

Are you missing important peaks?

Get better Monoclonal Antibody Resolution
with MAbPac RP columns for High Performance
Reverse Phase Chromatography

MABPac RP Column

The Thermo Scientific™ MABPac™ RP columns are designed for comprehensive characterization of mAb purity by reversed-phase (RP) liquid chromatography. High resolution of intact mAb, mAb fragments such as light chain (LC), heavy chain (HC), Fc and Fab, scFc and F(ab')₂, will reveal the location, as well as nature, of modification using LC/UV or LC/MS.

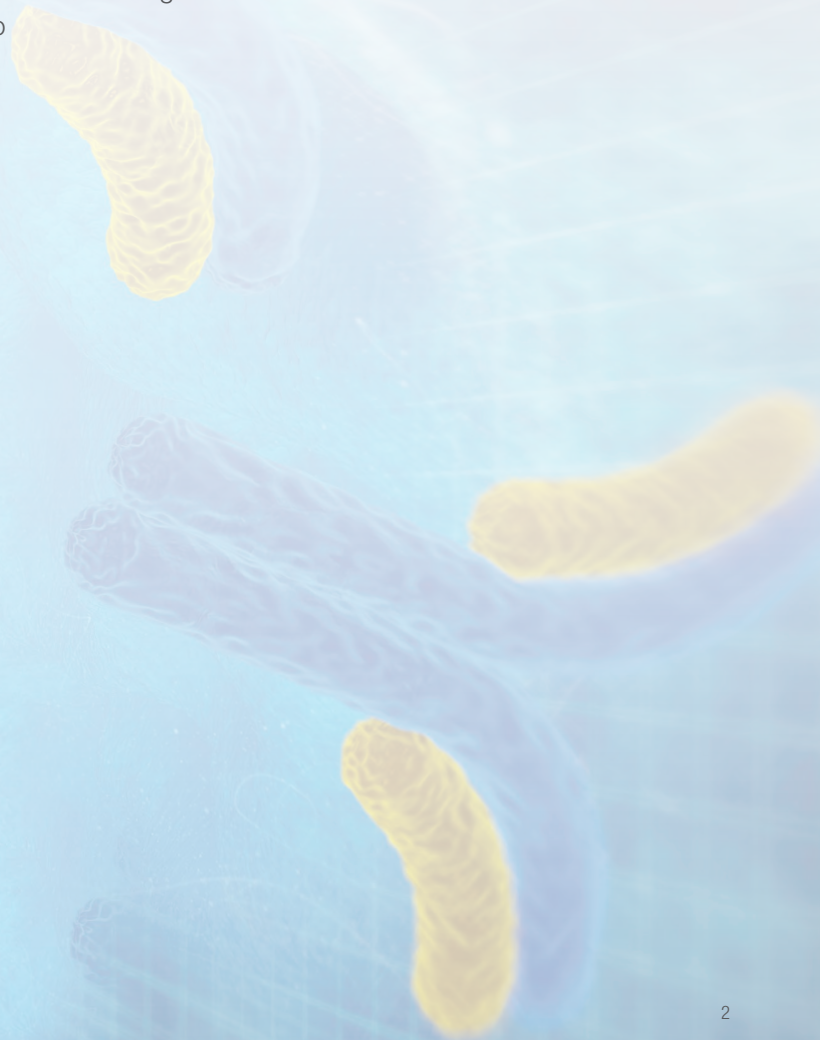


Product Highlights

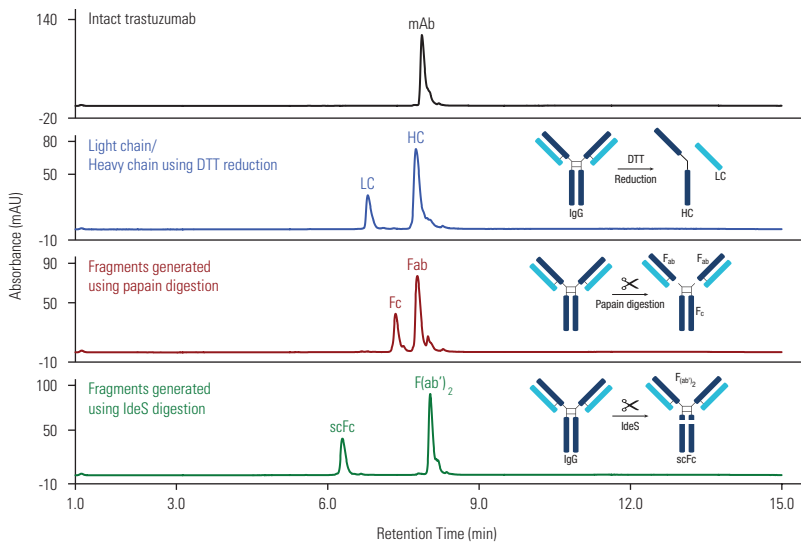
- Superior resolution of monoclonal antibodies and related substances: unique macroporous resin with faster mass transfer capabilities
- High efficiency columns with lower carry-over than silica based columns – engineered into the surface chemistry
- Excellent MS compatibility
- Wider operating pH range (0–14) easy cleaning with up to 800 mM NaOH (not possible with silica based columns)
- High temperature stability (up to 110 °C) for better chromatographic separation
- Fast separations capability for high throughput separations
- Wide loadability range with 3 orders of magnitude: 0.2 ng to 20 µg

Optimized Column Technology

