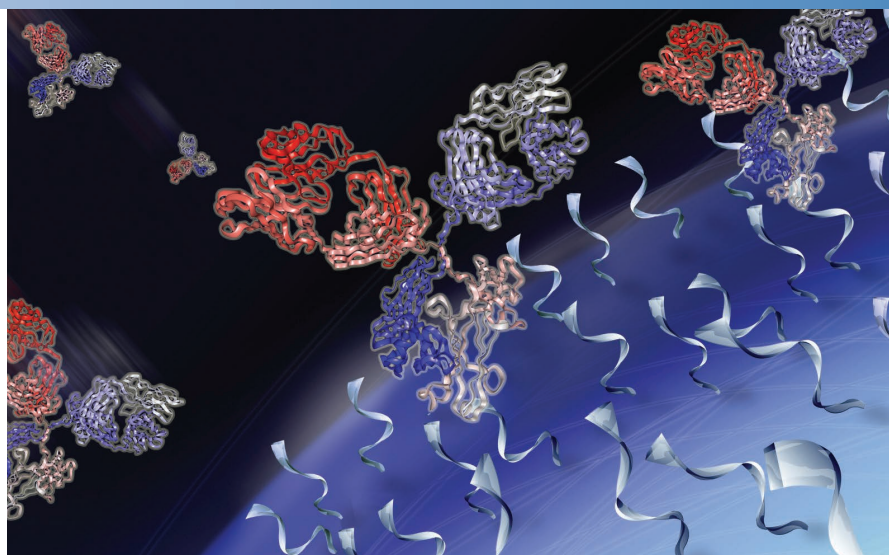
MAbPac HIC-10 Column

High Resolution HIC Column for Monoclonal Antibody Analysis

The Thermo Scientific™ MAbPac™ HIC-10 column is a high resolution silica column designed for separation of monoclonal antibodies (mAbs) and related biologics by hydrophobic interaction. The unique chemistry provides high resolution, excellent bio-compatibility, and selectivity that is different from other HIC columns on the market.

Product Highlights

- Unique chemistry designed for optimal selectivity for mAbs
- Excellent bio-compatibility and extremely low carryover
- High resolution and high efficiency
- Compatible with both organic solvent and aqueous mobile phase
- Rugged column stability



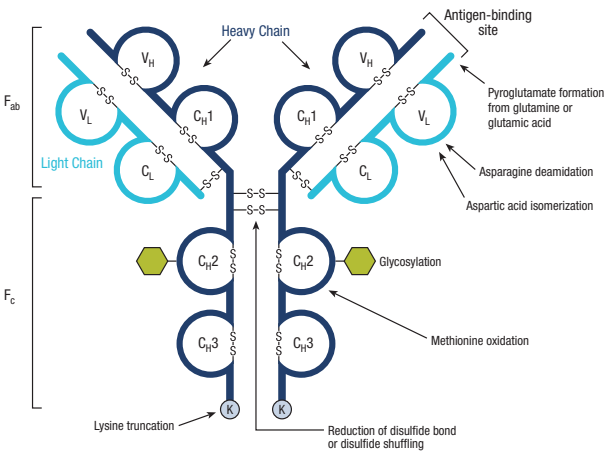


Figure 1: Structure of IgG and typical forms of heterogeneity

Introduction

Monoclonal antibodies are the most successful and prominent class of biotherapeutics, currently accounting for approximately one third of total biologics sales. With high specificity and excellent biocompatibility, they are expected to rise at the fastest rate within the total biologics market. Monoclonal antibodies have demonstrated effectiveness against autoimmune disorders, cardiovascular diseases, infectious diseases, and cancer. The proliferation of monoclonal antibody therapeutics and their susceptibility to various biochemical modifications has increased the emphasis on characterization of these highly heterogeneous products for their safety and efficacy. Monoclonal antibodies are highly heterogeneous due to various degradation mechanisms including oxidation, reduction, deamidation, isomerization, and lysine truncation (Figure 1).

Hydrophobic Interaction Chromatography (HIC) is a technique for separation of proteins including monoclonal antibodies by hydrophobic interaction. The HIC mobile phase usually consists of a salting-out agent, which at high concentration retains the protein by increasing hydrophobic interaction between the protein and the stationary phase. Bound proteins are eluted by decreasing the salt concentration. HIC has been used as an orthogonal method to size exclusion chromatography (SEC) and ion exchange chromatography for the characterization of mAb heterogeneity. Analysis of succinimides, antibody fragments, oxidated mAbs, C-terminal lysine modification, and drug-conjugated mAbs were successfully carried out using HIC to monitor the stability and in some cases the potency of the drug.

