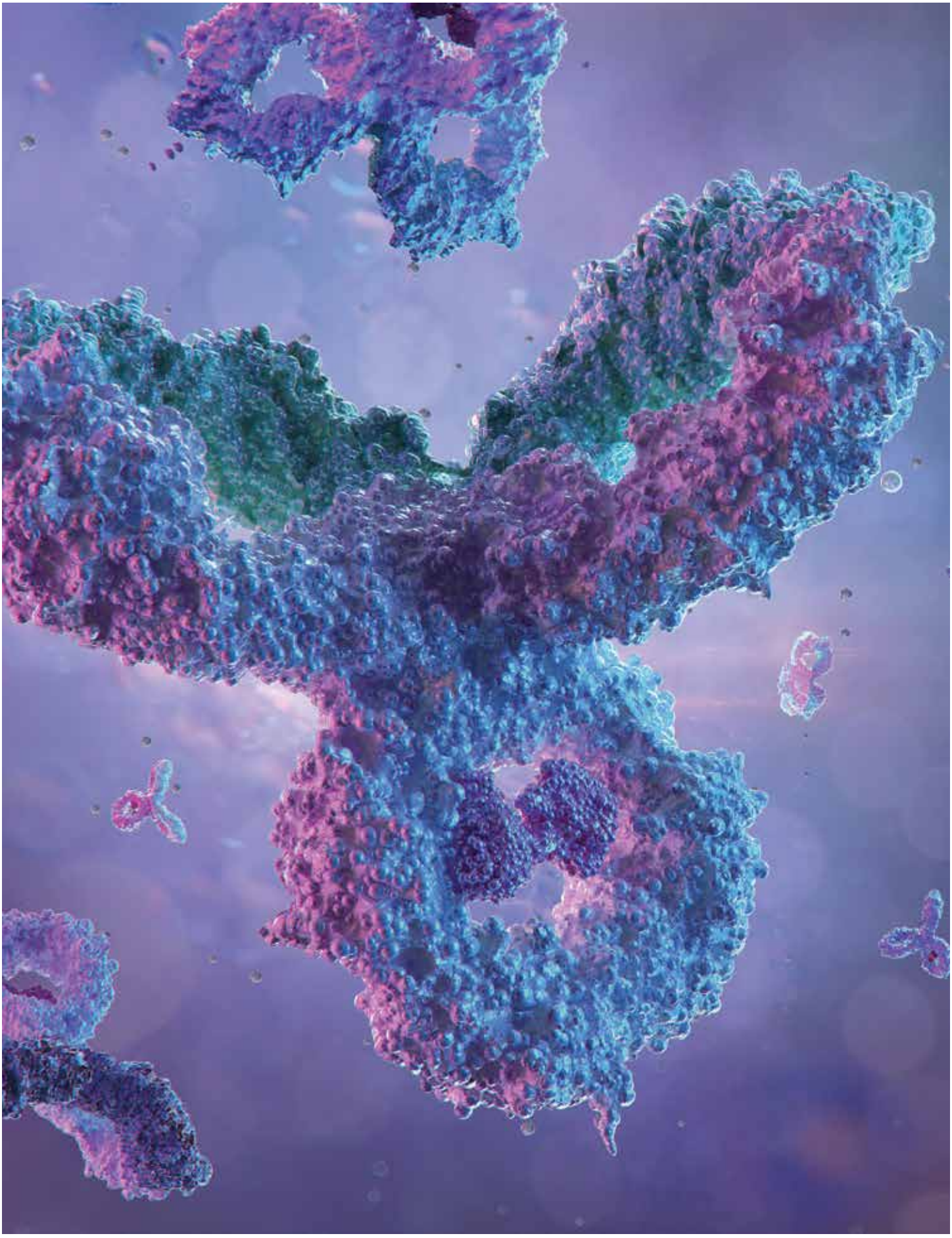
Agilent

Trusted Answers

High-Resolution, Fast, and Accurate Intact and Reduced mAb Analysis

Agilent AdvanceBio RP-mAb column for large molecule characterization



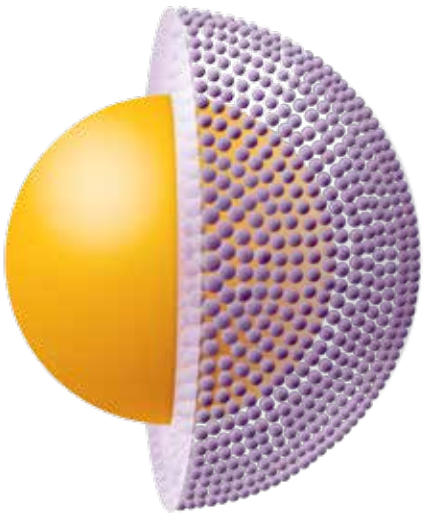
What makes Agilent Poroshell products better?

AdvanceBio RP-mAb columns with Poroshell 450Å particles are based on superficially porous particle technology, which features a solid silica core and a porous outer layer.

Compared to traditional totally porous particles of the same (or similar) size, Poroshell particles deliver higher chromatographic efficiencies and enable fast, high-resolution separations.

Poroshell superficially porous particles offer several advantages over totally porous particles:

- Uniform particles with smooth surfaces, ensuring smaller particle size distributions
- Short analyte diffusion path in/out of the porous layer
- Narrow particle size distributions, allowing more uniform packing of column particles

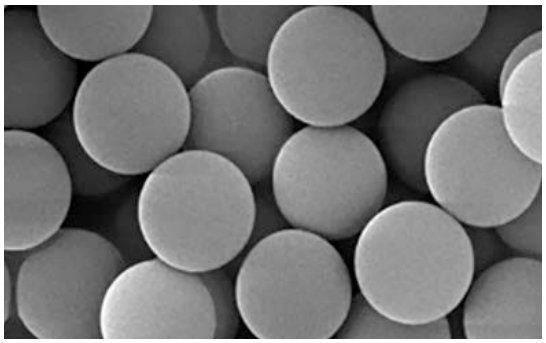


How is a Poroshell particle made?

Agilent uses a unique manufacturing process for Poroshell particles. Specifically, we minimize the number of manufacturing steps to ensure maximum particle—and chromatographic—reproducibility.

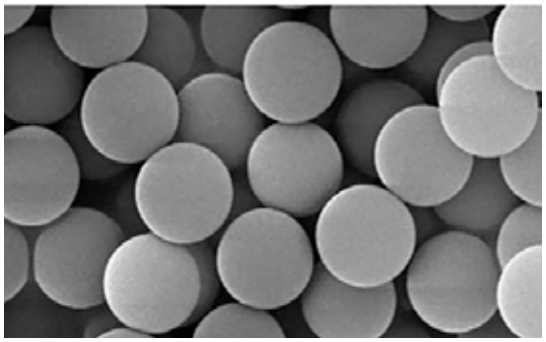