The MABPac RP column is a reversed-phase (RP) column based on a macroporous resin specifically designed for high resolution separation of intact monoclonal antibodies (mAbs) and mAb fragments. The stationary phase is fully compatible with mass spectrometry friendly organic solvents such as acetonitrile and isopropanol, as well as low pH eluents containing trifluoroacetic acid or formic acid.



Fast Separation of Intact Proteins/mAbs and Fragments



Column: MAbPac RP, 4 μ m
 Format: 3 \times 50 mm
 Mobile phase A: H₂O/FA/TFA (99.88 : 0.1:0.02 v/v/v)
 Mobile phase B: MeCN/ H₂O/FA/TFA (90: 9.88 : 0.1:0.02 v/v/v/v/v)
 Gradient:

Time (min)	%A	%B
0.0	80	20
1.0	80	20
11.0	55	45
12.0	55	45
14.0	80	20
15.0	80	20

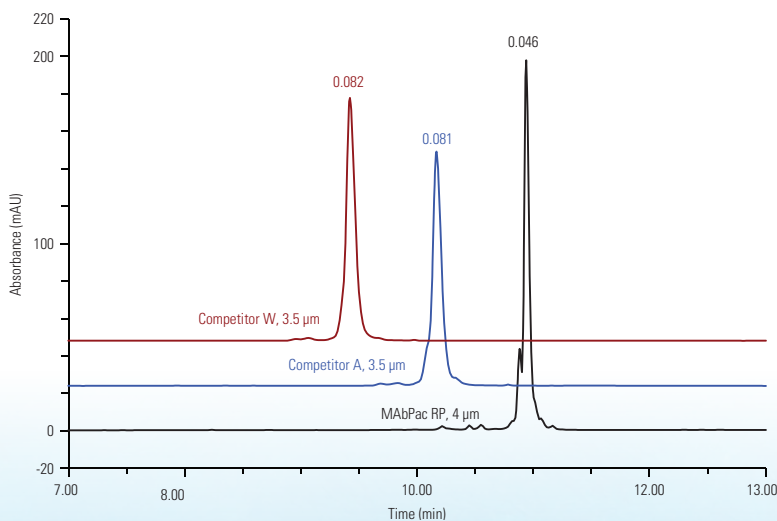
Temperature: 80 °C
 Flow Rate: 0.5 mL/min
 Inj. Volume: 5 μ L
 Detection: UV (280 nm)

Sample:
 (a) Trastuzumab (5 mg/mL)
 (b) Trastuzumab + DTT (4 mg/mL)
 (c) Trastuzumab + Pepsin (2 mg/mL)
 (d) Trastuzumab + IdeS (2 mg/mL)

Figure 1. Separation of mAb and mAb fragments.

Superior Separation of mAb

MAbPac RP chemistry and morphology provides superior resolution of intact mAb and variants, as shown in this comparison with other column suppliers. Twice as many variants, with almost half of the peak width half height, are clearly resolved using the MAbPac RP column: resulting in easier and better identification throughout the analysis (Figure 2).



