

For HPLC/UHPLC



**Develosil® FlexFire WP C4**

**NOMURA CHEMICAL CO., LTD.**



## Develosil® FlexFire WP C4

### For analysis of high molecular compounds such as proteins and antibodies!!

FlexFire WP C4 is a column targeting high molecular compounds such as proteins and antibodies. The pH durability and temperature durability have been significantly updated compared to the past so that they can be used under all analytical conditions, and will greatly contribute to future biopharmaceutical analysis.

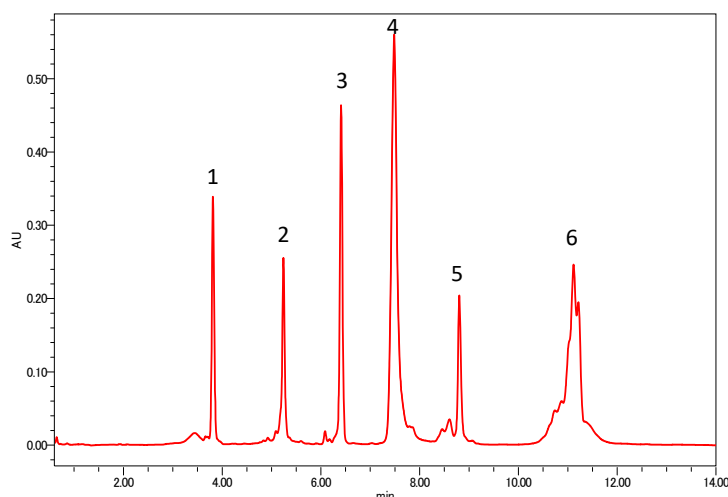
### Spec of FlexFire WP C4

	FlexFire WP C4
Particle size	2.6µm, 5µm
Chemistry	Butyl
Surface area	170m <sup>2</sup> /g
Pore volume	1.4mL/g
Pore diameter	30nm
Carbon	5.0%
End-cap	あり
pH	pH1-10
Temperature	~80°C
Max pressure	2.6µm: 600bar (=60MPa=8,702psi)
	5µm: 300bar (=30MPa=4,351psi)

FlexFire WP C4 elutes proteins with different properties based on their differences in hydrophobicity and is primarily aimed at characterizing the primary structure.

It can also be applied to the analysis of intact mAbs as well as proteins. Intact mAb requires high temperature conditions, but FlexFire WP C4 has a durability of 80°C, so a sharp peak can be eluted.

### ■ Analysis of Protein standard



#### Conditions:

Column: Develosil FlexFire WP C4, 2.6µm (2.0x50mm)  
 Mobile phase: A) Water + 0.1%TFA  
 B) Acetonitrile + 0.1%TFA

#### Gradient:

Time	mL/min	%A	%B	Curve
0.00	0.3	80	20	
12.60	0.3	40	60	6
12.63	0.3	80	20	6

#### Temperature:

40°C

#### Detection:

UV220nm

#### Sample:

- Ribonuclease A (0.15mg/mL) M.W.=13,683
- Cytochrome C (0.20mg/mL) M.W.=11,702
- Lysozyme (0.20mg/mL) M.W.=14,314
- BSA (0.68mg/mL) M.W.=66,296
- Myoglobin (0.20mg/mL) M.W.=11,199
- Ovalbumin (1.84mg/mL) M.W.=42,699

