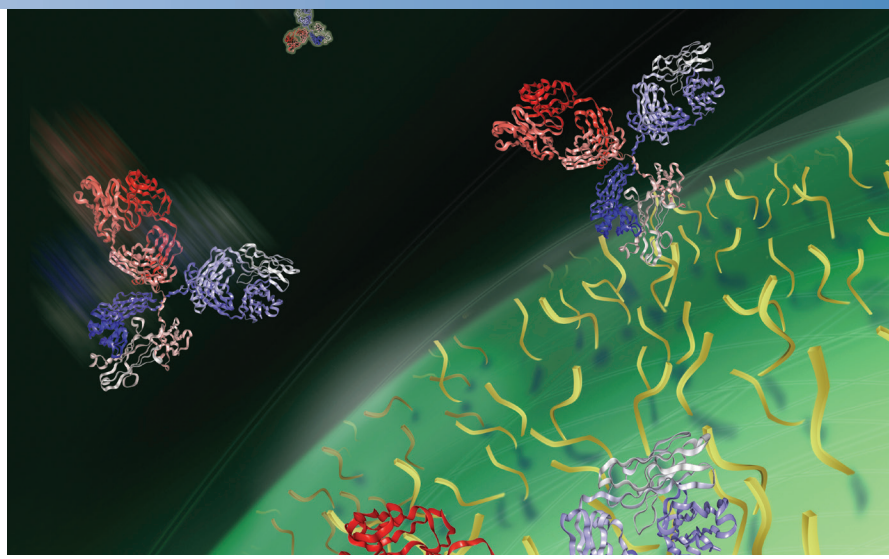
MAbPac HIC-20 Column

High-resolution HIC column for monoclonal antibody analysis

The Thermo Scientific™ MAbPac™ HIC-20 column is a high-resolution silica-based HIC column designed for the separation of mAbs and mAb variants. Its unique, proprietary column chemistry provides high resolution, rugged stability, and desired selectivity for the analysis of mAbs and related variants.

Product Highlights

- Optimal selectivity for mAbs and related variants
- Excellent separation for mAb fragments and oxidized variants
- High resolution and high efficiency
- Compatible with both organic solvent and aqueous mobile phase
- Rugged column stability



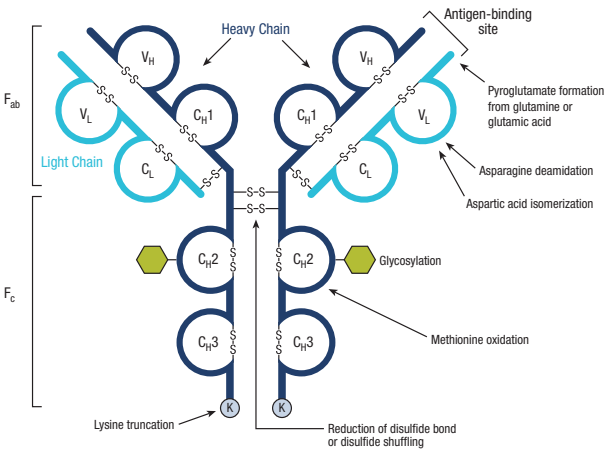


Figure 1: Structure of IgG and typical forms of heterogeneity

Introduction

Monoclonal antibodies (mAbs) have proved to be one of the most successful classes of biotherapeutics. The FDA has approved an increasing number of mAbs for clinical uses against cancer, autoimmune disorder, Crohn's disease and rheumatoid arthritis among other diseases. Recombinant mAbs are subject to a variety of biochemical modifications during processing, delivery and storage (Figure 1). Due to the potential impact of these modifications on mAb safety and efficacy, thorough characterization of mAb products has become increasingly important.

Hydrophobic interaction liquid chromatography (HIC) separates proteins and mAb molecules in order of increasing hydrophobicity. Analytes bind to the weakly hydrophobic stationary phase in the presence of high salt concentration and elute off the column as the salt concentration decreases. In contrast to reverse phase liquid chromatography, HIC typically preserves the biological activity of the protein, which is useful for downstream functional analysis such as binding and cell-based potency assays. In addition, HIC typically provides separation with little carryover. Due to these benefits, HIC is not only used for analysis of mAb variants but also has been widely used as a purification method for mAb products.

Column Technology

The MAbPac HIC-20 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 resolution, and unique selectivity optimized for mAbs and different from other HIC phases

Applications

MAbPac HIC-20 is designed for the separation of proteins including mAbs. This column is especially superior for the separation of mAb fragments and oxidized mAb variants.

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Separation of Proteins and mAbs

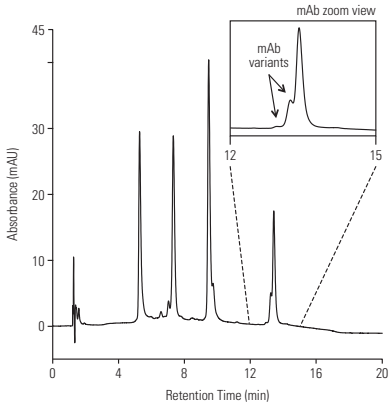
The MAbPac HIC-20 provides high resolution separation of proteins and a mAb in 20 minutes (Figure 2). The unique chemistry of the column allows the analysis of hydrophilic proteins such as myoblogin and ribonuclease A as well as more hydrophobic mAbs. Both smaller proteins and mAbs show excellent peak shape and peak width which demonstrates its biocompatibility for small to large proteins. In addition, MAbPac HIC-20 is able to separate minor variants of the mAb sample shown in Figure 2 inset. Further separation of these variants may be achieved by optimizing the mobile phase and gradient condition.