Column: RP columns
 Format: 3 \times 50 mm or 4.6 \times 50 mm
 Mobile phase A: H₂O/TFA (99.9 : 0.1 v/v)
 Mobile phase B: MeCN/ H₂O/TFA (90: 9.9 : 0.1 v/v/v)
 Gradient:

Time (min)	%A	%B
-3.0	80	20
1.0	80	20
11.0	50	50
12.0	50	50
12.1	80	20
15.0	80	20

Temperature: 80 °C
 Flow Rate: 0.5 mL/min or 1.0 mL/min
 Inj. Volume: 0.5 μ L or 1 μ L
 Detection: UV (280 nm)

Sample: NISTmAb (10.0 mg/mL)
 Peak Label: PWHH (min)

Figure 2. Comparison of NISTmAb separation.

LC/MS Analysis of Intact mAb and mAb Fragments

The MAbPac RP column can be directly coupled to the mass spectrometer for MS detection of mAb and mAb fragments. Combination with the Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ Mass Spectrometer enables accurate mass measurement of mAbs (Figure 3).

Column: MAbPac RP, 4 μm
 Format: 3 × 50 mm
 Mobile phase A: H₂O/FA/TFA (99.88 : 0.1:0.02 v/v/v)
 Mobile phase B: MeCN/ H₂O/FA/TFA (90: 9.88 : 0.1:0.02 v/v/v/v)
 Gradient:

Time (min)	%A	%B
0.0	80	20
1.0	80	20
11.0	55	45
12.0	55	45
14.0	80	20
15.0	80	20

Temperature: 80 °C
 Flow Rate: 0.5 mL/min
 Inj. Volume: 1 μL
 UV Detection: 280 nm
 MS Detection: Positive-ion mode
 Mass Spec: Q ExactivePlus

Sample: Reduced trastuzumab (4 mg/mL)

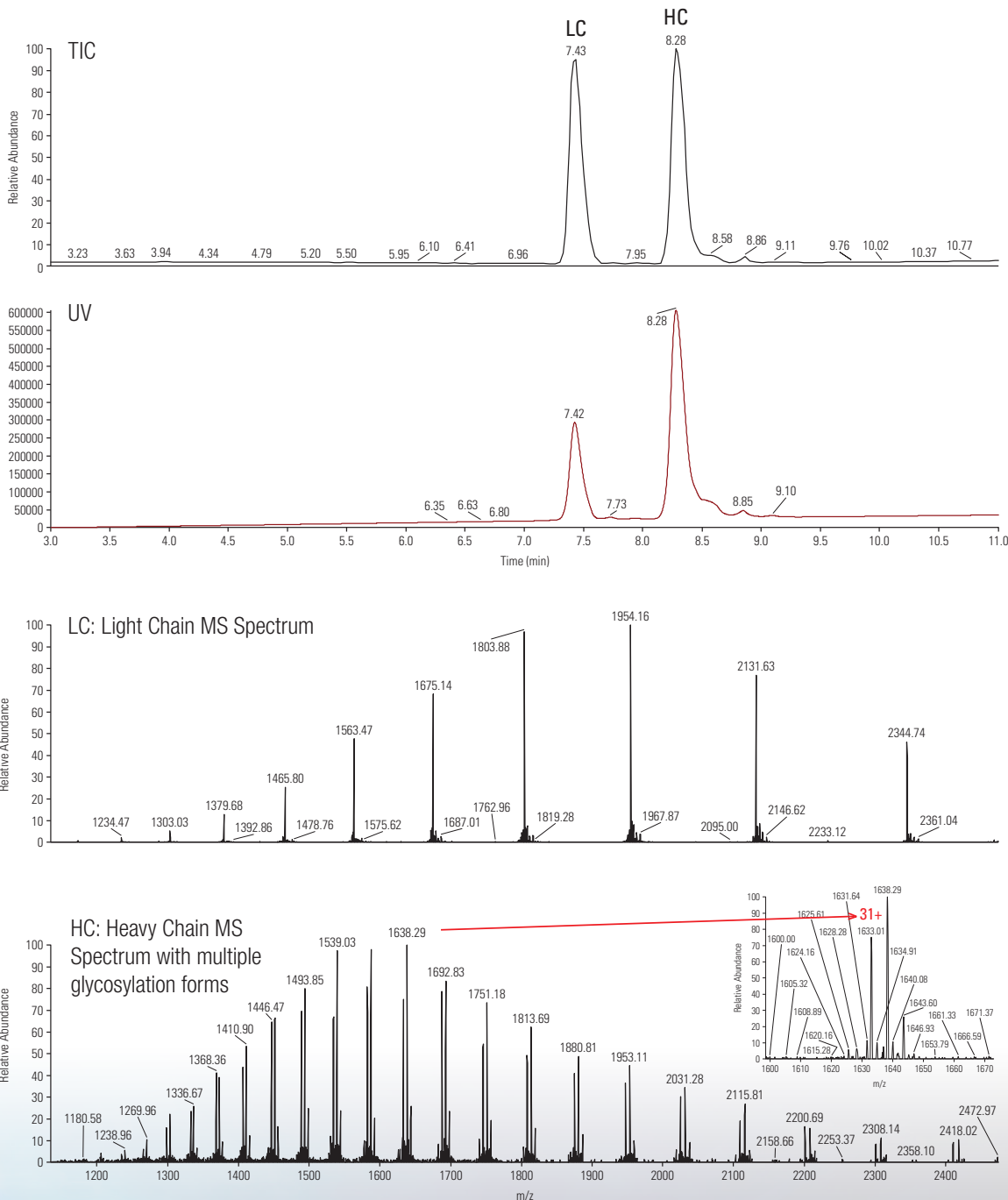
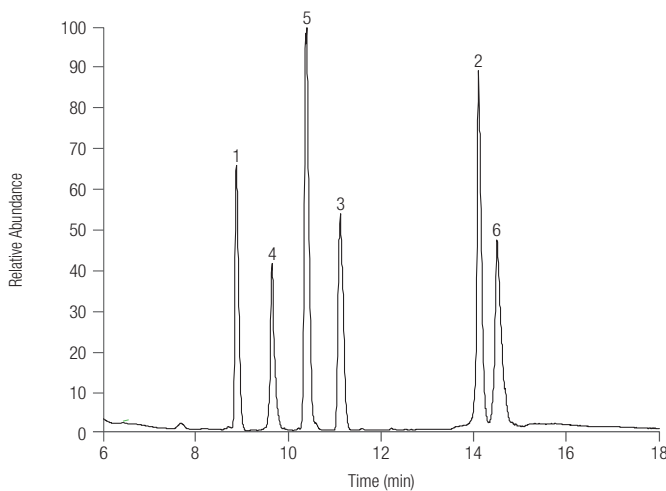