#### Injection volume:

5.0µL

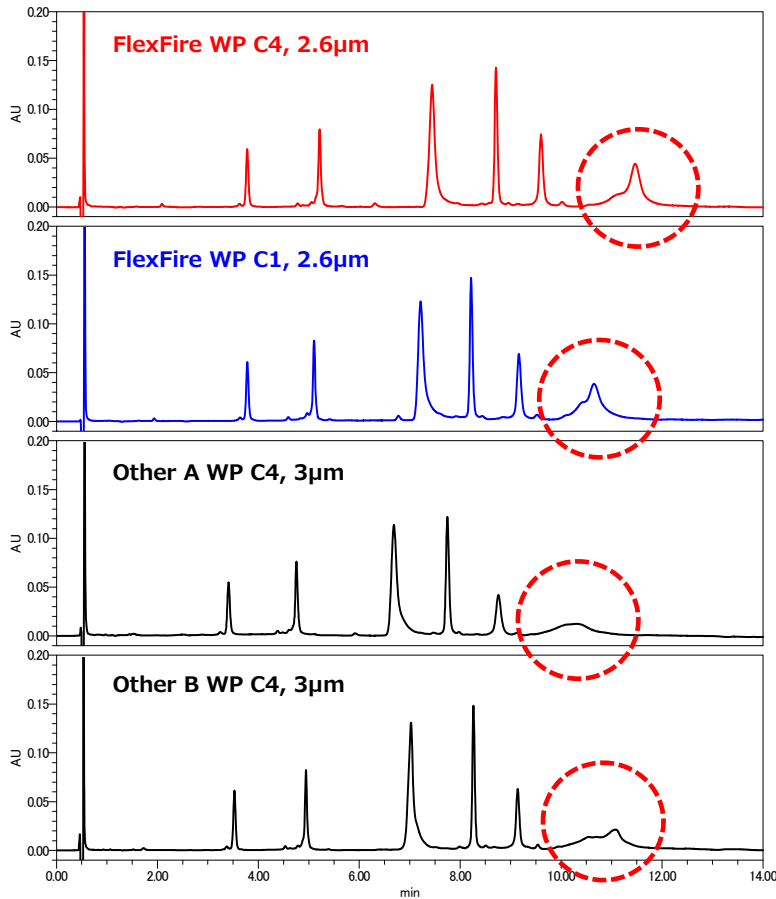
#### System:

Waters ACQUITY UPLC H-Class Plus

#### Mixer:

100µL

## Comparison of Protein separation



### Conditions:

Column: Develosil FlexFire WP C4, 2.6µm (2.0x50mm)  
 Develosil FlexFire WP C1, 2.6µm (2.0x50mm)  
 Other A WP C4, 3µm (2.0x50mm)  
 Other B WP C4, 3µm (2.0x50mm)

Mobile phase: A) Water + 0.1%TFA  
 B) Acetonitrile + 0.1%TFA

### Gradient:

Time	mL/min	%A	%B	Curve
0.00	0.3	80	20	
12.60	0.3	40	60	6
12.63	0.3	80	20	6

### Temperature:

40°C

### Detection:

UV220nm

### Sample:

- Ribonuclease A (0.15mg/mL) M.W.=13,683
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### Injection volume:

5.0µL

### System:

Waters ACQUITY UPLC H-Class Plus

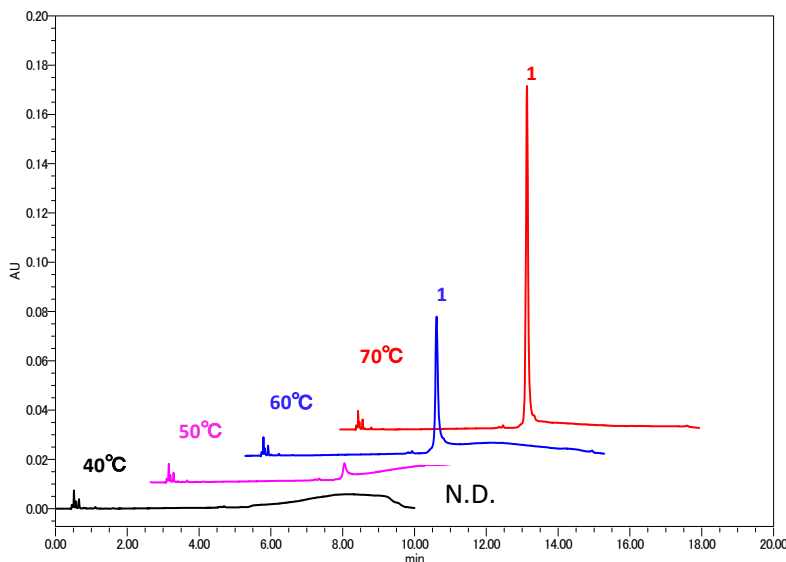
### Mixer:

100µL

Focusing on ovalbumin, we compared FlexFire WP C4 with other companies' columns.

The peak top can be recognized in FlexFire WP C4, C1, but it is broad in other companies A and B. As mentioned in the previous section, it is considered that they are affected by secondary effects.