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Column Technology

The MAbPac HIC-10 column is based on high-purity, spherical, wide-pore (1,000 Å), 5 µm silica particles functionalized with proprietary alkyl amide groups. The advanced surface bonding technology leads to excellent chemical stability, high efficiency, and unique selectivity optimized for mAbs and different from other HIC phases.

Applications

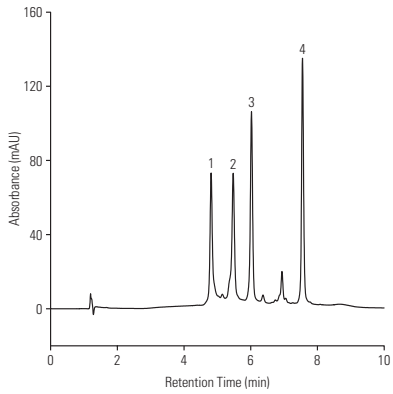
High Resolution and Fast Separation of Proteins

The MAbPac HIC-10 provides high-efficiency, high-resolution protein separation within 10 minutes, as shown in Figure 2. Such high resolution allows for integration and high-quality fraction collection. The ultra-high resolution can be achieved on a 4.6 × 250 mm column (Figure 3).

Separation of Antibody-Drug Conjugates

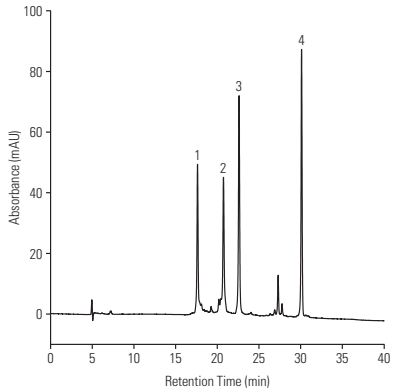
Antibody drug conjugates (ADCs) are a rapidly growing class of protein therapeutics that target cancer cells. ADCs utilize the exquisite selectivity of the antibody to achieve targeted delivery of cytotoxic drugs. An ADC molecule is often highly heterogeneous with respect to both the distribution and 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. Unconjugated mAb has significantly lower potency while the ADCs with high drug load are subject to rapid renal clearance. Therefore, it is critical to fully characterize and monitor the heterogeneity of ADCs during development and production.

Hydrophobic interaction chromatography is often used for the separation of ADCs since attachment of cytotoxin alters the hydrophobicity of the antibody. The least hydrophobic unconjugated antibody elutes first and as the number of attached drugs increases the elution time of each ADC increases as well. Therefore HIC is often used to characterize the distribution of ADC molecules with different drug-to-antibody ratios (DARs). Figure 4b shows the separation of a cysteine-conjugated ADC mimic sample on the MAbPac HIC-10 column. The ADC mimics were conjugates between a drug mimic and mAb via the sulfhydryl group of interchain cysteine residues which results in a mixture of drug-loaded antibody species with 0 to 8 drugs (Figure 4a). The unmodified mAb and ADCs with DAR values ranging from 2 to 8 are well resolved by the MAbPac HIC-10 column.



Column:	**MAbPac HIC-10**, 5 µm		
Format:	4.6 × 100 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
	-5.0	100	0
	0.0	100	0
	1.0	100	0
	7.0	0	100
	10.0	0	100
Flow Rate:	1.0 mL/min		
Inj. Volume:	10 µL		
Temp.:	30 °C		
Detection:	UV (280 nm)		
Sample:	Protein mixture		
Peaks:	1. Myoglobin 2. Ribonuclease A 3. Lysozyme 4. α-Chymotrypsinogen A		

Figure 2: Fast separation of standard protein mixture



Column:	**MAbPac HIC-10**, 5 µm		
Dimension:	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
	-10.0	100	0
	0.0	100	0
	2.0	100	0
	30.0	0	100
	40.0	0	100
Flow Rate:	0.6 mL/min		
Temp.:	30 °C		
Inj. Volume:	10 µL		
Detection:	UV (280 nm)		
Sample:	Protein Standard		
Peaks:	1. Myoglobin 2. Ribonuclease A 3. Lysozyme 4. α-Chymotrypsinogen A		