Step 1: make the solid core

The Poroshell 450Å cores in AdvanceBio RP-mAb columns have a very smooth surface and a uniform particle size—both of which contribute to a tight overall particle size distribution, improving analytical performance and reproducibility.



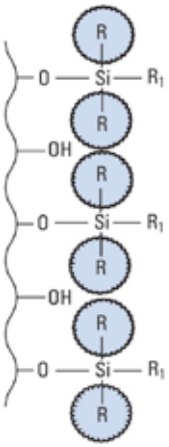
Step 2: apply the porous shell

At Agilent, we apply the porous shell in one single step. This unique process delivers more column-to-column reproducibility than other vendors' columns.



Step 3: apply the bonded phase

Three differentiated and robust bonded phases enable analysis of a variety of analytes in different LC modes, to solve even your toughest separation challenges.



A Tradition of Innovation and Efficiency

Poroshell technology: improving separation efficiency in LC

More than 15 years ago, Agilent introduced the industry's first superficially porous column technology with Poroshell 300 for large molecules, followed by Poroshell 120 for small molecules.

Today, the Poroshell family of products spans several pore sizes and nearly two dozen chemistries. Whether you use an HPLC system or a newer UPLC system, Poroshell products provide exceptional separation efficiency and significantly boost performance and throughput from every LC in the lab.

