

BioLC columns

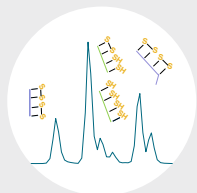
# Connected chromatography solutions

BioLC columns and accessories

# Introduction

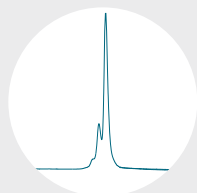
## Your complete tool kit

Thermo Fisher Scientific has innovative Thermo Scientific™ BioLC columns for each step of your therapeutic protein characterization, no matter how challenging your separation. Here is just one example, a fully characterized model sample of pertuzumab. Discover our full range in this catalogue.



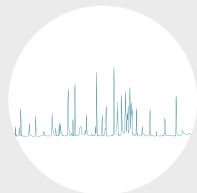
### Intact or subunit analysis

Thermo Scientific™ MAbPac™ RP columns are ideal for intact and subunit analysis by MS or UV detection. The polymeric packing material offers column longevity, high resolution and the wide pores to allow for low carryover profiling of your sample.



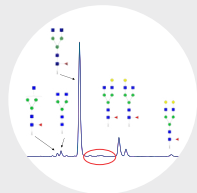
### Oxidation monitoring

Deduce protein folding errors or charge-neutral amino acid modifications with the Thermo Scientific™ MAbPac™ HIC-20 hydrophobic interaction column. Our range of innovative HIC chemistries deliver native separations not seen on other columns.



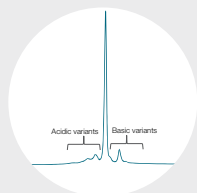
### Peptide mapping

Experience reproducible peptide mapping and quantitation. The combination of rapid digestion from the Thermo Scientific™ SMART Digest kit and separation with the high resolution Thermo Scientific™ Hypersil™ GOLD column delivers outstanding, reproducible and efficient peptide mapping separations.



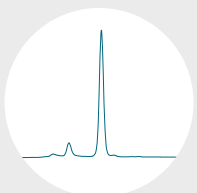
### Released glycan analysis

Fully characterize your released N-glycans with the Thermo Scientific™ Accucore™ 150 Amide-HILIC column. This solid core column offers high resolution, durability, and the ability to run separations at lower temperatures to reveal the complete glycan profile.



### Charge variant analysis

For charge variant analysis by LC-UV or LC-MS/MS Thermo Scientific™ ProPac™ 3R SCX and Thermo Scientific™ ProPac™ 3R SAX columns deliver outstanding resolution on a highly robust, reproducible and high-resolution platform. Combine ProPac 3R SCX columns with our proprietary Thermo Scientific™ CX-1 gradient buffers formulations to enable fast, robust and reproducible pH gradients that are simple to optimize and easily automated - without the need for time-consuming mobile phase adjustments.

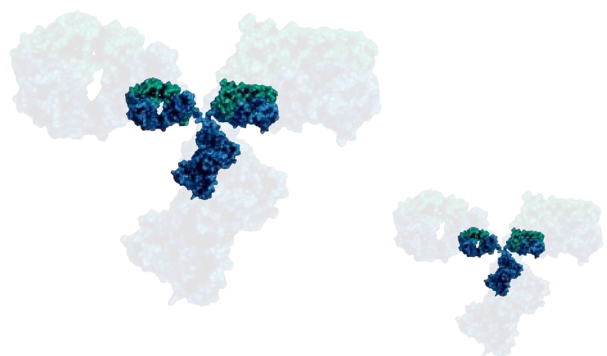


### Aggregate analysis

Thermo Scientific™ MAbPac™ SEC-1 offers excellent size exclusion separation even under challenging conditions for aggregate analysis. Compatible with mass spectrometry for native LC-MS/MS workflows.

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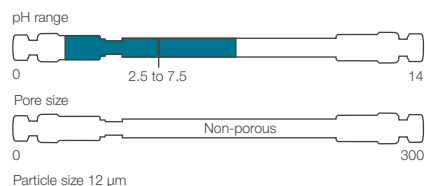
# BioLC column selection quick guide

Target applications	Column type	Mode of analysis	Recommended column	Particle size (µm)	Pore size (Å)	pH range	Maximum backpressure (psi)	Solvent compatibility
<b>Affinity</b>	Affinity columns	Affinity	<b>MABPac Protein A</b>	12	Non-porous	2.5-7.5	1,000	—
<b>Intact analysis by HIC</b>	Silica-based hydrophobic interaction chromatography columns	Hydrophobic interaction	<b>MABPac HIC-10</b>	5	1,000	2-8	4.6 × 100 mm = 6,000 4.6 × 250 mm = 8,000	Compatible with organic solvents and aqueous mobile phases
			<b>MABPac HIC-20</b>	5	1,000	2-9		
			<b>MABPac HIC Butyl</b>	5	Non-porous	2-12	4,000	Compatible with up to 50% organic solvents
<b>Released glycan analysis</b>	Silica based, mixed-mode columns	Mixed-mode	<b>GlycanPac AXH-1</b>	1.9	175	2-8	10,000	0 – 90% aqueous buffer; 10 – 100% acetonitrile or alcohols
				3	120	2-8	6,000	
			<b>GlycanPac AXR-1</b>	1.9	175	2-8	10,000	Compatible with 0 – 100% aqueous and common HPLC solvents (except acetone)
			<b>Accucore 150 Amide HILIC</b>	2.6	150	2-8	14,500	—
<b>Aggregate fragment analysis</b>	Silica-based size exclusion chromatography phases	Size exclusion	<b>MABPac SEC-1</b>	5	300	2.5-7.5	1,000 for 300 mm 600 for 150 mm	100% organic solvents
<b>Intact and subunit analysis</b>	Polymeric reversed-phase columns	Reversed-phase	<b>MABPac RP</b>	4	1,500	2.1, 3.0 mm (0-14) 1 mm (1-7)	4,000	Up to 100% ACN, IPA, MeOH
	Polymeric reversed-phase columns	Reversed-phase	<b>ProSwift RP-1S</b>	Monolith	Monolith	1-14	2,800	Most common organic solvents
			<b>ProSwift RP-2H</b>				2,800	
			<b>ProSwift RP-3U</b>				2,800	
			<b>ProSwift RP-4H</b>				1 × 50 mm = 2,000 2 × 250 mm = 3,000	
<b>Charge variant analysis</b>	Monolithic ion-exchange columns	Ion-exchange	<b>ProPac 3R SCX</b>	3	Non-porous	2-12*	4,500	—
			<b>ProPac 3R SAX</b>	3			4,500	—
			<b>MABPac SCX-10RS</b>	5			7,000	—
			<b>MABPac SCX-10</b>	5, 10			3,000 for 10 µm 5,000 for 5 µm	*Please consult column manual
			<b>ProPac SAX-10</b>	10			3,000	*Please consult column manual
			<b>ProPac Elite WCX</b>	5			4,500	*Please consult column manual
<b>Peptide mapping</b>	Silica based, reversed-phase columns	Reversed-phase	<b>Hypersil GOLD C18</b>	1.9	175	1-11	18,130	—
				3	175		5,800	—
			<b>Acclaim 120 C18</b>	2.2	120	2-8	Various	—
				3	120			—
				5	120			—
<b>Nucleic acids and oligonucleotides</b>	Polymeric ion-exchange columns	Ion-exchange	<b>DNAPac PA200</b>	8	Non-porous	2.5-12.5*	4,000	*Please consult column manual
			<b>DNAPac PA200RS</b>	4	Non-porous	2.5-12.5*	10,000	—
			<b>DNASwift SAX-1S</b>	Monolith	Monolith	3-14*	1,500	*Please consult column manual
	Polymeric reversed-phase	Reversed-phase	<b>DNAPac RP</b>	4	Proprietary wide pore	0-14	4,000	—

# Affinity columns

Providing fast, accurate titer analysis of monoclonal antibodies in harvest cell cultures, the nonporous, polymeric Thermo Scientific™ MAbPac™ Protein A HPLC Column delivers reproducible, highly efficient separations.

## MAbPac Protein A column

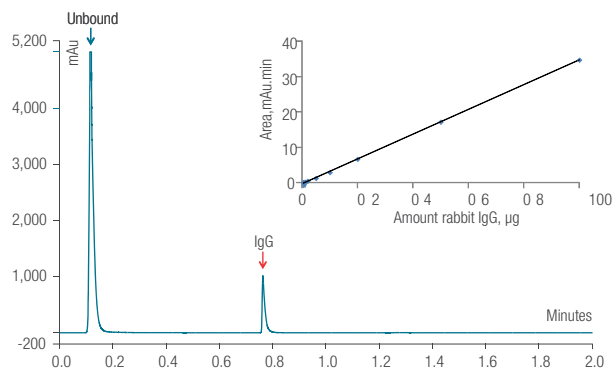


### Additional reading

Links	Type	Description
	Application note	MAbPac Protein A: A novel affinity Protein A column
	Learn more	<a href="https://www.thermofisher.com/biolc">thermofisher.com/biolc</a>

### Harvest cell culture titer analysis

MAbPac Protein A, 12 μm, 35 x 4.0 mm	
Flow rate	2 mL/min
Mobile phase A	50 mM sodium phosphate, 150 mM NaCl, 5% acetonitrile, pH 7.5
Mobile phase B	50 mM sodium phosphate, 150 mM NaCl, 5% acetonitrile, pH 2.5
Gradient	0% B for 0.2 mins, 100% B for 0.60 mins, 0% B for 1.20 mins
Temperature	30 °C
Injection volume	10 μL
Detection	UV at 280 nm
Sample	mAb B, 5 mg/mL harvest cell culture



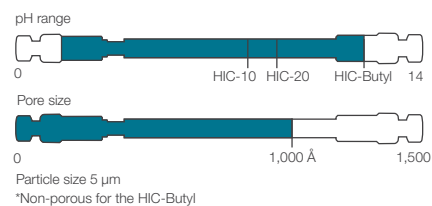
### MAbPac Protein A column

Particle size (μm)	Format	Length (mm)	4.0 mm ID
12	HPLC column	35	<a href="#">082539</a>

# Intact analysis by HIC

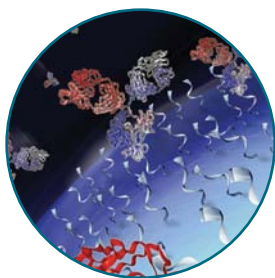
Orthogonal to IEX and SEC, Hydrophobic Interaction Chromatography (HIC) offers selectivity to resolve charge neutral protein oxidations and protein misfolds. Our proprietary 1000 Å silica Thermo Scientific™ MAbPac™ HIC-10 and Thermo Scientific™ MAbPac™ HIC-20 provide unique separation profiles offering high resolution for protein samples. For more hydrophobic samples, select the Thermo Scientific™ MAbPac™ HIC-Butyl column.