Figure 3: Separation of standard protein mixture

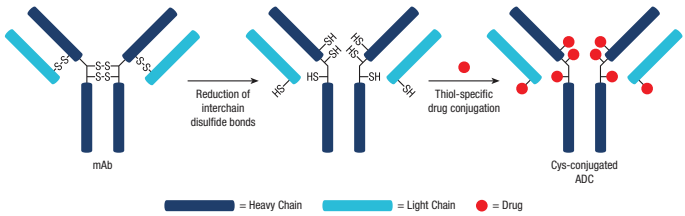
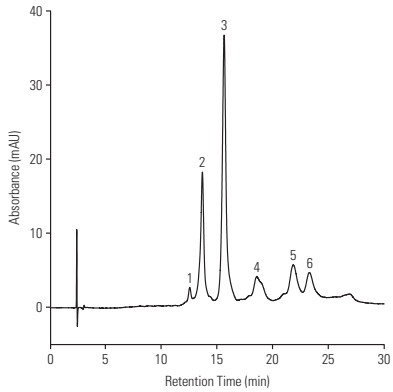


Figure 4a: Schematic representation of conjugation of drug mimic via interchain cysteine residues



Column:	**MAbPac HIC-10**, 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
	30.0	0	100
	35.0	0	100
Flow Rate:	0.5 mL/min		
Inj. Volume:	5 µL (5 mg/mL)		
Temp.:	25 °C		
Detection:	UV (280 nm)		
Sample:	Cys-conjugated ADC mimic		
Peaks:	1. Unconjugated mAb 2. DAR 2 3. DAR 4 4. DAR 6 5. DAR 6 6. DAR 8		

Figure 4b: Separation of Cys-conjugated ADC mimic

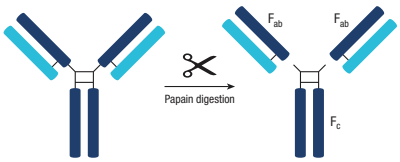
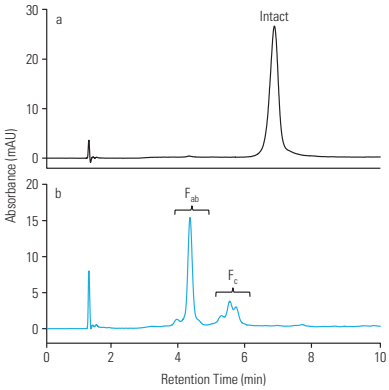
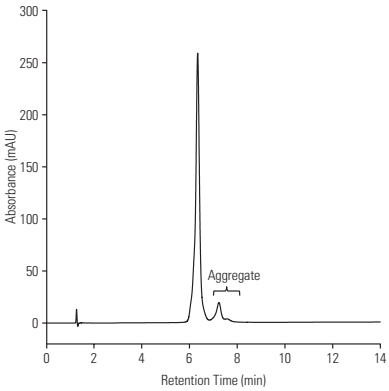


Figure 5a: Schematic representation of Papain digestion of monoclonal antibody



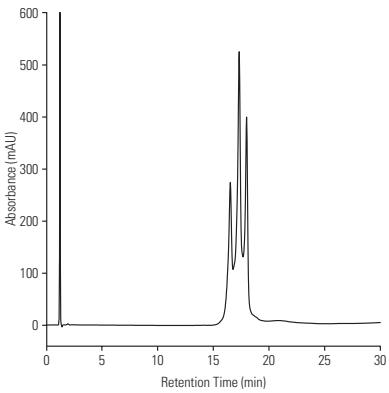
Column:	**MAbPac HIC-10**, 5 µm		
Format:	4.6 × 100 mm		
Mobile phase A:	1.5 M ammonium sulfate, 50 mM sodium phosphate, pH 7.0 / isopropanol (95:5 v/v)		
Mobile phase B:	50 mM sodium phosphate, pH 7.0 / isopropanol (80:20 v/v)		
Gradient:	Time (min)	%A	%B
	-5.0	100	0
	0.0	100	0
	1.0	100	0
	15.0	0	100
	20.0	0	100
Flow Rate:	1.0 mL/min		
Inj. Volume:	Papain digest:	10 µL 1 mg/mL)	
	Intact mAb:	2 µL (5 mg/mL)	
Temp.:	25 ºC		
Detection:	UV (280 nm)		
Sample:	a. Intact mAb b. Papain digest		