AdvanceBio RP-mAb columns with Poroshell 450Å particles

- **Excellent lot-to-lot reproducibility**

  A proprietary, single-step porous shell process dramatically reduces tiny differences between lots and columns.

- **Superior peak shape**

  High-purity silica and advanced bonding chemistries reduce peak tailing and give you faster, more accurate results.

- **Three innovative chemistries**

  They support optimal resolution across biologic compounds and orthogonal selectivity for confirmation analysis

- **Wide instrument compatibility**

  AdvanceBio RP-mAb wide pore 3.5 µm particles can provide benefits on either an HPLC or a UPLC system, allowing for easy transfer from discovery to QC

- **MS compatibility**

  AdvanceBio RP-mAb C4 and Diphenyl phases are compatible with formic acid, resulting in excellent peak shapes and MS data after desalting.

Agilent InfinityLab LC/MSD Series

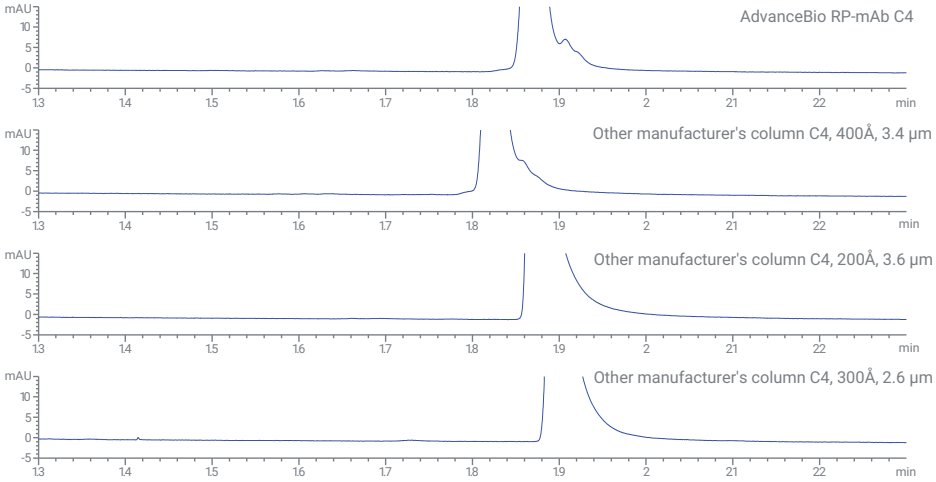
The new InfinityLab LC/MSD iQ provides essential mass detection to quickly confirm compounds and provide more specificity and selectivity than a UV detector. The InfinityLab Flex Bench MS enables mobility and optimizes your lab space with an easy to access stack.

The right bonded phases for fast, high-resolution characterization of intact mAbs and their subunits

Biotherapeutics such as monoclonal antibodies (mAbs) have become established over the last decade and continue to grow. While biotherapeutics provide distinct advantages over small molecules drugs, their quality control and characterization are inherently more complex. Poroshell technology, exclusive to Agilent, is the foundation of every AdvanceBio RP-mAb stationary phase. These reversed-phase columns have been designed to optimize intact and reduced mAb analysis when characterizing primary structure during biopharmaceutical discovery, development, and QA/QC. These columns focus on the complex challenges associated with monoclonal characterization and deliver higher resolution and faster run times when compared to fully porous alternatives, while providing accurate and reproducible results.

AdvanceBio RP-mAb outperforms the competition

Separation of intact humanized recombinant Herceptin IgG1 performed on an AdvanceBio RP-mAb C4 column and three competitive columns. AdvanceBio RP-mAb C4, optimized for mAb separations, provides better peak shape and resolution for intact protein separations than the leading competitive columns.



Specifically designed for mAb separations, AdvanceBio RP-mAb provides superior peak shape and resolution than other columns used for intact protein separations.

