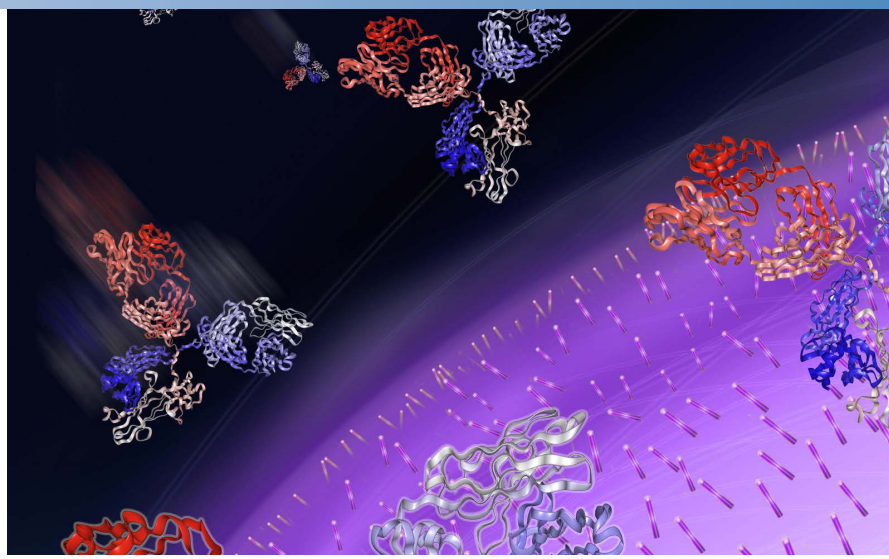
MAbPac HIC-Butyl Column

High resolution HIC column for monoclonal antibody analysis

The Thermo Scientific™ MAbPac™ HIC-Butyl column is a high-resolution polymer-based hydrophobic interaction chromatography (HIC) column designed for the separation of mAbs and antibody-drug conjugates (ADCs). The hydrophilic resin and optimal density of the butyl groups lead to excellent bio-compatibility and low carryover. In addition, MAbPac HIC-Butyl column provides complementary selectivity to MAbPac HIC-10 and MAbPac HIC-20 columns.

Product Highlights

- Optimal selectivity for ADCs
- High resolution and high efficiency
- Stable at wide range of pH (2–12)
- Low carryover
- Rugged column packing



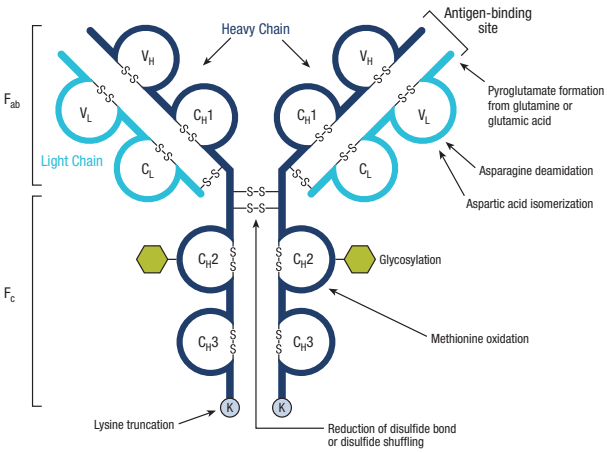


Figure 1: Structure of IgG and typical forms of heterogeneity

Introduction

Monoclonal antibodies (mAbs) have proven to be an excellent paradigm for highly selective and biocompatible drugs. Various types of mAb products including intact mAbs, mAb fragments, engineered variants, and ADCs are being developed for the treatment of cancer, infectious disease and inflammatory disease. MAbs are prone to degradation and biochemical modifications during production and storage, which may reduce stability and potency of the product The complexity of antibodies is illustrated in Figure 1. Therefore, thorough characterization of mAb purity, biochemical modifications, and mAb aggregation is critical to ensure the safety and efficacy of mAb based pharmaceuticals.

HIC is widely used as an orthogonal method to cation exchange chromatography (CEX) and size exclusion chromatography (SEC) for the characterization of mAbs. HIC separates proteins based on the hydrophobic interaction with the stationary phase. Proteins bind to the weakly hydrophobic stationary phase in the presence of high salt concentration and elute off the column as the concentration of salt decreases. In contrast to reverse phase liquid chromatography, HIC typically runs under non-denatured condition that preserves the biological activity of the protein, which is useful for downstream functional analysis such as binding and cell-based potency assays.