## MAbPac HIC-10, HIC-20, HIC-Butyl columns

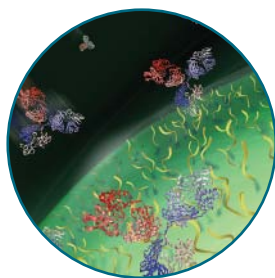


### Additional reading

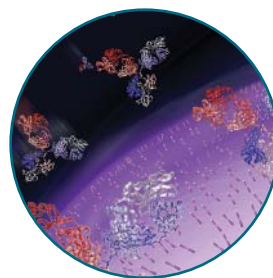
Links	Type	Description
	Application note	MAbPac HIC-10 High resolution separation of a fusion protein on MAbPac HIC-10 column
	Application note	HIC as a complementary, confirmatory tool to SEC for the analysis of mAb aggregates
	Application note	MAbPac HIC-20 High resolution separation of mAb fragments on MAbPac HIC-20 column
	Application note	High resolution separation of monoclonal antibody (mAb) oxidation variants
	Application note	High resolution separation of cysteine-conjugated antibody drug mimics
	Learn more <a href="https://www.thermofisher.com/biolc">thermofisher.com/biolc</a>	



MAbPac HIC-10



MAbPac HIC-20

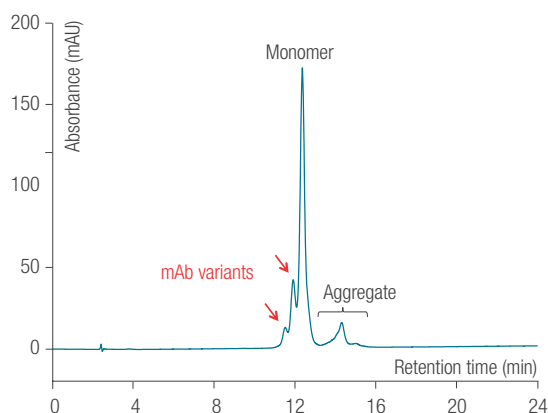


MAbPac HIC-Butyl

# Intact analysis by HIC continued

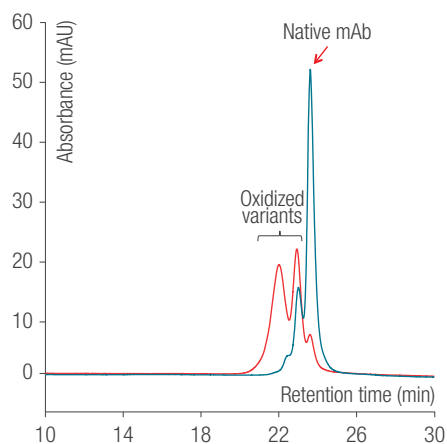
## Separation of mAb aggregates

MAbPac HIC-10, 5 $\mu$ m, 100 x 4.6 mm			
Flow rate	0.5 mL/min		
Mobile phase A	2 mM ammonium sulfate, 100 mM sodium phosphate, pH 7.0		
Mobile phase B	100 mM sodium phosphate, pH 7.0		
Temperature	20 $^{\circ}$ C		
Injection volume	15 $\mu$ L		
Detection	UV at 280 nm		
Sample	Monoclonal antibody (4 mg/mL)		
	Time (min)	%A	%B
	-5.0	60	40
Gradient	0.0	60	40
	1.0	60	40
	29.0	0	0
	34.0	0	0



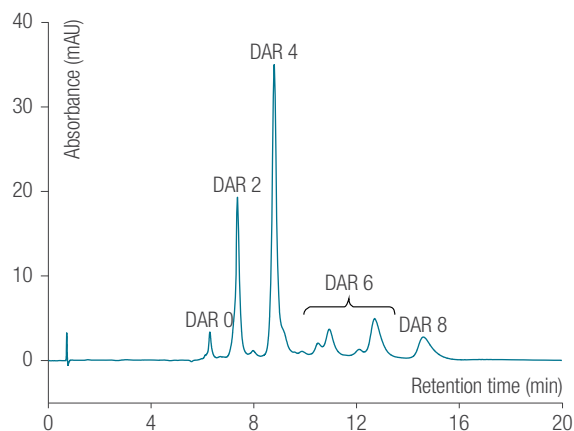
## Separation of mAb fragments

MAbPac HIC-20, 5 $\mu$ m, 250 x 4.6 mm			
Flow rate	0.5 mL/min		
Mobile phase A	2 mM ammonium sulfate, 100 mM sodium phosphate, pH 7.0		
Mobile phase B	100 mM sodium phosphate, pH 7.0		
Temperature	30 $^{\circ}$ C		
Injection volume	Untreated mAb: 20 $\mu$ L (1.25 mg/mL) Oxidized mAb: 20 $\mu$ L (1.25 mg/mL)		
Detection	UV at 280 nm		
Sample	Untreated mAb H <sub>2</sub> O <sub>2</sub> , oxidized mAb		
	Time (min)	A%	%B
	-6.0	50	50
Gradient	0.0	50	50
	2.0	50	50
	30.0	0	100
	35.0	0	100



## Separation of Antibody Drug Conjugates (ADCs)

MAbPac HIC-Butyl, 5 $\mu$ m, 100 x 4.6 mm			
Flow rate	1.0 mL/min		
Mobile phase A	1.5 mM 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)		
Temperature	25 $^{\circ}$ C		
Injection volume	5 $\mu$ L		
Detection	UV at 280 nm		
Sample	Cys-conjugated ADC mimic (5 mg/mL)		
	Time (min)	%A	%B
	-5.0	100	0
Gradient	0.0	100	0
	1.0	100	0
	15.0	0	100
	20.0	0	100



# Intact analysis by HIC continued

## MABPac HIC selection guide

Column	MABPac HIC-10	MABPac HIC-20	MABPac HIC-Butyl
Intact mAbs/proteins	++++	+++	++
mAb aggregates	++++	+++	++
mAb fragments (F <sub>ab</sub> and F <sub>c</sub> )	+++	++++	+++
Oxidized mAbs	+++	++++	+++
Antibody Drug Conjugates (ADCs)	+++	+++	++++
Bispecific mAbs	+++	++++	++

Greater number of ++++ denotes greater suitability



## MABPac HIC family columns

Description	Particle size (µm)	Format	Length (mm)	4.6 mm ID
MABPac HIC-10	5	Guard cartridges (2/pk)	10	<a href="#">088482</a>
		HPLC column	100	<a href="#">088480</a>
			250	<a href="#">088481</a>
MABPac HIC-20	5	Guard cartridges (2/pk)	10	<a href="#">088555</a>
		HPLC column	100	<a href="#">088553</a>
			250	<a href="#">088554</a>
MABPac HIC-Butyl	5	Guard cartridges (2/pk)	10	<a href="#">088559</a>
		HPLC column	100	<a href="#">088558</a>
Guard cartridge holder	—	—	—	<a href="#">069580</a>



### Video:

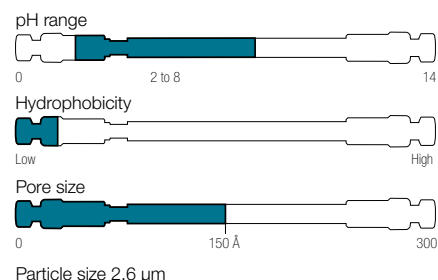
Introduction to hydrophobic interaction chromatography



# Released glycan analysis

For monoclonal antibodies, or protein samples with a lot of neutral glycans, the Thermo Scientific™ Accucore™ 150-Amide HILIC offers outstanding separation on a solid core particle. The low backpressure of this particle allows users to experiment with optimum temperature of their separation, to maximize the elucidation of their released glycan profile. For proteins with charged glycans, we offer two mixed mode column chemistries combining anion exchange with HILIC or RP separations. Thermo Scientific™ GlycanPac™ AXH-1 separates the glycan profile by charge, size, and hydrophilicity. Thermo Scientific™ GlycanPac™ AXR-1 separates the profile by charge, size, and branch isomers.