Method Parameters

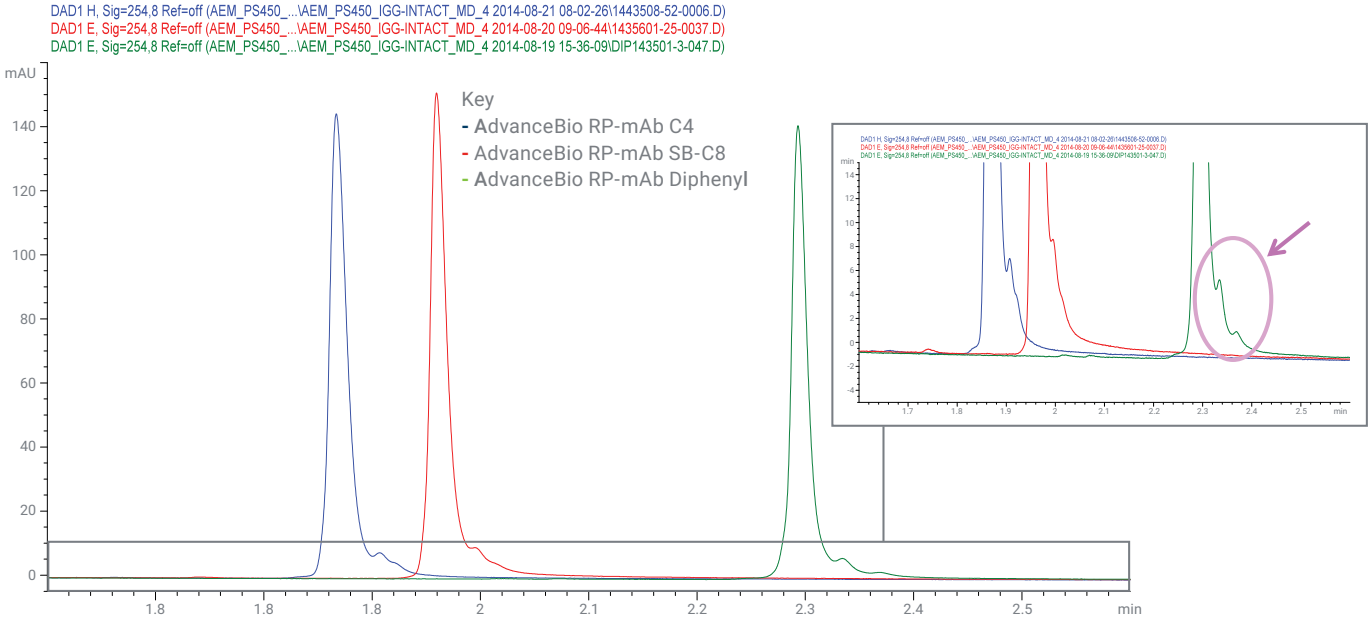
Column dimensions:	2.1 x 100 mm, 3.5 µm
Mobile phase A:	0.1% TFA in water:IPA (98:2)
Mobile phase	B: IPA:ACN:Mobile phase A (70:20:10)
Flow rate:	1.0 mL/min
Gradient:	10-58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B
Sample:	5 µL injection of humanized recombinant Herceptin Variant IgG1 intact from Creative Biolabs (1 mg/mL)
Temperature:	80 °C
Detection:	UV @ 254 nm

For intact analysis, choose from three bonded phases

When choosing a stationary phase, it is important to start with the chemical characteristics of your target molecule and its impurities. This understanding will help to leverage your phase choice for optimal results. As a general rule, the table below acts as a guide for selectivity, but as best practice, screening multiple phases and ion pairing reagents is recommended during the method optimization process.

Select the right bonded phase for optimal results

C4:	Best for fast, high-resolution separations, hydrophobic proteins
SB-C8:	Fast, high-resolution separations for less hydrophobic proteins, increased resolution between closely related impurities (similar hydrophobicity profiles)
Diphenyl:	Orthogonal selectivity (confirmational), or when separating species based on aromatic or pi-pi properties



Discover the influence of different bonding chemistries: Unveiling the effects on IgG1 (trastuzumab) chromatograms