Separation of mAb Fragments

Analysis of Fab and Fc subdomains resulting from papain digestion is a common procedure to obtain further information about the mAb sample (Figure 3a). Papain fragmentation often provides resolution of mAb variants that are not clearly detected when analyzing the intact mAb. In addition, one can easily tell whether the source of the variation is on the Fab domain or the Fc domain. HIC can provide the resolution required for the separation of Fab and Fc fragments and related variants. Figure 3b shows a comparison of an intact mAb and its papain digest on MAbPac HIC-20. The MAbPac HIC-20 column efficiently separates Fab and Fc fragments and further separates variants of these fragments.

Separation of Oxidized mAb Variants

Oxidation of therapeutic mAbs during production or storage is a common degradation mechanism and has become a major concern in mAb production. In many cases, oxidized mAbs have less to no potency compared to its native form. Therefore analysis of such degradation is critical to ensure the therapeutic efficacy of the mAb products. Oxidation of amino acid residues on a mAb can alter the hydrophobic nature of the mAb either by the increase in polarity of the oxidized form or due to resulting conformational change. Hydrophobicity-based HPLC methods such as reverse phase chromatography and HIC are typically used to characterize oxidized mAb products. The MAbPac HIC-20 provides superior resolution of oxidized mAb variants from unmodified mAb. As shown in Figure 4, the MAbPac HIC-20 can resolve oxidized mAb variants without fragmentation or other sample preparation.



Column:	**MAbPac HIC-20**, 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
	15.0	0	100
	20.0	0	100
Flow Rate:	1.0 mL/min		
Inj. Volume:	10 µL		
Temp.:	30 °C		
Detection:	UV (280 nm)		
Sample:	Proteins + mAb		
Peaks:	1. Myoglobin 2. Ribonuclease A 3. Lysozyme 4. mAb		

Figure 2: Separation of a mixture of proteins and a mAb

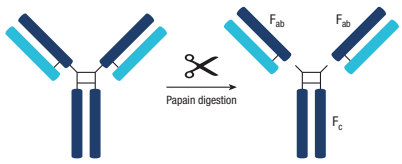
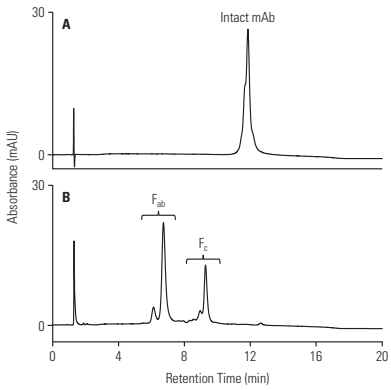
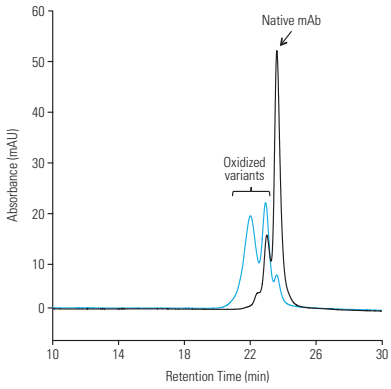


Figure 3a: Schematic representation of papain digestion of monoclonal antibody



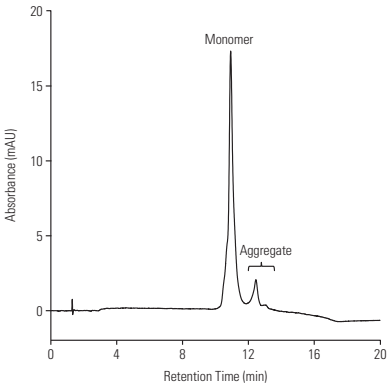
Column:	**MAbPac HIC-20**, 5 µm		
Format:	4.6 ×100 mm		
Mobile phase A:	2 M ammonium sulfate, 100 mM sodium phosphate, pH 7.0		
Mobile phase B:	100 mM sodium phosphate, pH 7.0		
Gradient:	Time (min)	%A	%B
	-5.0	60	40
	0.0	60	40
	1.0	60	40
	15.0	0	100
	20.0	0	100
Flow Rate:	1.0 mL/min		
Inj. Volume:	Intact mAb:	12 µL (1 mg/mL)	
	Papain digest:	12 µL (1 mg/mL)	
Temp.:	30 °C		
Detection:	UV (280 nm)		
Sample:	A. Intact mAb B. Papain digest		