## Accucore 150-Amide-HILIC column

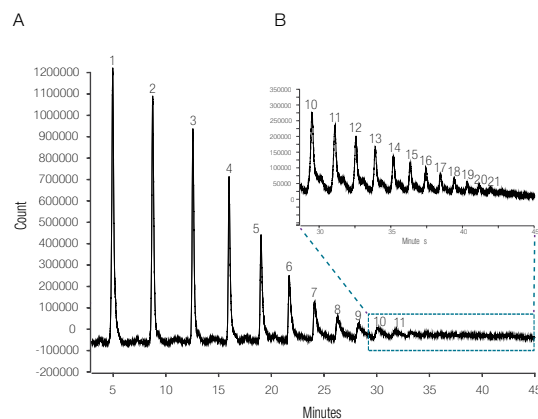


### Additional reading

Links	Type	Description
	Application note	Analysis of human IgG glycans on a solid core amide HILIC stationary phase
	Learn more	<a href="https://www.thermofisher.com/biolc">thermofisher.com/biolc</a>

### 2-AB labeled dextran ladder

Accucore 150-Amide-HILIC, 2.6 μm, 100 x 2.1 mm	
Flow rate	500 μL/min
Mobile phase A	Acetonitrile
Mobile phase B	50 mM ammonium formate, pH 4.5
Temperature	60 °C
Injection volume	2 μL to 5 μL
Backpressure at starting conditions	110 bar
Injection wash solvent	80:20 (v/v) acetonitrile:water
Detector	Fluorescence, 330 nm excitation wavelength; 420 nm emission wavelength; acquisition start after 3 min from gradient start
Run time	50 min
Gradient	20–50% B in 40.0 minutes; 50% B for 5.0 minutes 50–20% B in 0.5 minutes; 50% B for 4.5 minutes



(A) 2 μL injection of sample, where 11 glycans were separated  
(B) 5 μL injection of sample, zoomed-in to the later part of the gradient rise. A further 10 glycans were detected

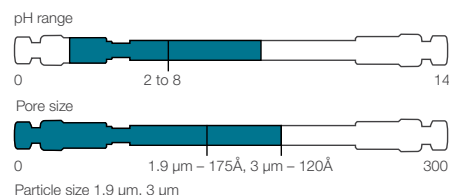


### Accucore 150-Amide-HILIC columns

Particle size (μm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
2.6	Defender guard (4/pk) HPLC column	10	<a href="#">16726-012105</a>	—	—
		50	<a href="#">16726-052130</a>	<a href="#">16726-053030</a>	—
		100	<a href="#">16726-102130</a>	<a href="#">16726-103030</a>	<a href="#">16726-104630</a>
		150	<a href="#">16726-152130</a>	<a href="#">16726-153030</a>	<a href="#">16726-154630</a>
		250	<a href="#">16726-252130</a>	—	—
—	Guard cartridge holder	—	<a href="#">852-00</a>	<a href="#">852-00</a>	<a href="#">850-00</a>

# Released glycan analysis continued

## GlycanPac AXH-1 column

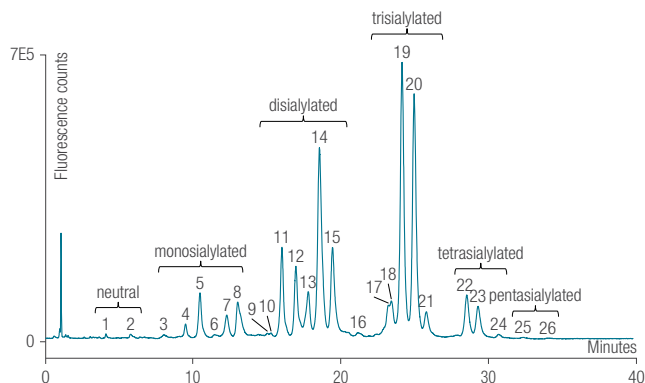


### Additional reading

Links	Type	Description
	Application note	Separation of 2AB-labeled N-linked glycans from bovine fetuin
	Application note	Separation of 2AA-labeled N-linked glycans from human IgG
	Application note	Separation of 2AA-labeled N-linked glycans from glycoproteins
	Learn more <a href="https://www.thermofisher.com/biolc">thermofisher.com/biolc</a>	

### Separation of 2AB labeled N-glycans from bovine fetuin by charge, size and polarity

GlycanPac AXH-1, 1.9 μm, 150 x 2.1 mm				
Flow rate	0.4 mL/min			
Mobile phase A	Acetonitrile (100%)			
Mobile phase B	Water			
Mobile phase C	Ammonium formate (100 mM, pH = 4.4)			
Temperature	30 °C			
Injection volume	5 μL			
Detection	Fluorescence, 320/420 nm			
Sample	2AB labeled N-glycan from bovine fetuin			
Curve	5			
Gradient	Time (min)	%A	%B	%C
	-10.0	78	20	2
	0.0	78	20	2
	30.0	70	20	10
	35.0	60	20	20
	40.0	50	20	30

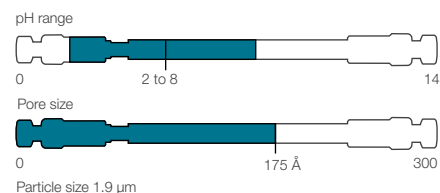


### GlycanPac AXH-1 columns

Particle size (μm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
1.9	UHPLC column	100	<a href="#">082473</a>	—	—
		150	<a href="#">082472</a>	—	—
		250	<a href="#">082521</a>	—	—
3	Guard cartridges (2/pk)	10	<a href="#">082476</a>	<a href="#">082475</a>	<a href="#">082474</a>
	HPLC column	150	<a href="#">082470</a>	<a href="#">082469</a>	<a href="#">082468</a>
—	Guard cartridge holder	—	<a href="#">069580</a>	<a href="#">069580</a>	<a href="#">069580</a>

# Released glycan analysis continued

## GlycanPac AXR-1 column

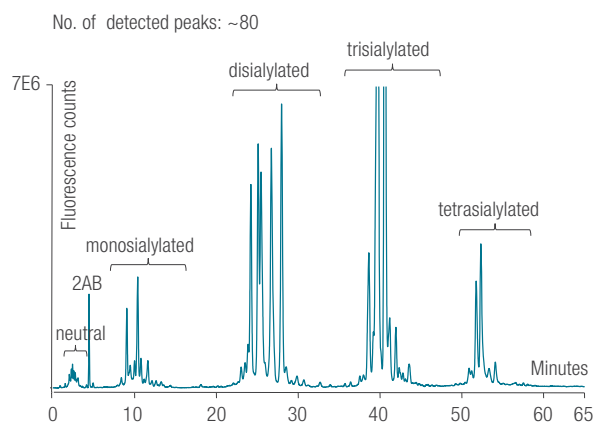


### Additional reading

Links	Type	Description
	Application note	Separation of 2AB labeled N-glycans from bovine fetuin
	Application note	Structural analysis of native N-glycans released from proteins
	Learn more <a href="https://thermofisher.com/biolc">thermofisher.com/biolc</a>	

### Separation of 2AB labeled N-glycans from bovine fetuin

GlycanPac AXR-1, 1.9 μm, 150 x 2.1 mm				
Flow rate	0.4 mL/min			
Mobile phase A	Acetonitrile			
Mobile phase B	Water			
Mobile phase C	Ammonium formate (100 mM, pH = 4.4)			
Temperature	40 °C			
Sample load	100 pmoles			
Detection	Fluorescence, 320/420 nm			
Sample	2AB labeled N-glycan from bovine fetuin			
Curve	5			
	Time (min)	%A	%B	%C
	-10.0	0	95	5
	0.0	0	95	5
Gradient	1.0	0	95	15
	30.0	1	74	25
	65.0	20	50	30



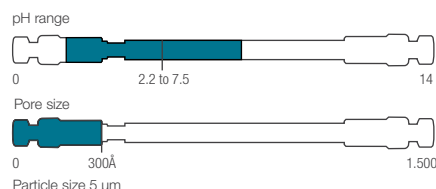
### GlycanPac AXR-1 columns

Particle size (μm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
1.9	UHPLC column	150	<a href="#">088136</a>	—	—
		250	<a href="#">088135</a>	—	—
—	Guard cartridge holder	—	<a href="#">069580</a>	<a href="#">069580</a>	<a href="#">069580</a>

# Aggregate fragment analysis

For mAb samples, our 300 Å silica Thermo Scientific™ MAbPac™ SEC-1 provides separation of aggregates and fragments samples to characterize your analyte by LC-UV or LC-MS.

## MAbPac SEC-1 column

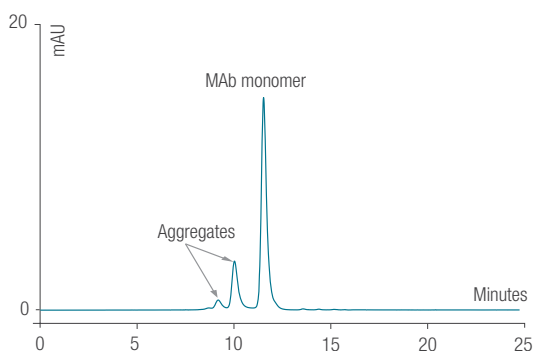


### Additional reading

Links	Type	Description
	Application note	Lifetime stability of size-exclusion chromatography columns for protein aggregate analysis
	Application note	Analysis of monoclonal antibodies and their fragments
	Learn more <a href="https://www.thermofisher.com/biolc">thermofisher.com/biolc</a>	

### Monoclonal antibody aggregate separation

MAbPac SEC-1, 5 µm, 300 x 4.0 mm (PEEK)	
Flow rate	0.20 mL/min
Mobile phase	0.3 mM NaCl in 50 mM phosphate buffer pH 6.8
Gradient	0% B for 0.2 mins, 100% B for 0.60 mins, 0% B for 1.20 mins
Temperature	30 °C
Injection volume	2 µL
Detection	280 nM
Sample	mAb (10 mg/mL)



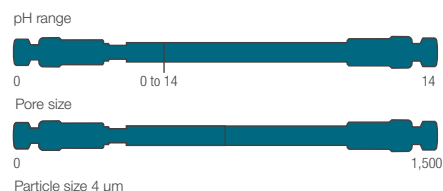
### MAbPac SEC-1 columns

Particle size (µm)	Format	Length (mm)	2.1 mm ID	4.0 mm ID	7.8 mm ID
5	Guard column	50	—	<a href="#">074697</a>	—
—	HPLC column	150	<a href="#">088790</a>	<a href="#">075592</a>	—
—		300	<a href="#">088789</a>	<a href="#">074696</a>	<a href="#">088460</a>

# Intact and subunit analysis (RP)

The wide pore (1500 Å) polymeric Thermo Scientific™ MAbPac™ RP columns offers high resolution separation and minimal carryover for monoclonal antibody samples. Excellent lifetime and ability to separate intact and protein subunits, compatible with LC-UV and LC-MS/MS applications. The monolithic Thermo Scientific™ ProSwift™ RP columns offer unique selectivity, high throughput separations for a wide range of protein sizes. These columns provide high loadability and operate under very low backpressure.