Method Parameters

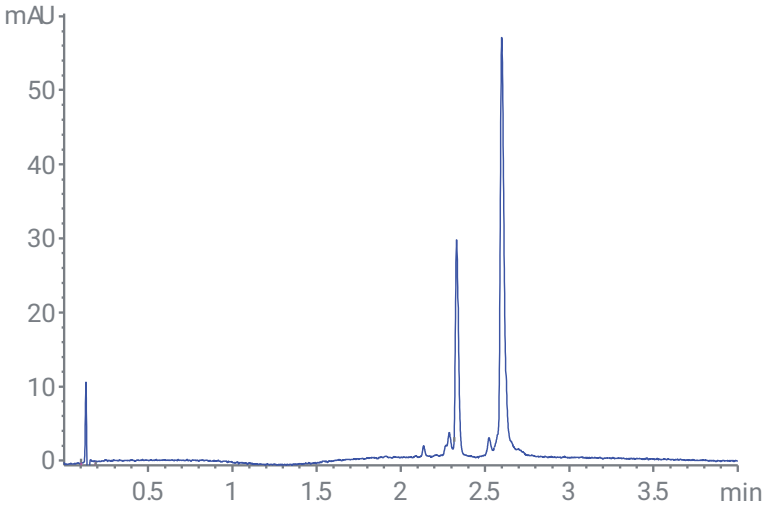
Column dimensions:	2.1 x 100 mm
Mobile phase A:	0.1% TFA in water:IPA (98:2)
Mobile phase B:	IPA:acetonitrile:MPA* (70:20:10)
Flow rate:	1.0 mL/min
Gradient:	10-58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B
Sample:	5 µL injection of Humanized Recombinant Herceptin Variant IgG1 Intact from Creative Biolabs (1 mg/mL)
Temperature:	80 °C
Detection:	UV @ 254 nm

Increase your resolution for fragment analysis

Whether speed, retention, resolution, or a balance of these factors is your goal – AdvanceBio RP-mAb columns are specifically designed to result in an optimized separation for intact and reduced mAbs – whether you are looking at the Fab/Fc regions or heavy and light chain separations.

Method Parameters

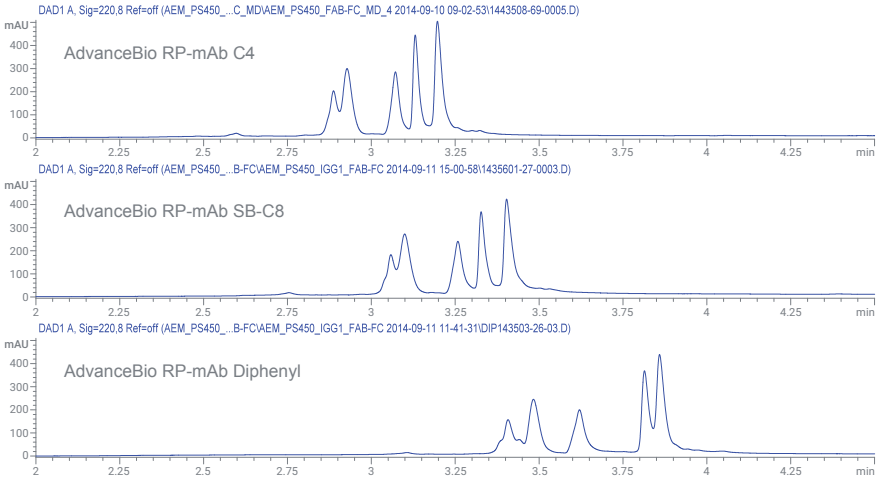
Column:	AdvanceBio RP-mAb Diphenyl, 2.1 x 50 mm, 3.5 µm
Eluent A:	0.1% TFA in water
Eluent B:	IPA:ACN:water (70:20:10) + 0.09 % TFA
Flow rate:	1 mL/min
Gradient:	0 min, 15% B; 0.5 min, 25% B; 1.5 min, 35% B; 1.51 min, 35% B; 3 min, 60% B; 4 min, 60% B
Injection:	1 µL (1 mg/mL) (TECP reduction)
Sample:	Rituximab innovator
Temperature:	80 °C
Detection:	UV, 220 nm



mAb fragment analysis, chemical digestion – heavy chain/light chain.