Figure 3. Reduced mAb LC/MS analysis.

Top-down LC/MS/MS Analysis of Intact Protein Standards

Thermo Scientific™ Pierce™ Intact Protein Mix consists of six recombinant proteins: IGF-I LR3 (9 kDa), thioredoxin (12 kDa), protein G (21 kDa), carbonic anhydrase II (29 kDa), protein AF (51 kDa), and exo klenow (68 kDa). These proteins are selected to satisfy the following criteria: 1) evenly covering a MW range of 10kD–66kD, 2) presenting mostly clean, modification and adduct-free ESI spectra, and 3) having ESI charge state distributions covered a wide m/z range from 500–2,000. A 1 mm ID MAbPac RP column was chosen to analyze these proteins because it provides higher sensitivity than larger internal diameter columns, when sample size is limited.

Figure 4a shows baseline separation of all six proteins using an acetonitrile/H₂O/formic acid mobile phase. Top-down MS/MS spectra were acquired on a Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer using top 3–5 DDA method. OT MS1 data was acquired at resolution settings of 15 at m/z 200 and OTMS2 at a resolution of 120K at m/z 200. Figure 4b shows deconvolution results from Thermo Scientific™ Protein Deconvolution™ 4.0 software and Figure 4c shows top-down results from ProSight PD 1.1 node in Proteome Discoverer 2.1 software.



Column: MAbPac RP, 4 μm
 Format: 1.0 × 150 mm
 Mobile phase A: H₂O/FA (99.9 : 0.1 v/v)
 Mobile phase B: MeCN/FA (99.0: 0.1 v/v)
 Gradient: Time (min) %A %B
 0.0 90 10
 1.0 80 20
 15.0 55 45
 16.0 10 90
 20.0 10 90
 20.1 90 10
 25.0 90 10
 Temperature: 60 °C
 Flow Rate: 0.1 mL/min
 Inj. Volume: 1 μL
 MS Detection: positive-ion mode
 Mass Spec: Q Exactive HF
 Sample: Pierce Intact Protein Standard Mix, 500 ng/μL (P/N A35526)
 1. IGF-I LR3 (9 kDa)
 2. Thioredoxin (12 kDa)
 3. Protein G (21 kDa)
 4. Carbonic Anhydrase II (29 kDa)
 5. Protein AF (51 kDa)
 6. Exo Klenow (68 kDa)

Figure 4a. Total ion chromatogram of the LC/MS analysis of the Intact Protein Standard Mix.