Column Technology

The MAbPac HIC-Butyl column is based on hydrophilic, non-porous, 5 µm polymer particles functionalized with butyl groups. The hydrophilic resin provides biocompatibility and low carryover while the optimal density of the butyl groups provides high resolution separation.

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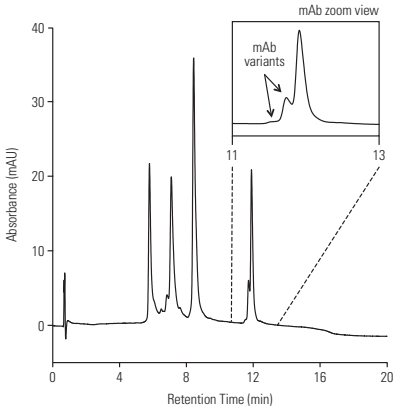
Applications

Separation of Proteins and mAbs

The MAbPac HIC-Butyl provides high resolution separation of proteins and a mAb in 20 minutes as shown in Figure 2. Both smaller proteins and the mAb show excellent peak shape and peak width which demonstrates its biocompatibility for small to large proteins. In addition, MAbPac HIC-Butyl is able to separate a few minor variants of the mAb sample.

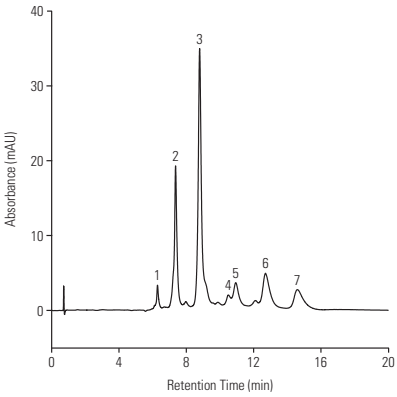
Separation of Antibody-Drug Conjugates

ADC is an emerging class of biotherapeutics due to its high selectivity and high potency. ADCs are cytotoxic drugs attached to mAbs by chemical linkers with labile bonds. The antibody part of the ADC specifically recognizes a tumor marker and once the ADC is internalized into the target cell, the toxic drug is released which in turn kills tumor cells. There are two most common methods to attach the cytotoxic drug. The first method is to attach the drug to free cysteine residues after reduction. And the second method is to attach the drug to lysine residues that are positioned throughout the antibody. These conjugation methods yield heterogeneous mixtures of ADC variants since there is more than one cysteine or lysine residues present in an antibody molecule. The heterogeneity of ADC molecules may potentially cause safety and efficacy issues when administered into patients. Therefore purification and full characterization of ADCs is critical during development and production. Since the attachment of a drug molecule alters the hydrophobicity of the mAb, hydrophobicity based HPLC methods such as reverse phase chromatography and HIC are commonly used to characterize ADCs. Opposed to reverse phase chromatography, HIC does not denature the protein analytes. Therefore HIC is ideal for further functional analysis of separated ADC molecules.



Column:	**MAbPac HIC-Butyl**, 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:	2 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



Column:	**MAbPac HIC-Butyl**, 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		
Temp.:	25 °C		
Detection:	UV (280 nm)		
Sample:	Cys-conjugated ADC mimic (5 mg/mL)		
Peaks:	1. Unconjugated mAb 2. DAR 2 3. DAR 4 4. DAR 6 5. DAR 6 6. DAR 6 7. DAR 8		

Figure 3a: Separation of Cys-conjugated ADC mimic

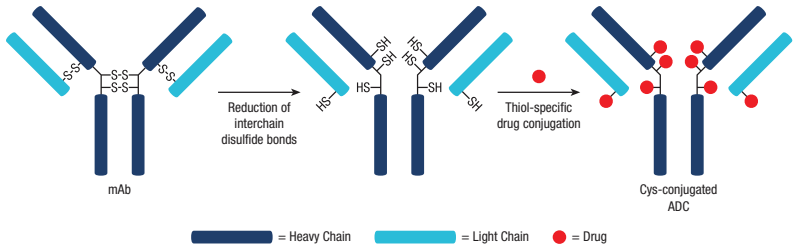
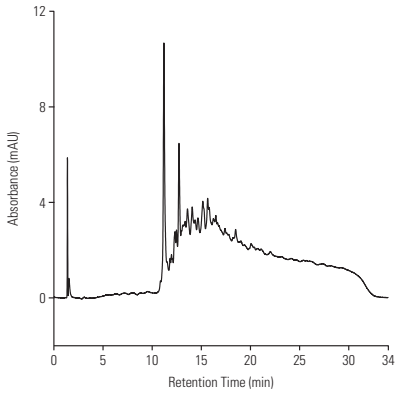