## MAbPac RP column

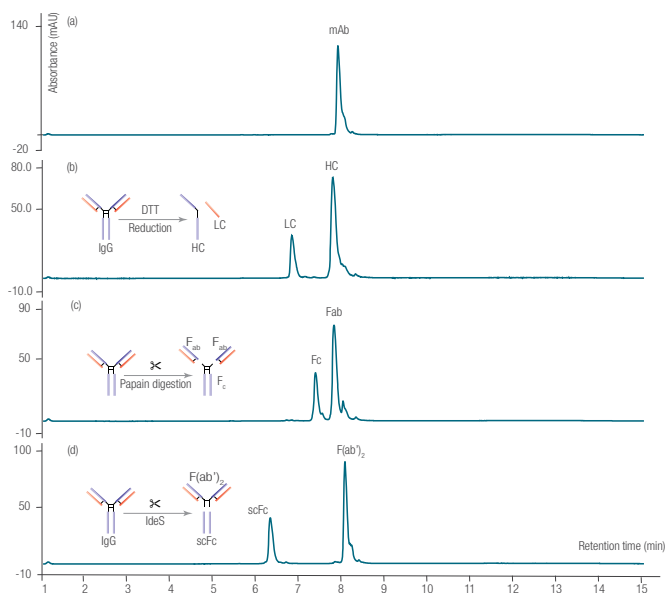


### Additional reading

Links	Type	Description
	Application note	Confident monoclonal antibody sequence verification by complementary LC-MS techniques
	Application note	Fast analysis of therapeutic monoclonal antibody fragments
	Learn more <a href="https://thermofisher.com/biolc">thermofisher.com/biolc</a>	

### mAb and mAb fragments analysis

MAbPac RP, 4 μm, 50 x 3.0 mm			
Flow rate	0.5 mL/min		
Mobile phase A	H <sub>2</sub> O/FA/TFA (99.88 : 0.1 : 0.02 v/v/v)		
Mobile phase B	ACN/H <sub>2</sub> O/FA/TFA 90 : 9.88 : 0.1 : 0.02 v/v/v/v/v		
Temperature	80 °C		
Injection volume	5 μL		
Detection	UV at 280 nm		
Sample	(a) trastuzumab (5 mg/mL)		
	(b) trastuzumab + DTT (4 mg/mL)		
	(c) trastuzumab + Papain (2 mg/mL)		
	(d) trastuzumab + IdeS (2 mg/mL)		
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	
16.0	80	20	



# Intact and subunit analysis (RP) continued



## MABPac RP columns

Particle size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID
4	Guard cartridges (2/pk)	10	<a href="#">088649</a>	<a href="#">088646</a>
		50	<a href="#">088648</a>	<a href="#">088645</a>
	HPLC column	100	<a href="#">088647</a>	<a href="#">088644</a>
		150	<a href="#">303270</a>	<a href="#">303269</a>
—	Guard cartridge holder	—	<a href="#">069580</a>	<a href="#">069580</a>

## MABPac RP 1 mm columns

Particle size (µm)	Length (mm)	1 mm ID
4	50	<a href="#">303182</a>
	100	<a href="#">303183</a>
	150	<a href="#">303184</a>



### Webinars

Analytical and life science webinars live and on-demand

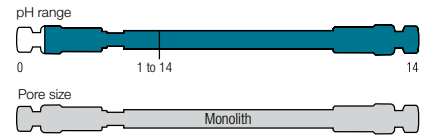


### NIBRT collaboration information

A collaboration built for Biopharma between the National Institute for Bioprocessing Research and Training (NIBRT) and Thermo Fisher Scientific [thermofisher.com/nibrt](https://www.thermofisher.com/nibrt)

# Intact and subunit analysis (RP) continued

## ProSwift RP column



### Additional reading

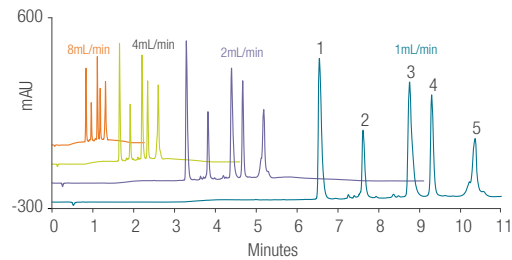
Links	Description
-------	-------------

Learn more [thermofisher.com/biolc](https://www.thermofisher.com/biolc)

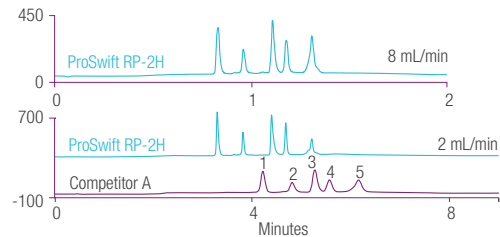
### ProSwift column

ProSwift RP-2H, 50 x 4.6 mm	
Flow rate	1, 2, 4, or 8 mL/min
Mobile phase A	H <sub>2</sub> O/ACN (95:5; V/V) + 0.1% TFA
Mobile phase B	H <sub>2</sub> O/ACN (5:95; V/V) + 0.1% TFA
Injection volume	2 µL
Detection	UV at 214 nm
Sample	Mixture of five proteins
Gradient	1 mL/min: 1-75% B in 12 min 2 mL/min: 1-75% B in 6 min 4 mL/min: 1-75% B in 3 min 8 mL/min: 1-75% B in 1.5 min
Analytes	1. Ribonuclease A 1.5 mg/mL 2. Cytochrome C 0.5 mg/mL 3. BSA 1.5 mg/mL 4. Carbonic anhydrase 0.9 mg/mL 5. Ovalbumin 1.5 mg/mL

### Proteins



### Competitive comparison



### ProSwift RP columns

Functional group	Length (mm)	1.0 mm ID	4.6 mm ID
RP-1S	50	—	<a href="#">064297</a>
RP-2H	50	—	<a href="#">064296</a>
RP-3U	50	—	<a href="#">064298</a>
RP-4H	50	<a href="#">069477</a>	—
	250	<a href="#">066640</a>	—

# Charge variant analysis

For charge variant analysis by LC-UV or LC-MS/MS Thermo Scientific™ ProPac™ 3R SAX and Thermo Scientific™ ProPac™ 3R SCX columns deliver outstanding resolution on a highly robust, reproducible and high-resolution platform. Combine ProPac 3R SCX columns with our proprietary CX-1 buffers formulations to enable fast, robust and reproducible pH gradients that are simple to optimize and easily automated - without the need for time-consuming mobile phase adjustments.

## Protein isoelectric point (pI)

<7

>7

### ProPac 3R SAX column

- Works well with salt and pH gradient buffers
- Best choice for proteins with acidic pI
- Analyze full/empty AAV capsid ratios

### ProPac 3R SCX column

- Highest resolution with excellent reproducibility
- Works well with CX-1 buffers

### MABPac SCX-10 column

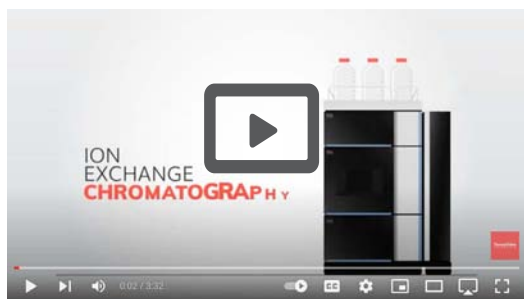
- Alternative selectivity to WCX, scalable from short methods analysis to semi-prep formats
- Works well with CX-1 buffers

### ProPac WCX-10 column

- Industry GOLD standard – widely used and published

### ProPac Elite WCX column

- Improved resolution, speed and reproducibility over ProPac WCX-10 column
- Works well with CX-1 buffers



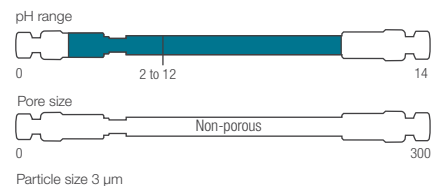
### Video:

Tips to improve your charge variant analysis by ion exchange



# Charge variant analysis continued

## ProPac 3R SCX column

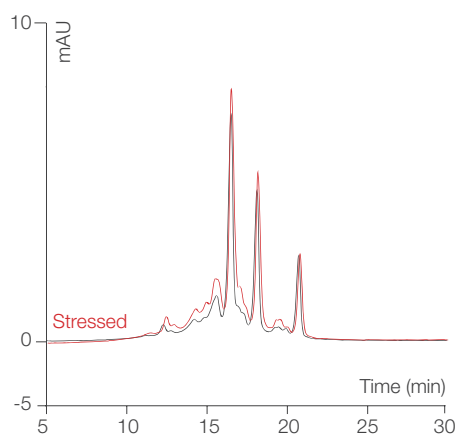


### Additional reading

Links	Type	Description
	Application note	Salt gradient analysis of monoclonal antibodies using a 3 μm monodisperse SCX column
	Application note	Method development for pH gradient analysis of monoclonal antibodies using SCX column
	Learn more <a href="https://thermofisher.com/propac">thermofisher.com/propac</a>	

