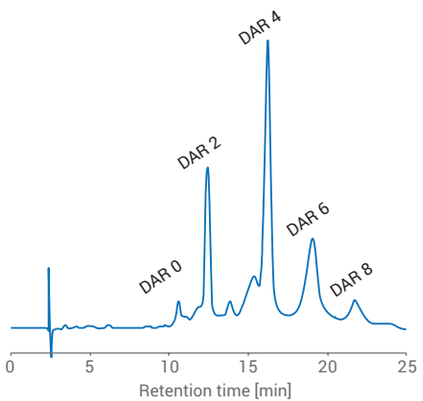
High-resolution separation of ADC, showing DAR profile				
Column	AdvanceBio HIC, 4.6 x 100 mm, 3.5 µm			
Eluent A	2M ammonium sulfate, 50 mM sodium phosphate, pH 7.0			
Eluent B	50 mM sodium phosphate, pH 7.0			
Eluent C	propan-2-ol			
Gradient	Time (min) 0 20 25 30 40	% A 50 0 0 50 50	% B 45 75 75 45 45	% C 5 25 25 5 5
Temperature	30 °C			
Flow rate	0.5 mL/min			
Injection volume	5 µL			
Detection	UV, 220 nm			

After all samples have been completed, flush the column with eluent A.



These results were generated on an Agilent 1260 Infinity II Bio-inert LC system. Results on other instruments may vary.

Operating parameters	
Mobile-phase compatibility	AdvanceBio HIC columns are compatible with all commonly used mobile phase conditions for HIC. Ammonium sulfate, in the presence of sodium phosphate buffer, is recommended. *Use of organic modifiers such as isopropanol is possible, but care should be taken to avoid column damage due to increased eluent viscosity, solvent immiscibility, or salt precipitation.* ***Salt precipitation is likely if methanol is introduced.***
pH stability	2.0 to 8.0; column lifetime is likely to be affected outside of this range. Note: HIC is typically performed at near neutral pH (pH 7.0).
Operating temperature	25-30 °C (recommended), 60 °C (maximum)
Maximum pressure	400 bar (5,000 psi) Typical Operating pressure below 200 bar at 1 mL/min
Flow rate	Typical operating flow rates are 0.5 to 1.0 mL/min for columns with an internal diameter of 4.6 mm

Working at extremes of the operating parameters is likely to reduce column lifetime.

Column care

An increase in backpressure and decrease in performance may occur over time. If the pressure has increased, first identify if this is due to the LC instrument or to the column. If the increase in pressure is caused by a system component, such as tubing or a filter, replace and retest.

Column cleaning instructions

Understanding the likely source of contamination is important to determine the type of cleaning solution that may be required. Often, it is sufficient to simply flush the column sequentially with water, isopropanol (propan-2-ol), and then water. Washing should be performed at a reduced flow rate with a minimum of 20 column volumes per solvent. Flushing the column with water containing a chaotropic salt (to disrupt protein structure) may be beneficial, but should be followed with a water wash.

Storage recommendations

Never store your AdvanceBio HIC column with a high salt-containing mobile phase used for gradient elution. For overnight storage, it is advisable to flush your column with low salt-containing buffer. **For long-term storage, it is essential to flush the salt containing mobile phase from the column and to transfer the column into 100% acetonitrile.** Take particular care to ensure there is no possibility of salt precipitation when re-installing the column by flushing out the acetonitrile with water or low buffer concentration mobile phase first. In the case of organic modifiers, monitor backpressure due to increased viscosity, and also ensure that steps are taken to avoid salt precipitation.

Ordering details

Description	Part Number
AdvanceBio HIC column 450 Å, 4.6 x 30 mm, 3.5 µm	681975-908
AdvanceBio HIC column 450 Å, 2.1 x 100 mm, 3.5 µm	685975-908

Standards

Regularly monitoring the performance of your AdvanceBio HIC column using standards is highly recommended. Since the results of column efficiency testing (plate counts, tailing factors, and resolution factors) can be affected by instrumentation, operating conditions, and other external factors, it is important to consider this.

www.agilent.com/chem/advancebio

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Agilent AdvanceBio HIC column

User guide

Application-specific columns for hydrophobic interaction chromatography (HIC)

Built using the capabilities of the ZORBAX fully porous particles and proprietary bonding technology, the 450 Å pore size, 3.5 µm AdvanceBio HIC columns provide new levels of hydrophobicity and resolution.