Method Parameters

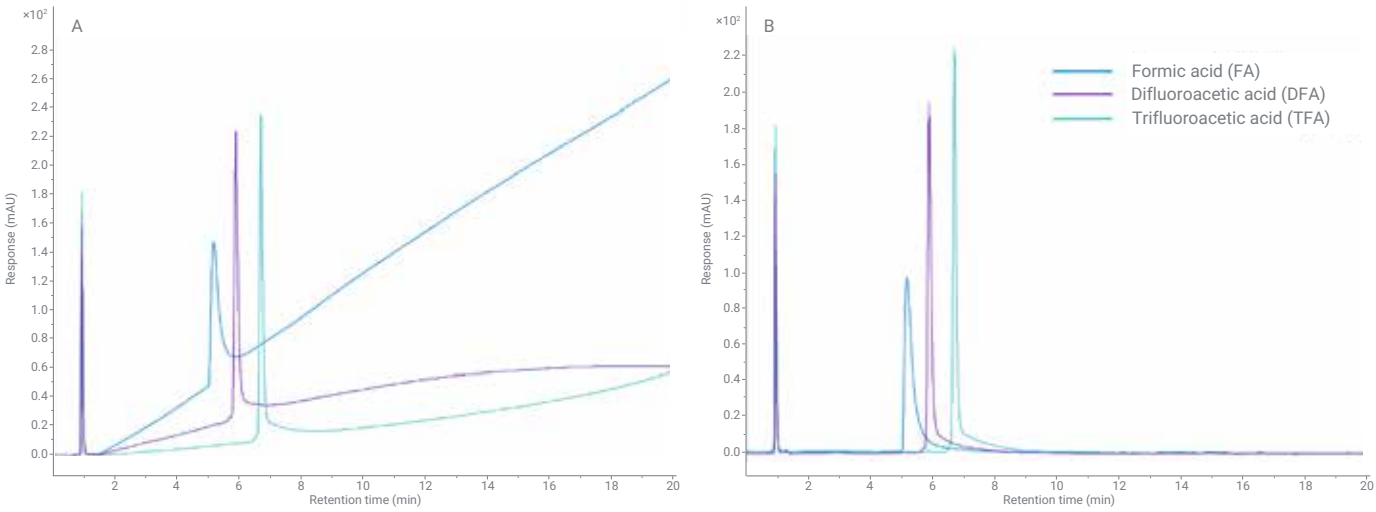
Column dimensions:	2.1 x 100 mm
Mobile phase A:	0.1% TFA in water
Mobile phase B:	n-propanol: acetonitrile: MPA* (80:10:10)
Flow rate:	0.8 mL/min
Gradient:	5-40% B in 5 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B
Sample:	1 µL injection of Fc/Fab, Papain Digested Humanized Recombinant Herceptin Variant IgG1 from Creative Biolabs (2 mg/mL)
Temperature:	60 °C
Detection:	UV @ 220 nm



Fast, high resolution mAb fragment analysis, AdvanceBio RP-mAb Diphenyl provides additional detail through a unique selectivity.