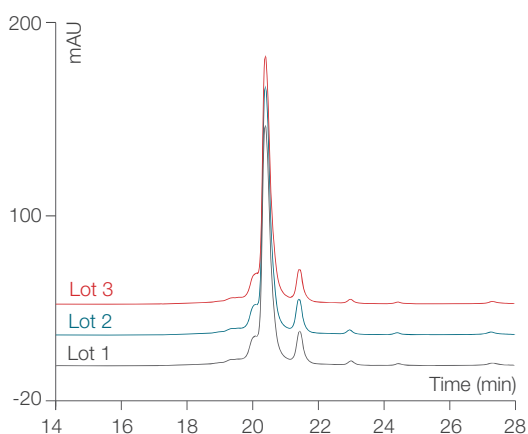
### Salt gradient analysis of infliximab

ProPac 3R SCX column, 3 μm			
Format	4 × 100 mm		
Mobile phase	A: 20 mM MES, pH 6.5 B: 20 mM MES, pH 6.5 + 0.5 M NaCl		
Flow rate	0.3 mL/min		
Injection	2 μL		
Temp	30 °C		
Detection	UV, 280 nm		
Sample	Infliximab – 5 mg/mL		
Gradient	%A	%B	
Time (min)	0.0	93	7
	30.0	78	22
	30.1	20	80
	33.0	20	80
	33.1	93	7
	40.0	93	7



### Lot-to-lot reproducibility of NISTmAb salt gradient separation

ProPac 3R SCX column, 3 μm			
Format	4 × 100 mm		
Mobile phase	A: 20 mM MES, pH 6.5 B: 20 mM MES, pH 6.5 + 0.5 M NaCl		
Flow rate	0.3 mL/min		
Injection	2 μL		
Temp	30 °C		
Detection	UV, 280 nm		
Sample	NISTmAb – 10 mg/mL		
Gradient	%A	%B	
Time (min)	0.0	95	10
	30.0	75	30
	30.1	20	80
	33.0	20	80
	33.1	95	10
	40.0	95	10

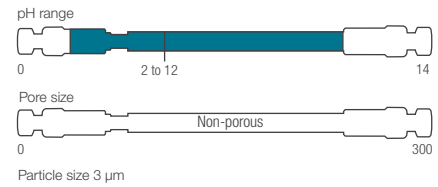


### ProPac 3R SCX 3 μm columns

Particle size (μm)	Length (mm)	2.0 mm ID	4.0 mm ID
3	50	<a href="#">43103-052068</a>	<a href="#">43103-054068</a>
	100	<a href="#">43103-102068</a>	<a href="#">43103-104068</a>

# Charge variant analysis continued

## ProPac 3R SAX column

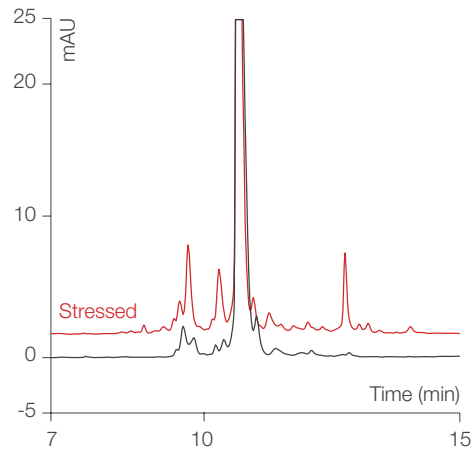


### Additional reading

Links	Type	Description
	Application note	Salt gradient analysis of Protein G using a 3 µm monodisperse SAX column
	Application note	Salt gradient separation and analysis of adeno-associated virus samples using SAX column
	Learn more <a href="https://www.thermofisher.com/propac">thermofisher.com/propac</a>	

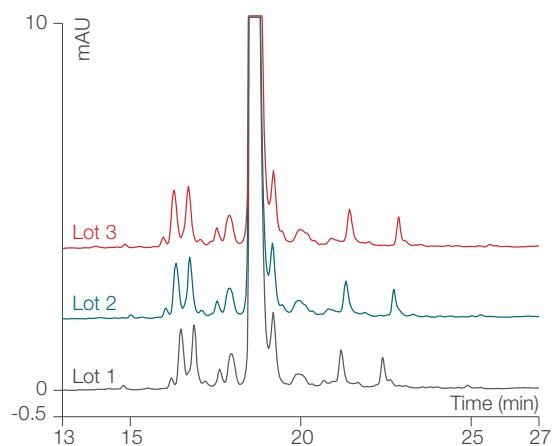
### Salt gradient analysis of protein G

ProPac 3R SAX column, 3 µm			
Format	4 × 100 mm		
Mobile phase	A: 20 mM Tris, pH 8.0 B: 20 mM Tris + 500 mM NaCl, pH 8.0		
Flow rate	0.5 mL/min		
Injection	1 µL		
Temp	30 °C		
Detection	UV, 280 nm		
Sample	Protein G – 5 mg/mL		
Gradient	%A	%B	
Time (min)	0.0	88	12
	1.0	88	12
	16.0	58	42
	16.1	0	100
	18.0	0	100
	18.1	88	12
	30.0	88	12



### Lot-to-lot reproducibility of protein G salt gradient separation

ProPac 3R SAX column, 3 µm			
Format	4 × 100 mm		
Mobile phase	A: 20 mM Tris, pH 8.0 B: 20 mM Tris + 500 mM NaCl, pH 8.0		
Flow rate	0.5 mL/min		
Injection	1 µL		
Temp	30 °C		
Detection	UV, 280 nm		
Sample	Protein G – 5 mg/mL		
Gradient	%A	%B	
Time (min)	0.0	88	12
	1.0	88	12
	31.0	58	42
	31.1	0	100
	33.0	0	100
	33.1	88	12
	45.0	88	12

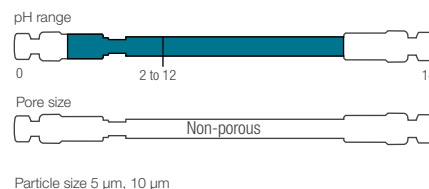


### ProPac 3R SAX 3 µm columns

Particle size (µm)	Length (mm)	2.0 mm ID	4.0 mm ID
3	50	<a href="#">43203-052068</a>	<a href="#">43203-054068</a>
	100	<a href="#">43203-102068</a>	<a href="#">43203-104068</a>

# Charge variant analysis continued

## MABPac SCX-10 column

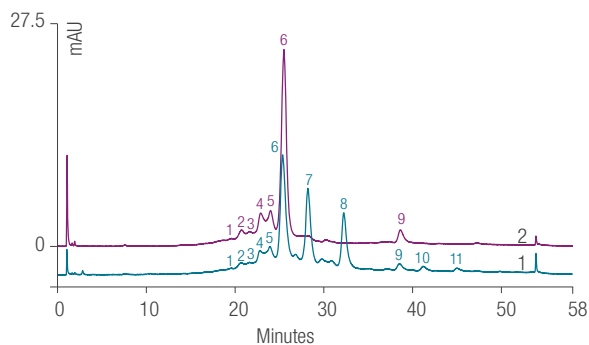


### Additional reading

Links	Type	Description
	Application note	A global pH-gradient based charge variant analysis
	Application note	High throughput, high resolution monoclonal antibody analysis
	Learn more <a href="https://www.thermofisher.com/biolc">thermofisher.com/biolc</a>	

### Baseline resolution of C-terminal lysine variants of a monoclonal antibody

MABPac SCX-10, 5 µm, 250 x 4.0 mm	
Flow rate	1 mL/min
Mobile phase A	20 mM MES (pH 5.6) + 60 mM NaCl
Mobile phase B	20 mM MES (pH 5.6) + 300 mM NaCl
Gradient	15–36% B in 50 min
Temperature	30 °C
Injection volume	5 µL
Detection	UV at 280 nm
Sample	1. mAb B, 900 µg in 100 µL (no carboxypeptidase) 2. mAb B, 900 µg in 100 µL + carboxypeptidase, 50 µg, incubation at 37 °C for 3 h
Both chromatograms	Peaks 1–5: acidic variants
Sample 1	Peaks 6-8: C-Terminal lysine truncation variants of main peak. Peaks 9-11: C-Terminal lysine truncation variants of minor variant peak
Sample 2	Peak 6 results from peaks 6, 7, and 8 after CBP treatment. Peak 9 results from peaks 9, 10, and 11 after CBP treatment

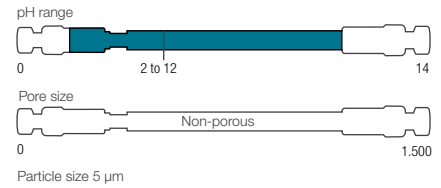


### MABPac SCX-10 columns

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID
5	HPLC column	50	—	<a href="#">078656</a>	—
		150	—	<a href="#">085198</a>	—
		250	—	<a href="#">078655</a>	—
10	HPLC column	50	<a href="#">075749</a>	<a href="#">074631</a>	—
		150	—	<a href="#">075603</a>	—
		250	<a href="#">075604</a>	<a href="#">074625</a>	<a href="#">088784</a>