Figure 5b: Separation of papain digested monoclonal antibody



Column:	**MAbPac HIC-10**, 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 (8 mg/mL)		
Temp.:	25 ºC		
Detection:	UV (280 nm)		
Sample:	Monoclonal antibody		

Figure 6: Separation of monoclonal antibody aggregate



Column:	**MAbPac HIC-10**, 5 µm		
Format:	4.6 × 100 mm		
Mobile phase A:	2.0 M ammonium sulfate, 100 mM sodium phosphate, pH 7.0 / isopropanol (93:7 v/v)		
Mobile phase B:	100 mM sodium phosphate, pH 7.0 / isopropanol (93:7 v/v)		
Gradient:	Time (min)	%A	%B
	-5.0	95	5
	0.0	95	5
	30.0	0	100
	40.0	0	100
Flow Rate:	1.0 mL/min		
Inj. Volume:	10 µL (1 mg/mL)		
Temp.:	30 ºC		
Detection:	UV (214 nm)		
Sample:	Fusion protein		

Figure 7: Separation of fusion protein

Analysis of mAb Fragments

Analysis of antibody fragments is important for both characterization of F_{ab} or F_c (Figure 5a) based biotherapeutics and localization of the sources of heterogeneities on a monoclonal antibody molecule. HIC can provide the resolution required for the separation of F_{ab} and F_c fragments and their hydrophilic or hydrophobic variants. Figure 5b shows a comparison of an intact mAb and its papain digest on MAbPac HIC-10. The MAbPac HIC-10 column efficiently separates F_{ab} and F_c fragments and further separates variants of these fragments. These variant peaks imply oxidation or other modifications in these fragments.

Separation of mAb Aggregates

Protein and antibody aggregates are formed either during product expression in cell culture, downstream processing or storage. These aggregates may cause undesirable immune reactions which affect the safety of the drug. SEC is the most widely used technique for detection and quantification of protein aggregates in biological drug products. However several researchers have reported the use of HIC for removal of protein aggregates. Figure 6 demonstrates the separation of monoclonal antibody aggregates from the non-aggregated form on the MAbPac HIC-10 column. Unlike SEC aggregates typically elute later than the main peak on an HIC column due to increased hydrophobicity.

Separation of Fusion Proteins

Fusion proteins are proteins created through the joining of two or more genes that originally form separate proteins. Recombinant fusion proteins are commonly used in biological research and therapeutics. For example, GFP proteins have been fused to numerous proteins as a biological tool to localize and monitor the protein of interest in cells. Among fusion protein therapeutics, F_c fusion proteins are the most promising with already six approved F_c fusion drugs on the market. Researchers have attached F_c region of a monoclonal antibody onto a therapeutic protein to increase its stability and deliverability. Figure 7 shows the separation of a fusion protein containing three fractions: the fusion protein, the fusion protein with loss of several amino acid residues, and the fusion protein with some tertiary structure distortion. All three components can be successfully separated on the MAbPac HIC-10 column while other HIC phases fail to resolve them.

Physical Data

Product Name	MAbPac HIC-10
Column Chemistry	Proprietary alkyl amide
Substrate	Spherical, high purity, wide-pore (1,000 Å)
Particle Size	5 µm
Surface Area	20 m^2/g
Pore Size	1,000 Å

Specifications and Operational Parameters

Dimension (mm)	Recommended Flow Rate (mL/min)	Maximum Flow Rate (mL/min)	Maximum Pressure (psi)	Temperature Limit (°C)	pH Range	Solvent Compatibility
4.6 × 100 mm	0.4–1.0	1.5	6,000	60	2.0–8.0	Compatible with up to 100% organic solvent
4.6 × 250 mm	0.4–1.0	1.5	8,000	60	2.0–8.0	
4.6 × 10 mm	0.4–1.0	2.0	6,000	60	2.0–8.0	

Ordering Information

Description	Particle Size	Part Number
MAbPac HIC-10, Analytical 4.6 × 100 mm	5 µm	088480
MAbPac HIC-10, Analytical 4.6 × 250 mm	5 µm	088481
MAbPac HIC-10, Guard Cartridges 4.6 × 10 mm (2/pk)	5 µm	088482
Guard Cartridge Holder		069580

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