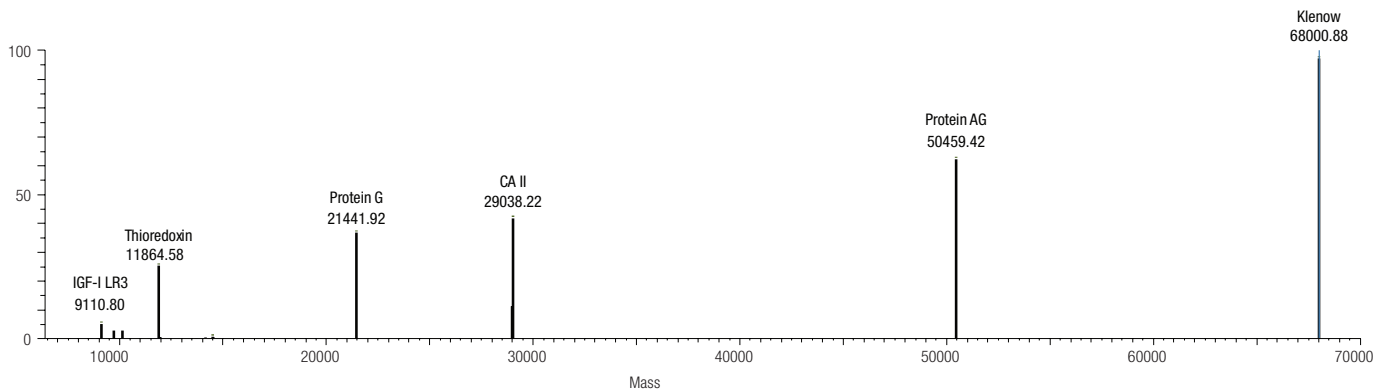


Figure 4b. Average masses for all six proteins in the Intact Protein Standard Mix.

	Checked	Master	Accession	Description	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pt	# Peptides ProSightPD Absolute Mass Search
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	ProteinG	ProteinG Immunoglobulin G-binding protein G	100%	2	17	2	1	198	21.4	4.75	2
2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	rCA	rCA Carbonic anhydrase 2	100%	1	9	1	1	260	29.1	7.12	1
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	M_trx	Thioredoxin, mitochondrial	99%	1	2	1	1	108	12.0	5.08	1
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	ProteinAG	ProteinAG Immunoglobulin G-binding protein A	100%	1	8	1	1	455	50.6	4.77	1
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	IGF_LR3	Insulin-like growth factor I	100%	1	2	1	1	83	9.1	8.28	1
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Klenow	Klenow DNA polymerase [ECO.0000256]	100%	1	4	1	1	605	68.0	6.11	1

Figure 4c. List of identified proteins using Proteome Discoverer 2.1 software.

Physical Data

Chemistry	Phenyl
Polymer substrate	DVB particles
Particle size	4 μm
Pore size	1,500 \AA
Column housing	Stainless steel for the 3.0 and 2.1 mm ID Glass Lined Stainless Steel for 1.0 mm ID

Operational Specifications

Column	Column ID (mm)	Flow Rate (mL/min)	Pressure Limit (psi)	Temperature ($^{\circ}\text{C}$)	pH Range
MABPac RP	3.0	0.50–1.00	4,000	< 110	0–14
MABPac RP	2.1	0.30–0.60	4,000	< 110	0–14
MABPac RP	1.0	0.075–0.15	4,000	< 110	1–7

Ordering Information

Description		Particle Size (μm)	Length (mm)	1.0 mm ID	2.1 mm ID	3.0 mm ID
MABPac RP Columns	Analytical	4	50	303182	088648	088645
			100	303183	088647	088644
			150	303184	303270	303269
	Guard Cartridges (2/pk)*		10		088649	088646

* Standard Guard Cartridge Holder Required: P/N 069580

Find out more at thermofisher.com/mabpac

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