# Charge variant analysis continued

## MABPac SCX-10 RS column

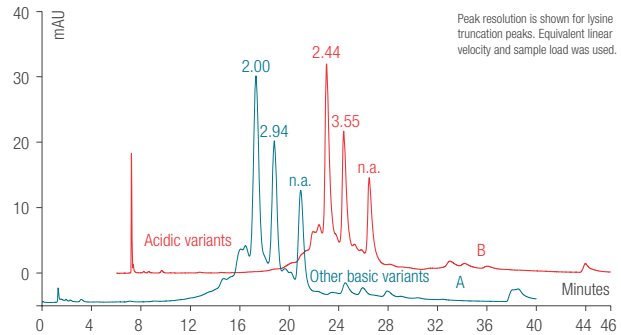


### Additional reading

Links	Description
 <a href="https://www.thermofisher.com/biolc">Learn more thermofisher.com/biolc</a>	

### Lysine variants

MABPac SCX, 5 µm, 250 x 4.6 mm	
Flow rate	1.5 mL/min
Mobile phase A	20 mM MES pH 5.6 + 60 mM
Mobile phase B	20 mM MES pH 5.6 + 3 mM NaCl
Injection volume	15 µL
Detection	UV at 280 nm
Sample	mAb 5 mg/mL
Both chromatograms	Peaks 1–5: acidic variants
Chromatogram A	Gradient: 33-53% B in 30 min
Chromatogram B	Gradient: 33-53% B in 20 min

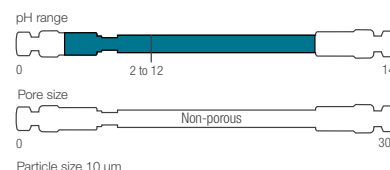


### MABPac SCX-10 RS columns

Particle size (µm)	Format	Length (mm)	2.1 mm ID	4.6 mm ID
5	UHPLC column	50	<a href="#">082675</a>	<a href="#">082674</a>
		150	<a href="#">088242</a>	<a href="#">085209</a>
		250	<a href="#">082515</a>	<a href="#">082673</a>

# Charge variant analysis continued

## ProPac SAX-10 column

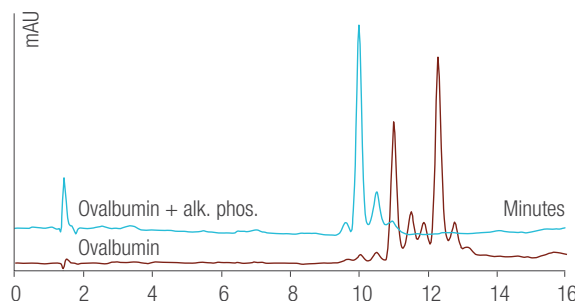


### Additional reading

Links	Description
 <a href="https://thermofisher.com/biolc">Learn more thermofisher.com/biolc</a>	

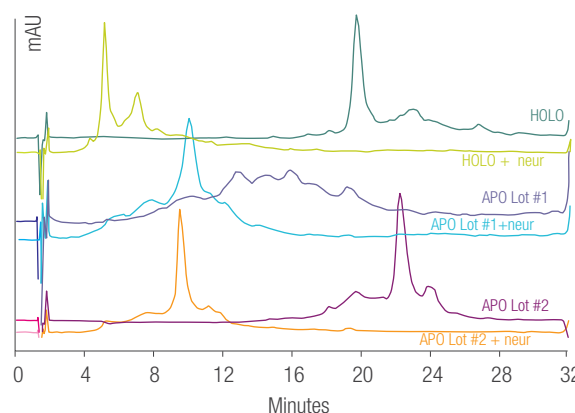
### Resolution of phosphorylation variants of ovalbumin

ProPac SAX-10, 10 µm, 250 x 4.0 mm				
Flow rate	1.0 mL/min			
Mobile phase A	Water			
Mobile phase B	2.0 mM NaCl			
Mobile phase C	0.1 mM Tris/HCl (pH 8.5)			
Injection volume	1.0 µL			
Detection	UV at 214 nm			
Sample	Ovalbumin before and after alkaline phosphatase treatment			
Gradient	Time (min)	%A	%B	%C
	0.0	80	0	20
	15.0	67.5	12.5	20



### Effect of sialylation on transferrin chromatography

ProPac SAX-10, 10 µm, 250 x 4.0 mm				
Flow rate	1.0 mL/min			
Mobile phase A	Water			
Mobile phase B	2.0 mM NaCl			
Mobile phase C	0.2 mM Tris/HCl (pH 9)			
Injection volume	50.0 µL			
Detection	UV at 214 nm			
Sample	HOLO (iron rich) and APO (iron poor) human transferrin samples before and after neuraminidase treatment. Digestions were carried out overnight at 37 °C in sodium acetate buffer at pH 5.			
Gradient	Time (min)	%A	%B	%C
	0.0	87	3	10
	30.0	83	7	10

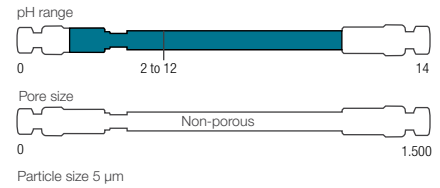


### ProPac SAX-10 columns

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22.0 mm ID	4 x 50 mm ID
10	Guard column	50	<a href="#">063454</a>	<a href="#">054998</a>	–	–	–
	HPLC column	250	<a href="#">063448</a>	<a href="#">054997</a>	<a href="#">063703</a>	<a href="#">088770</a>	<a href="#">078990</a>

# Charge variant analysis continued

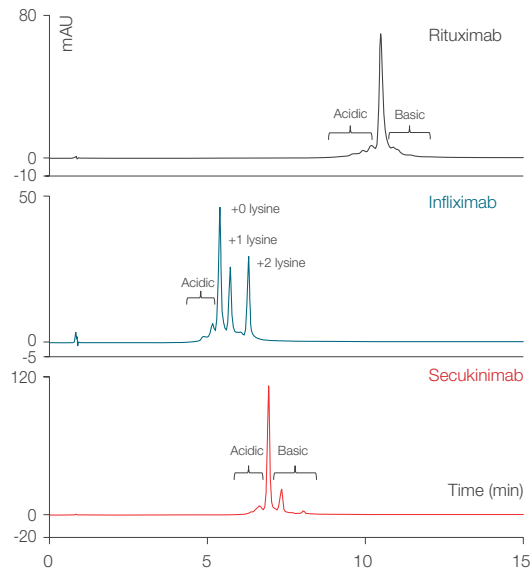
## ProPac Elite WCX column



### Additional reading

Links	Type	Description
	Application note	Confident monoclonal antibody sequence verification by complementary LC-MS techniques
	Application note	Fast analysis of therapeutic monoclonal antibody fragments
	Learn more <a href="https://www.thermofisher.com/biolc">thermofisher.com/biolc</a>	

ProPac Elite WCX, 5 µm, 150 x 4.0 mm			
Flow rate	1.0 mL/min		
Mobile phase A	1x CX-1 pH Gradient buffer A		
Mobile phase B	1x CX-1 pH Gradient buffer B		
Temperature	30 °C		
Injection volume	2 µL		
Detection	UV at 280 nm		
Sample	Top: rituximab, 5 mg/mL		
	Middle: infliximab, 5 mg/mL		
	Bottom: secukinimab, 5 mg/mL		
	Time (min)	%A	%B
Gradient	0.0	80	20
	15.0	20	80
	15.1	0	100
	17.0	0	100
	17.1	80	20
25.0	80	20	



### ProPac Elite WCX columns

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID
5	HPLC column	50	<a href="#">303028</a>	<a href="#">302973</a>
		100	<a href="#">303027</a>	<a href="#">302972</a>
		250	<a href="#">303026</a>	<a href="#">303025</a>

### ProPac Elite WCX kits

Particle size (µm)	Set contents	Length (mm)	4.0 mm ID
5	3 columns from 1 lot	150	<a href="#">302976</a>
	3 columns from 3 lots	150	<a href="#">302977</a>
	3 columns from 1 lot	250	<a href="#">303061</a>
	3 columns from 3 lots	250	<a href="#">303062</a>

# Charge variant analysis continued

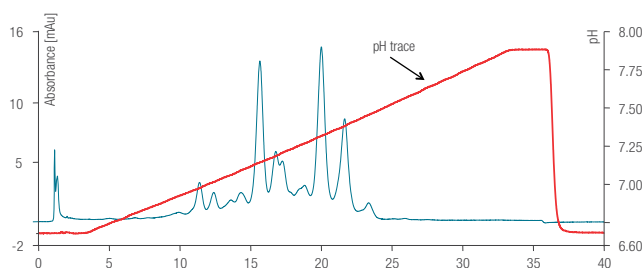
## pH gradient buffers

Ready-to-use buffers for simple method development during charge variant characterization

Thermo Scientific™ pH gradient platform accelerates method development and facilitates method transfer to QA/QC for a wide range of protein and mAb charge variants through a generic LC-based approach to charge variant characterization.

- Patented buffer formulations enable fast, robust and reproducible pH gradients that are simple to optimize and easily automated
- Ready to use with existing LC columns and systems, without the need for time consuming mobile phase adjustments
- Applicable to the majority of mAbs

Optimization of mAb charge variant separation using a linear pH gradient: 25% B (pH 6.75) to 50% B (pH 7.9)



### pH gradient buffers

Description	Buffer bottle size			
	125 mL	250 mL	500 mL	1000 mL
CX-1 pH gradient buffer A (pH 5.6)	<a href="#">083273</a>	<a href="#">085346</a>	<a href="#">302779</a>	<a href="#">303274</a>
CX-1 pH gradient buffer B (pH 10.2)	<a href="#">083275</a>	<a href="#">085348</a>	<a href="#">302780</a>	<a href="#">303275</a>



#### NIBRT collaboration information




A collaboration built for Biopharma between the National Institute for Bioprocessing Research and Training (NIBRT) and Thermo Fisher Scientific

[thermofisher.com/nibr](http://thermofisher.com/nibr)

# Peptide mapping and MAM

Thermo Scientific™ Hypersil GOLD™ VANQUISH™ C18 UHPLC columns are an excellent column choice for a broad range of peptides, offering high resolution for all critical quality attributes, without extremely long retention for more hydrophobic peptides. For faster separation of peptide samples select the Thermo Scientific™ Accucore™ C18 VANQUISH™ column. The column offers sub-2 µm particles providing ultra-short diffusion paths that result in extremely efficient separations.