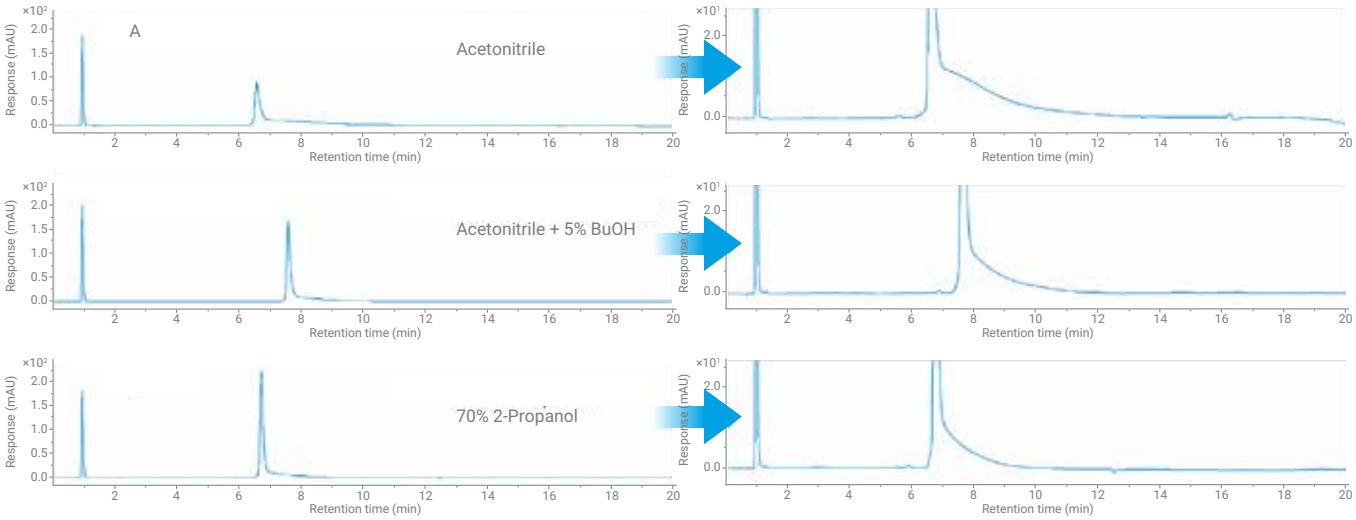
Fast and flexible method development

These particles offer method development flexibility – optimize your method with mobile phase selection, ion pair reagents, high temperature compatibility (up to 80 °C), and detection method. .



A) Chromatograms of an IgG1 (Ramucirumab) showing the effect of different ion pair reagents on reversed-phase. Concentration 0.1% in all cases. All the columns were operated at a constant temperature of 80 °C, flow rate 0.210 mL/min. Detection at 220 nm. The AdvanceBio RP-mAb Diphenyl column show excellent retention and sharper peak than C4 and C8 columns.

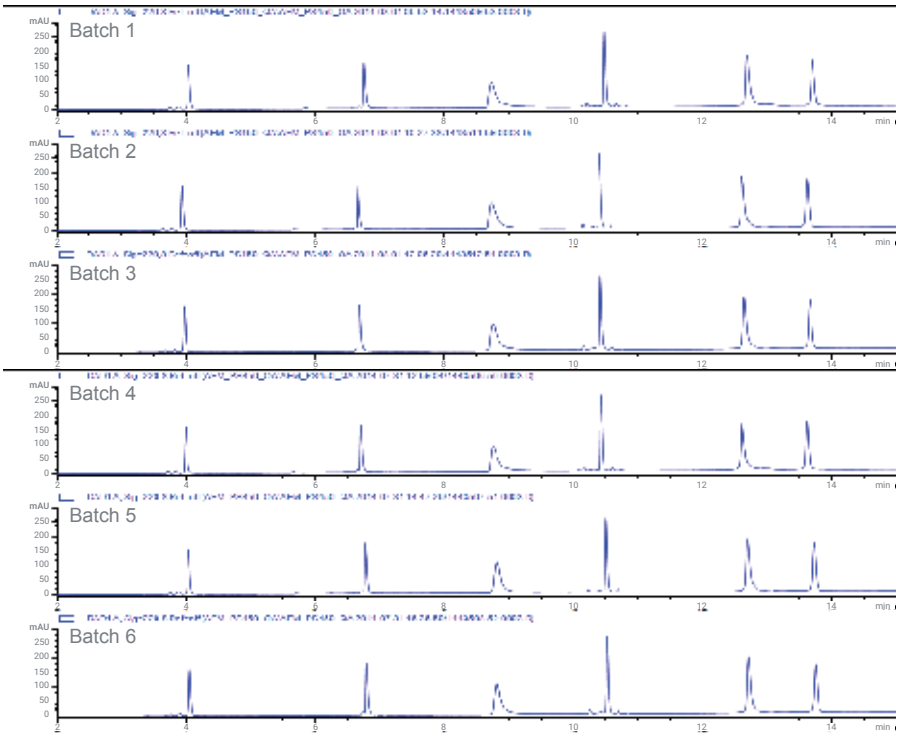
B) Chromatograms showing the baseline subtraction from the blank at 220 nm.



Chromatograms showing the effect of the mobile phase composition of an IgG1 as Ramucirumab on an AdvanceBio RP-mAb Diphenyl column. The addition of organic solvents with higher eluotropic values make an important effect on the mAbs recovery and tailing.