Figure 3b: Schematic representation of conjugation of drug mimic via interchain cysteine residues



Column:	**MAbPac HIC-Butyl**, 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
	29.0	0	100
	34.0	0	100
Flow Rate:	0.5 mL/min		
Inj. Volume:	5 µL		
Temp.:	35 °C		
Detection:	UV (280 nm)		
Sample:	Lys-conjugated ADC mimic (5 mg/mL)		

Figure 4: Separation of Lys-conjugated ADC mimic

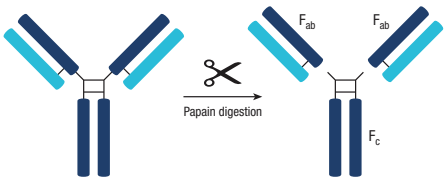
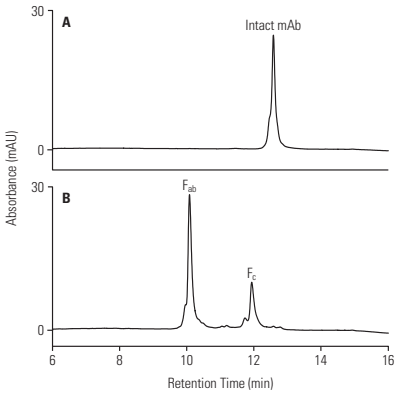


Figure 5a: Schematic representation of papain digestion of monoclonal antibody



Column:	**MAbPac HIC-Butyl**, 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:	2 µL	
	Papain digest:	5 µL	
Temp.:	30 °C		
Detection:	UV (280 nm)		
Sample:	a) Intact mAb (2.5 mg/mL) b) Papain digest (1 mg/mL)		

Figure 5b: Separation of (a) intact mAb and (b) papain digested mAb

Figure 3a shows the separation of a cysteine-conjugated ADC mimic sample on the MAbPac HIC-Butyl column. The ADC mimics were conjugates between a drug mimic and a 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 3b). The unmodified mAb and ADCs with DAR values ranging from 2 to 8 are well resolved on the MAbPac HIC-Butyl column.

Figure 4 shows the separation of a lysine-conjugated ADC mimic sample on the MAbPac HIC-Butyl column. The drug mimic is conjugated to lysine residues of the mAb. Typically lysine-linked ADCs are highly heterogenic due to multiple lysine residues within a mAb. MAbPac HIC-Butyl column is able to provide a reasonable separation of this complex mixture based on the hydrophobicity of the species.

Separation of mAb Fragments

Fab and Fc fragments generated by papain digestion of mAbs (Figure 5a) are often analyzed to obtain further information on the mAb heterogeneity. HIC is a promising tool for this purpose since it provides the resolution for the separation of Fab and Fc fragments and their variants. Figure 5b shows a comparison of an intact mAb and its papain digest on MAbPac HIC-Butyl. The MAbPac HIC-Butyl column efficiently separates Fab and Fc fragments and further separates variants of these fragments.

Reproducible Manufacturing

Each MAbPac HIC-Butyl 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-Butyl
Column Chemistry	Butyl
Substrate	Hydrophilic Polymer
Particle size	5 µm
Pore size	Non-porous

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	4,000	60	2.0–12.0	Compatible with up to 50% organic solvent
4.6 × 10 mm	0.5–1.0	2.0	4,000	60	2.0–12.0	

Ordering Information

Description	Particle Size	Part Number
MAbPac HIC-Butyl, Analytical 4.6 × 100 mm	5 µm	088558
MAbPac HIC-Butyl, Guard Cartridges 4.6 × 10 mm (2/pk)	5 µm	088559
Guard Cartridge Holder		069580

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