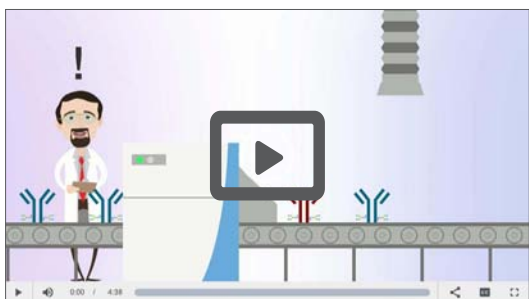
## Additional reading

Links	Type	Description
	Landing page	Multi-Attribute Method (MAM): Straight through to breakthrough
	Learning centre	Biopharmaceutical Multi-Attribute Method (MAM)
	Learn more <a href="https://thermofisher.com/biolc">thermofisher.com/biolc</a>	



### Video:

End-to-end MAM solution to move biopharma forward






### Video:

Learn how innovation and monitoring strategies can reduce the number of tests and enhance the methodology of validating impurity



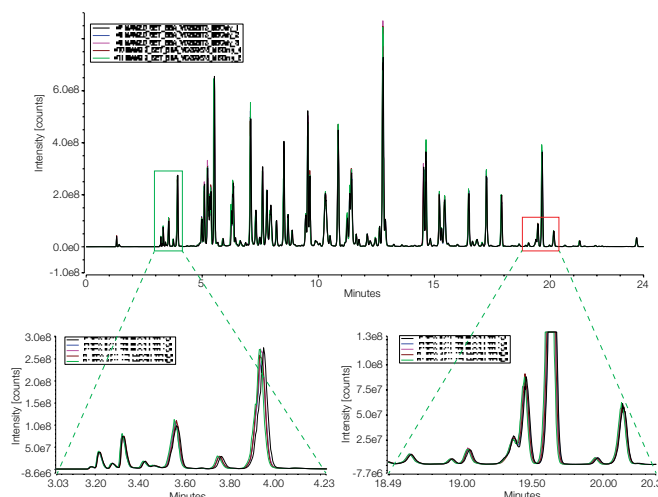
## Hypersil GOLD VANQUISH column

### Additional reading

Links	Type	Description
	Flyer	VANQUISH UHPLC columns. Delivering powerful separations
	Application note	An integrated LC-MS system performance evaluation test
	Learn more <a href="https://thermofisher.com/biolc">thermofisher.com/biolc</a>	

### Overlay of 5 TIC traces from the SET injection sequence

Hypersil GOLD VANQUISH C18 UHPLC column, 150 x 2.1 mm, 1.9 µm	
Flow rate	0.25 mL/min
Mobile phase A	H <sub>2</sub> O + 0.1% FA
Mobile phase B	ACN + 0.1% FA
Injection volume	5 µL
Detection	Mass spectrometer – Full scan
Sample	Pierce BSA protein digest standard, MS grade, UD294474 (P/N 88341)
Chromatogram B	Gradient: 33-53% B in 20 min







### Hypersil GOLD Vanquish columns

Particle size (µm)	Length (mm)	2.1 mm ID
1.9	50	<a href="#">25002-052130-V</a>
	100	<a href="#">25002-102130-V</a>
	150	<a href="#">25002-152130-V</a>

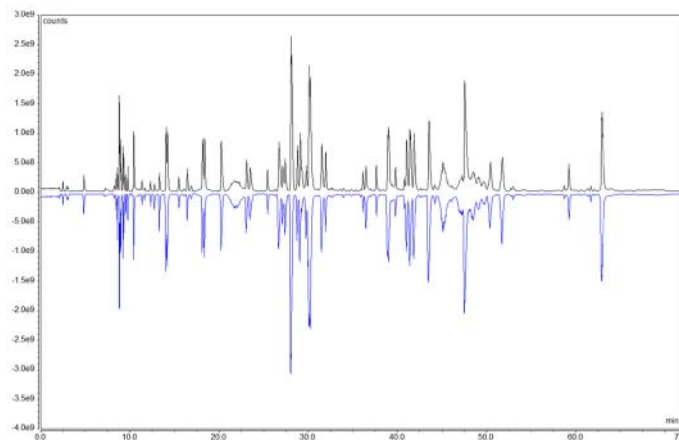
## Accucore VANQUISH C18+ column

### Additional reading

Links	Type	Description
	Application note	Comparative analysis of innovator and biosimilar monoclonal antibodies using MAM method
	Technical guide	Powerful separations are our core performance
	Poster	Application of a MS in QC method for characterization and attribute monitoring
	Learn more <a href="https://thermofisher.com/biolc">thermofisher.com/biolc</a>	

### Mirrored base peak chromatograms of rituximab innovator (black) and its biosimilar product (blue)

Accucore Vanquish C18+ UHPLC column, 1.5 µm, 2.1 × 150 mm	
Flow rate	0.25 mL/min
Mobile phase A	H <sub>2</sub> O + 0.1% FA
Mobile phase B	ACN + 0.1% FA
Injection volume	8 µL
Detection	Mass spectrometer
Sample	Rituximab innovator
Temperature	50 °C



### Accucore Vanquish C18+ columns

Particle size (µm)	Length (mm)	2.1 mm ID
1.5 µm	50 mm	<a href="#">27101-052130</a>
	100 mm	<a href="#">27101-102130</a>
	150 mm	<a href="#">27101-152130</a>

# Nucleic acids/oligonucleotides

Thermo Scientific™ DNAPac™ RP column offers ion-pair reversed phase separations of nucleic acid mixtures. Samples from siRNA to mRNA easily resolve on this polymer chemistry. Compatible with LC-UV and LC-MS/MS methodologies this column delivers outstanding separations.

Thermo Scientific™ DNAPac™ PA200 and Thermo Scientific™ DNAPac™ PA200RS columns are strong anion exchange columns for n-1 separation of oligo samples. Compatible with LC-UV, these columns offer orthogonal separation to reversed phase columns, separating the oligonucleotide sample by size and charge.

Thermo Scientific™ DNASwift™ column is a monolithic column designed for users who would like to do SAX purification of oligonucleotide samples using their analytical HPLC. These monolithic columns offer high loadability, with slightly less resolution than our analytical columns.



## Webinars

Analytical and life science webinars live and on-demand



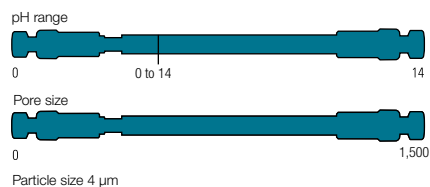
## NIBRT collaboration information

A collaboration built for Biopharma between the National Institute for Bioprocessing Research and Training (NIBRT) and Thermo Fisher Scientific

[thermofisher.com/nibr](https://thermofisher.com/nibr)

# Nucleic acids/oligonucleotides continued

## DNAPac RP column

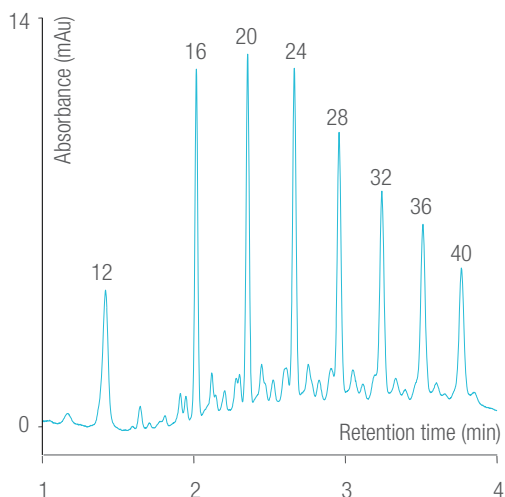


### Additional reading

Links	Description
 <a href="https://www.thermofisher.com/biolc">Learn more thermofisher.com/biolc</a>	

### Fast analysis of mixed base DNA

DNAPac RP, 4 μm, 50 x 2.1 mm			
Flow rate	0.8 mL/min		
Mobile phase A	25 mM HAA, pH 8.5		
Mobile phase B	25 mM HAA, pH 8.5/acetonitrile (50:50 v/v)		
Temperature	65 °C		
Injection volume	4 μL		
Detection	UV at 260 nm		
Sample	8-Combo DNA		
Gradient curve	3		
Peak label	Length of DNA		
	Time (min)	%A	%B
	-0.1	67	33
	0.0	67	33
Gradient	3.0	41	59
	3.1	5	95
	4.9	5	95
	5.0	67	33
	8.0	67	33

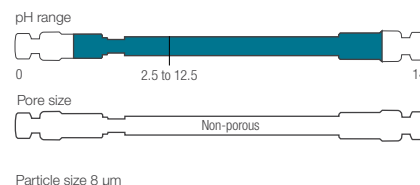


### DNAPac RP columns

Particle size (μm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID
4	Guard cartridges (2/pk)	10	<a href="#">088925</a>	<a href="#">088921</a>
		50	<a href="#">088924</a>	<a href="#">088920</a>
	HPLC column	100	<a href="#">088923</a>	<a href="#">088919</a>
		250	<a href="#">303324</a>	—
—	Guard cartridge holder	—	<a href="#">069580</a>	<a href="#">069580</a>