Quality and reproducibility you can count on

Since all Agilent columns, including the AdvanceBio brand, are manufactured under ISO9001 quality certification process, AdvanceBio RP-mAb products deliver the same lot to lot reproducibility and column lifetime that customers have come to expect.



QC results from six batches of RP-mAb C4

Method Parameters

Column:	AdvanceBio RP-mAb C4 2.1 x 100 mm
Mobile phase A:	0.1% TFA in water
Mobile phase B:	0.1% TFA in acetonitrile
Flow rate:	0.5 mL/min
Gradient:	20-50% B in 10 min
Sample:	2 µL injection of protein test standard
Temperature:	60 °C
Detection:	UV @ 220 nm

Sample

Batch 1. Ribonuclease A 13.7 kDa
Batch 2. Cytochrome C 12 kDa
Batch 3. Holo-Transferrin 80 kDa
Batch 4. a-Lactalbumin 14.2 kDa
Batch 5. Catalase 240 kDa
Batch 6. Carbonic Anhydase 29 kDa

Ordering Information

One-click ordering

You can always count on Agilent to support your entire workflow—including sample preparation, columns, supplies, standards, and instruments. To add items to your shopping cart at the Agilent online store, simply click the part number links. Then, enter the quantities for the products you need.

AdvanceBio RP-mAb 3.5 µm columns

Dimensions	C4	SB-C8	Diphenyl
2.1 x 50 mm	799775-904	789775-906	799775-944
2.1 x 75 mm	797775-904	787775-906	797775-944
2.1 x 100 mm	795775-904	785775-906	795775-944
2.1 x 150 mm	793775-904	783775-906	793775-944
4.6 x 50 mm	799975-904	789975-906	799975-944
4.6 x 100 mm	795975-904	785975-906	795975-944
4.6 x 150 mm	793975-904	783975-906	793975-944

InfinityLab LC supplies

A perfect fit for your biomolecule analysis

Agilent InfinityLab supplies are innovative consumables designed to work seamlessly together with Agilent LC instruments and columns for maximum efficiency and performance for your bio HPLC analysis..

Protect your column against particles

Particulates can lead to column clogging, poor chromatographic results and increased downtime. To safeguard against these issues, employing effective filtration techniques is vital. LC filtration assemblies can be used to filter mobile phases, especially when using water-based buffer solutions, to remove residue of undissolved salt crystals and microbes.

Inline filters can be installed to capture any particles in the flow path that come from solvents, samples or worn system parts. InfinityLab Quick Change inline filter offer tool-free replacement of filter discs and “click and seal” feedback to ensure ultimate ease of use.

Perfect connection for your bio application

Within the InfinityLab family, Agilent offers HPLC capillaries in a variety of materials to meet your needs. Capillaries made of MP35N, PEEK-lined stainless steel and titanium are inert and corrosion resistant and are particularly suitable for bio-applications. In combination with InfinityLab Quick Connect fittings, you can create a perfect finger-tight connection up to 1300 bar. For the best analytical performance.

Reduce chemical vapor in the lab

Acetonitrile and methanol are just two of the many toxic compounds you may be exposed to every day. The InfinityLab Stay Safe caps stop solvents from leaching into the air. Combined with the innovative InfinityLab Stay Safe purging bottle the purging of an HPLC with up to four solvent lines becomes a safe task.

Your sample’s journey starts in a vial – make it the right one!

Agilent offers a comprehensive line of vials, caps and inserts; whether a standard borosilicate glass vial will suffice, a surface-deactivated glass vial or polypropylene vial we have the containment solution for you. Beyond vial composition we offer vials in various designs reflecting the nature and sample volume available.

Tips and tools

Download our vials catalog (5994-4803EN) to help guide you in making the final decision on vials, supported by our vial selection tool.

Agilent CrossLab services

CrossLab is an Agilent capability that integrates services and consumables to support workflow success and important outcomes like improved productivity and operational efficiency. Through CrossLab, Agilent strives to provide insight in every interaction to help you achieve your goals. CrossLab offers method optimization, flexible service plans, and training for all skill levels. We have many other products and services to help you manage your instruments and your lab for best performance.

Learn more about Agilent CrossLab, and see examples of insight that leads to great outcomes, at www.agilent.com/crosslab

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