Figure 3b: Separation of (A) intact mAb and (B) papain digested mAb



Column:	**MAbPac HIC-20**, 5 µm		
Format:	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
	-6.0	50	50
	0.0	50	50
	2.0	50	50
	30.0	0	100
	35.0	0	100
Flow Rate:	0.5 mL/min		
Inj. Volume:	Untreated mAb:	20 µL (1.25 mg/mL)	
	Oxidized mAb:	20 µL (1.25 mg/mL)	
Temp.:	30 °C		
Detection:	UV (280 nm)		
Sample:	Untreated mAb H_2O_2 oxidized mAb		

Figure 4: Comparison of untreated mAb and H_2O_2 oxidized mAb



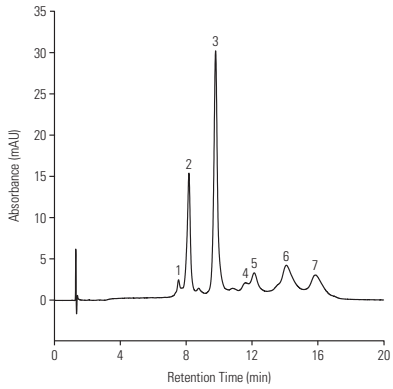
Column: **MAbPac HIC-20**, 5 µm
Format: 4.6 ×100 mm
Mobile phase A: 2.0 M ammonium sulfate, 100 mM sodium phosphate, pH 7.0
Mobile phase B: 100 mM sodium phosphate, pH 7.0
Gradient:

Time (min)	%A	%B
-5.0	60	40
0.0	60	40
1.0	60	40
15.0	0	100
20.0	0	100

Flow Rate: 1.0 mL/min
Inj. Volume: 2 µL (4 mg/mL)
Temp.: 30 °C
Detection: UV (280 nm)

Sample: mAb

Figure 5: Separation of mAb aggregates



Column: **MAbPac HIC-20**, 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 (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 6
7. DAR 8

Figure 6a: Separation of Cys-conjugated ADC mimic

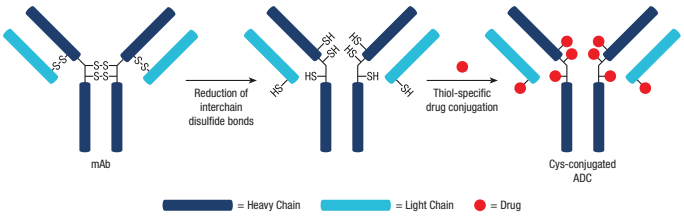


Figure 6b: Schematic representation of conjugation of drug mimic via interchain cysteine residues

Separation of mAb Aggregates

Aggregation is a common issue during manufacturing processes of therapeutic mAbs. Aggregation of mAbs compromises the safety, and efficacy of mAb products. Thus effective detection and removal of mAb aggregates is critical for the overall drug quality. SEC has been the most widely used HPLC method for detection and quantification of aggregation in mAb samples. However several studies have also shown the use of cation exchange chromatography and HIC as alternative techniques for the separation of mAb aggregates from the native mAb.
Figure 5 demonstrates the separation of mAb aggregates from the monomer on the MAbPac HIC-20 column. In HIC, aggregates typically elute later than the main peak due to the increased hydrophobicity.

Separation of Antibody-Drug Conjugates

Antibody drug conjugates (ADCs) are a highly potent class of biopharmaceuticals that selectively target cancer cells. An ADC molecule consists of a mAb that specifically recognizes a tumor marker and a cytotoxic drug that is released once internalized into the target tumor cell. The conjugation of a toxic payload to the antibody often results in an ADC molecule that is 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. Therefore, it is essential to fully characterize and monitor the heterogeneity of ADCs during development and production.

Since anticancer drugs are typically hydrophobic, the number of drugs attached to a mAb molecule correlates to the overall hydrophobicity of the ADC. Therefore, HIC is the most effective technique for the separation of ADCs based on their drug-to-antibody ratio (DARs). Figure 6a shows the separation of a cysteine-conjugated ADC mimic sample on the MAbPac HIC-20 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 6b). The unmodified mAb and ADCs with DAR values ranging from 2 to 8 are well resolved by the MAbPac HIC-20 column.

Reproducible Manufacturing

Each MAbPac HIC-20 column is manufactured to strict specifications to ensure column-to-column reproducibility. Each column is individually tested and shipped with a qualification assurance report.

Physical Data

Product Name	MAbPac HIC-20
Column Chemistry	Proprietary alkyl amide
Substrate	Spherical, high purity silica particles
Particle size	5 µm
Pore size	1,000 Å

Operational Specifications

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	0.5–1.0	1.5	6,000	60	2.0–9.0	Compatible with up to 100% organic solvent
4.6 × 250 mm	0.5–1.0	1.5	8,000	60	2.0–9.0	
4.6 × 10 mm	0.5–1.0	2.0	6,000	60	2.0–9.0	

Ordering Information

Description	Particle Size	Part Number
MAbPac HIC-20, Analytical 4.6 × 100 mm	5 µm	088553
MAbPac HIC-20, Analytical 4.6 × 250 mm	5 µm	088554
MAbPac HIC-20, Guard Cartridges 4.6 × 10 mm (2/pk)	5 µm	088555
Guard Cartridge Holder		069580

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