# Nucleic acids/oligonucleotides continued

## DNAPac PA200 column



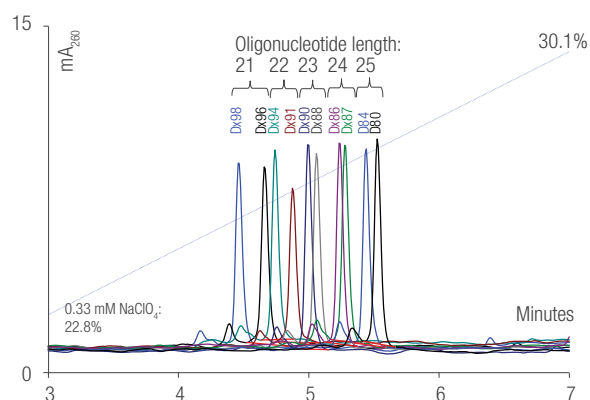
### Additional reading

Links	Description
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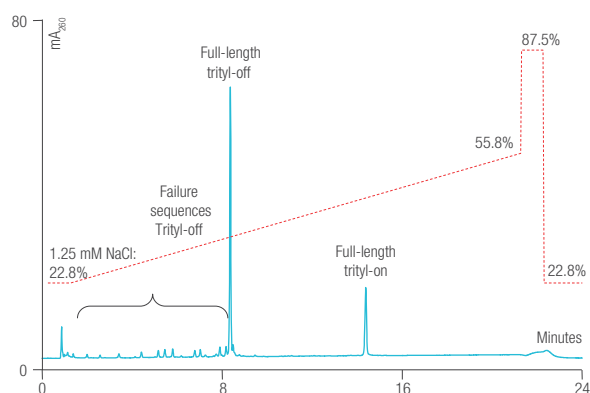
Learn more [thermofisher.com/biolc](https://www.thermofisher.com/biolc)

DNAPac PA200, 8 $\mu\text{m}$ , 250 x 4.0 mm	
Flow rate	1.2 mL/min
Mobile phase	$\text{NaClO}_4$ , pH 6.5 with 20% ACN
Detection	UV at 260 nm
Flow rate	1.2 mL/min

### Separation of oligonucleotides by length



### Target, failure and trityl-on oligonucleotides

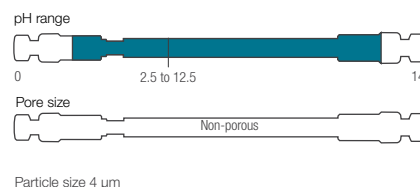


### DNAPac PA200 columns

Particle size ( $\mu\text{m}$ )	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22.0 mm ID
8	Guard column	50	<a href="#">063423</a>	<a href="#">062998</a>	<a href="#">063419</a>	<a href="#">088780</a>
	HPLC column	250	<a href="#">063425</a>	<a href="#">063000</a>	<a href="#">063421</a>	<a href="#">088781</a>

# Nucleic acids/oligonucleotides continued

## DNAPac PA200 RS column



### Additional reading

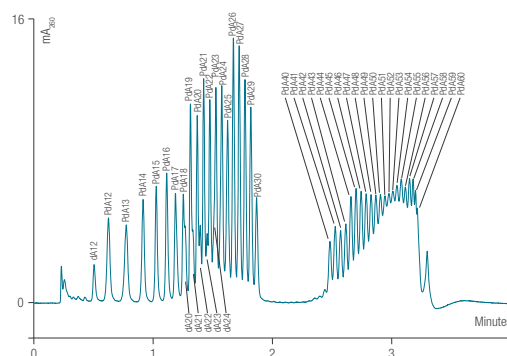
Links	Type	Description
	Brochure	Superior oligonucleotide analysis
	Application note	High resolution separation of oligonucleotides
	Application note	Ultra-high-resolution separation of oligonucleotides by UHPLC
	Application note	Separation of mixed-base oligonucleotides
	Learn more <a href="https://www.thermofisher.com/biolc">thermofisher.com/biolc</a>	

### Partial resolution of 46 oligonucleotides

DNAPac PA200 RS, 4 μm, 50 x 4.6 mm	
Flow rate	1.30 mL/min
Mobile phase A	20 mM Tris pH 8
Mobile phase B	A + 1.25 mM NaCl
Temperature	30 °C
Injection volume	2.5 μL
Gradient	28–43% B in 4 CV* (2.56 min) curve 3**
Sample	PdA12–30, 40–60

\*CV = column volumes

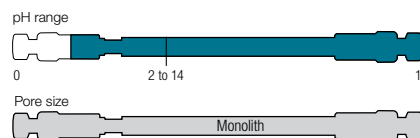
\*\*Curve 3 indicates continuously changing gradient, asymptotically approaching a maximum salt concentration. Programed in Thermo Scientific™ Chromeleon™ 6.8.



### DNAPac PA200 RS columns

Particle size (μm)	Format	Length (mm)	4.6 mm ID
4	BioRS column	50	<a href="#">082508</a>
		150	<a href="#">082509</a>
		250	<a href="#">082510</a>

## DNASwift SAX-1S column

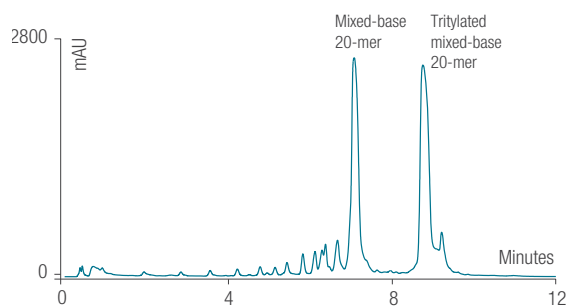


### Additional reading

Links	Description
<a href="https://www.thermofisher.com/biolc">Learn more thermofisher.com/biolc</a>	

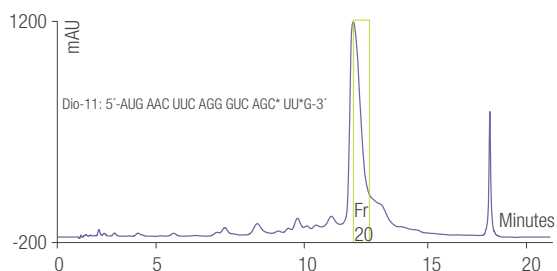
### Tritylated oligonucleotide

DNASwift SAX-1S, 150 x 5.0 mm	
Flow rate	1.5 mL/min
Mobile phase A	15 mM Tris, pH 8
Mobile phase B	15 mM Tris, pH 8, 1.25 M NaCl
Temperature	30 °C
Injection volume	20 µL
Detection	UV at 260 nm
Gradient	8–64% B in 10 min



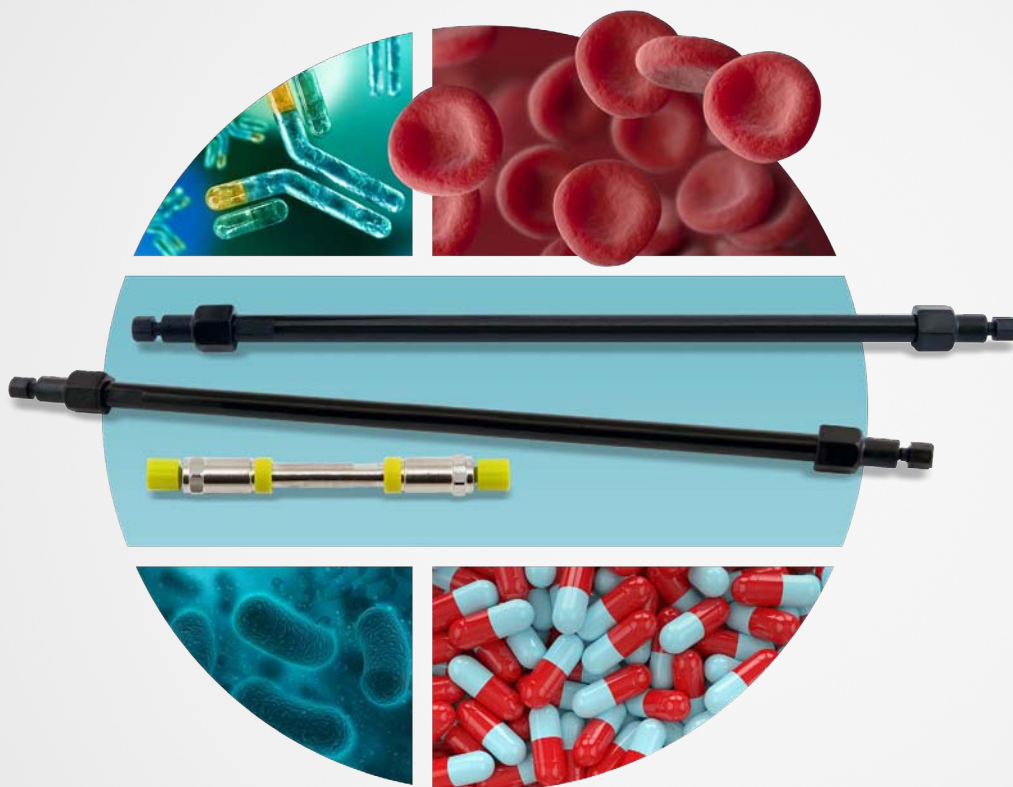
### Purification of a 21-base RNA sample with aberrant 2'-5' linkages at the 1 and 3 positions from the 3' end

DNASwift SAX-1S, 150 x 5.0 mm	
Flow rate	1.5 mL/min
Mobile phase A	40 mM Tris, pH 7
Mobile phase B	40 mM Tris, pH 7 + 1.25 M NaCl
Temperature	30 °C
Injection volume	125 µg
Detection	UV at 260 nm
Gradient	26–42% B in 10 column volumes



### DNASwift SAX-1S column

Length (mm)	5.0 mm ID
150	<b>066766</b>



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