Getting started

A column performance report, including a column-specific QC test chromatogram and a batch-specific protein separation, is enclosed with every Agilent column. The QC test system has been modified from a standard system to minimize system dead volume, so it may vary from the system used in your lab. This enables a better evaluation of the column efficiency and ensures a more consistent product. An optimized LC system will generate similar results to the chromatogram on your QC performance report.

If you have specific questions, contact Technical Support at **www.agilent.com/chem/techsupport**.

Mobile phase selection and operating temperature

Shipping solvents and compatibility: AdvanceBio HIC columns are shipped containing **100 % acetonitrile**. They should be flushed before use (see **Column conditioning**). Columns are compatible with buffers and salts commonly used for HIC.

Recommended starting gradient: Gradients should typically last from 10 to 20 column volumes for good resolution. Flushing and re-equilibration should be for at least 5 column volumes. For monoclonal antibodies, the initial ammonium sulfate concentration should be around 1.5 M or less (plus 20 to 100 mM sodium phosphate buffer). The gradient should run to 0 M ammonium sulfate (plus 20 to 100 mM sodium phosphate buffer).

Please see the "Quick Start Guide" (pub no. 5991-9514EN) at **www.agilent.com/chem/advancebio-hic-quickstart**, for further information.

Important safety considerations

- All points of connection in an LC system are potential sources of leaks. Users of liquid chromatography instruments should be aware of the potential toxicity or flammability of their mobile phases.
- Do not remove the column end fittings.
- Please adhere to operating pressure limits noted for each column. Exceeding these limits will compromise chromatographic performance and column lifetime, and could be unsafe.

Using your column

Installation

Before installing your column, ensure that your LC system is flushed thoroughly with water to alleviate any risk of precipitation from a high salt-containing mobile phase. Turn off the pump before connecting the column.

The direction of flow is marked on the column. Remove both end plugs and ensure that the system flow direction matches the arrow on the column. Do not use the column with the flow in the reverse direction.

Use of Agilent InfinityLab Quick Connect fittings (part number 5067-5957) or Quick Turn fittings (part number 5067-5966) produces simple and reliable connections to your HPLC or UHPLC instrument. No tools are needed to make leak-free connections that fit the column perfectly.

- Start the pump at a very low flow rate. Slowly increase the flow rate while monitoring the pressure. Ideally, set the pump ramp rate to the lowest possible value (for example 0.1 mL/min). In addition, set the pressure limit to a level that will protect the column from damage should a blockage occur (for example 200 bar).

Column conditioning

Every column is tested before shipment. Therefore, for first use, the shipping solvent must be replaced with eluent, taking care that all components are miscible and soluble. An intermediate flush with water before introducing a mobile phase containing buffer and low salt concentration is recommended. Finally, you should switch to the mobile phase containing buffer and high salt concentration.

Flushing with 10 to 20 column volumes should help in transitioning to your mobile phase. Care should be taken to make sure that the column has been properly equilibrated before use and the backpressure and UV baseline have stabilized.

- When changing eluents, always take the viscosity and risk of salt precipitation into account. If you are unsure, use a reduced flow rate and flush the column first with high-purity water before introducing a new eluent.
- If exposed to methanol, ammonium sulfate present in the mobile phase will precipitate, so steps should be taken to ensure that methanol is not present in the LC system.

Instructions for use

Mix your buffers freshly using high-purity components and ultrahigh purity water such as Milli-Q or Nanopure. Filter buffers through a 0.2 or 0.45 µm filter and degas before use. This will remove particulates and help reduce the risk of bacterial growth, which could otherwise damage the column and your UHPLC or HPLC system.

- It is important to ensure that your sample is fully dissolved and does not precipitate in the high salt concentration mobile phase. Protein concentrations of around 1 mg/mL will give excellent results.

For best peak shape, prepare samples in conditions as close to the initial mobile phase conditions as possible, including matching pH

High-resolution separation of mAb sample

Column	AdvanceBio HIC, 4.6 x 100 mm, 3.5 µm		
Eluent A	2M ammonium sulfate, 50 mM sodium phosphate, pH 7.0		
Eluent B	50 mM sodium phosphate, pH 7.0		
Gradient program	Time (min)	% A	% B
	0	50	50
	20	0	100
	25	0	100
	30	50	50
	40	50	50
Temperature	30 °C		
Flow rate	0.5 mL/min		
Injection volume	5 µL		
Detection	UV, 220 nm