## Analysis of Intact mab – Peak detection by temperature condition–



### Conditions:

Column: Develosil FlexFire WP C4, 2.6µm (2.0x50mm)  
 Mobile phase: A) Water + 0.1%TFA  
 B) Acetonitrile + 0.1%TFA

### Gradient:

Time	mL/min	%A	%B	Curve
0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

### Temperature:

40°C, 50°C, 60°C, 70°C

### Detection:

UV280nm

### Sample:

- Intact Mouse IgG1 (5.0mg/mL)

### Injection volume:

1.0µL

### System:

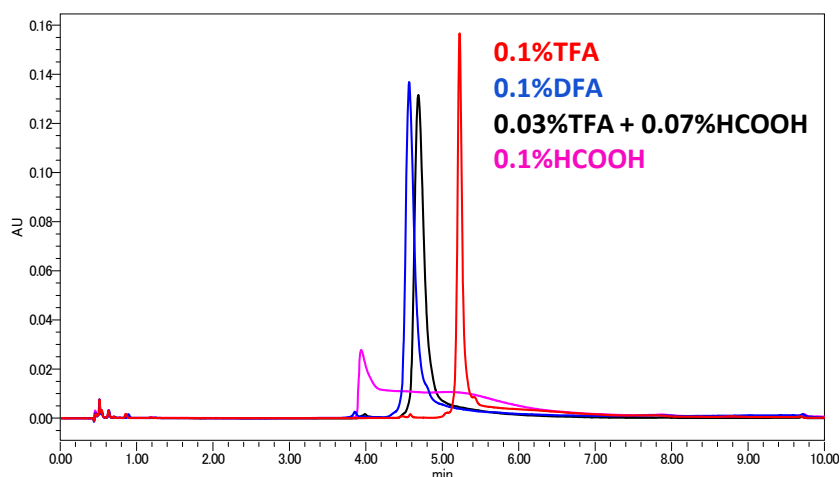
Waters ACQUITY UPLC H-Class

### Mixer:

100µL

Temperature setting is an important parameter in the analysis of intact mAbs. Although it was not detected at 40°C, the peak came to be detected by increasing the temperature, and an extremely sharp peak could be obtained at 70°C.

## Analysis of Intact mAb – Peak detection due to difference in mobile phase–



**Conditions:**  
 column: Develosil FlexFire WP C4, 2.6 $\mu$ m (2.0x50mm)  
 Mobile phase: A) Water + 0.1%TFA  
 B) Acetonitrile + 0.1%TFA  
 A) Water + 0.1%DFA  
 B) Acetonitrile + 0.1%DFA  
 A) Water + 0.03%TFA+0.07%HCOOH  
 B) Acetonitrile + 0.03%TFA+0.07%HCOOH  
 A) Water + 0.1%HCOOH  
 B) Acetonitrile + 0.1%HCOOH

Gradient:	min	Flow	%A	%B	Curve
	0.00	0.3	80	20	6
	8.40	0.3	40	60	6
	8.42	0.3	80	20	6

Temperature: 70 $^{\circ}$ C  
 Detection: UV280nm  
 Sample: Intact Mouse IgG1  
 Injection volume: 0.2 $\mu$ L  
 System: Waters ACQUITY UPLC H-Class Plus  
 Mixer: 100 $\mu$ L

Intact mAbs were analyzed with different mobile phases to reduce the risk when using LC/MS. It is difficult to maintain the peak shape with 0.1% formic acid, but a small amount of TFA and most of formic acid showed almost the same results as 0.1% DFA.

By using very few ion pair reagents, risk can be reduced and detection range can be extended.

## Order Information

Product	Particle	Size (i.d.XL)	Sutainless	Metal-Free
Flex Fire WP C4	2.6 $\mu$ m	2.0x35mm	310-L20035W	310-L20035MFW
		2.0x50mm	310-L20050W	310-L20050MFW
		2.0x75mm	310-L20075W	310-L20075MFW
		2.0x100mm	310-L20100W	310-L20100MFW
		2.0x150mm	310-L20150W	310-L20150MFW
		2.0x250mm	ASK	ASK
	5 $\mu$ m	2.0x35mm	310-520035W	310-520035MFW
		2.0x50mm	310-520050W	310-520050MFW
		2.0x75mm	310-520075W	310-520075MFW
		2.0x100mm	310-520100W	310-520100MFW
		2.0x150mm	310-520150W	310-520150MFW
		2.0x250mm	ASK	ASK

### ■ Contact us



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