mAb Subunit Standard

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I. INTRODUCTION

Waters® mAb Subunit Standard can be used in the benchmarking of LC and LC-MS techniques, proficiency testing among different instruments and laboratories, and system suitability. This standard is a filtered and stabilized formulation of reduced, IdeS-digested NIST Reference Material 8671 (NIST mAb), a humanized IgG1κ expressed from a murine cell line. Each vial of the standard is provided in an approximately 25 µg quantity.

Note: Reduced and denatured samples of IdeS-digested mAbs are usually prone to degradation due to their exposure to high concentrations of reductant and denaturant. For this reason, the mAb Subunit Standard is filtered prior to lyophilization to be free of such complications. In addition, it is carefully formulated with stabilizing excipients to extend its long-term stability. These specific excipients were selected for this standard to avoid interference with LC separations, whether they are performed via reversed-phase or hydrophilic interaction chromatography.

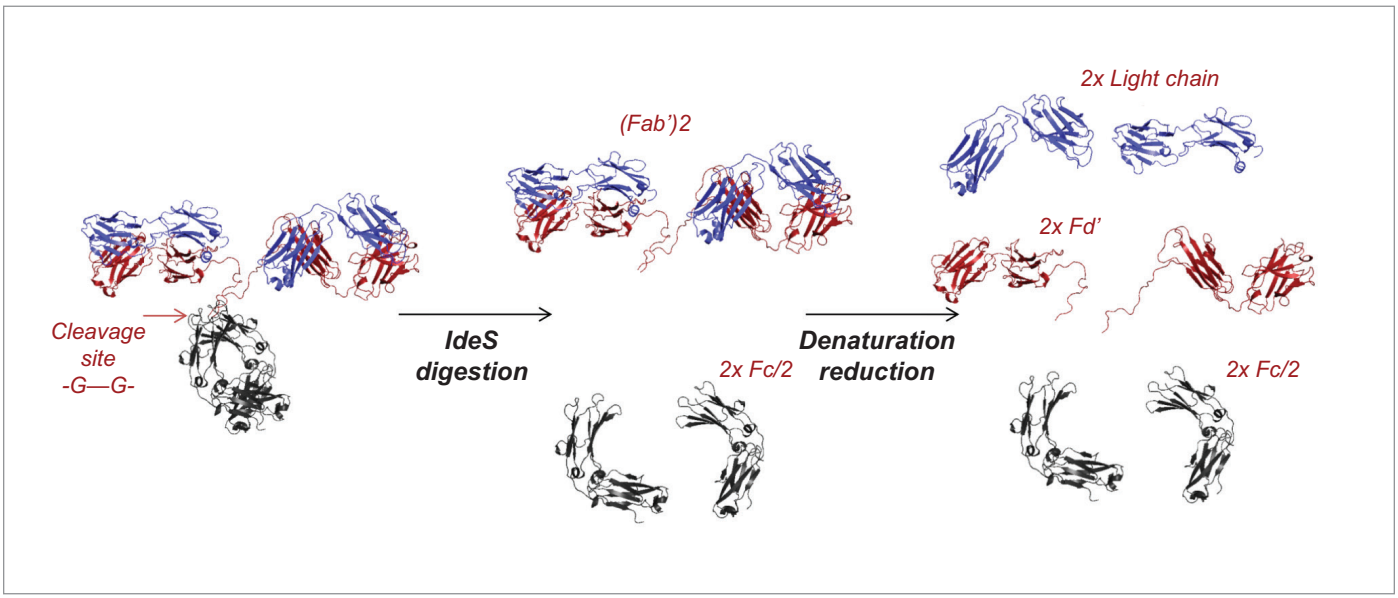

Figure 1. IdeS digestion and reduction of a monoclonal antibody.

II. RECOMMENDED RECONSTITUTION

It is recommended that the standard be dissolved in a 50 to 100 µL volume of aqueous diluent while carefully aspirating or vortexing to mix. Injection volumes between 0.5 and 4 µL, and mass loads between 0.25 to 1.00 µg, are recommended for 2.1 mm I.D. reversed-phase (RP) columns. If performing hydrophilic interaction chromatography (HILIC), smaller volumes should be injected to minimize solvent effects.

III. STORAGE AND STABILITY

Upon arrival and prior to reconstitution, please store the standard in its original packaging at -20 °C up until its marked expiration date. After reconstitution, the standard can be stored at 4-8 ˚C for seven days without concern of degradation.

IV. SEQUENCE AND MASS INFORMATION

Fc/2 Sequence

GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQY**N**STYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPG

The canonical site of IgG glycosylation is marked in bold.

LC Sequence

DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPG
KAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTISSLQPDDF
ATYYCFQGSGYPFTFGGGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV
TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS
PVTKSFNRGEC

Fd' Sequence

pQVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIR
QPPGKALEWLADIWWDDKKHYNPSLKDRLTISKDTSKNQ
VVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG

V. EXAMPLES OF RPLC CONDITIONS AND REPRESENTATIVE DATA

System:	ACQUITY™ UPLC™ H-Class Bio
Column:	ACQUITY UPLC Protein BEH C_4, 300Å 1.7 µm, 2.1 x 50 mm (p/n: 186004495)
Temp.:	80 °C
Mobile phase A:	0.1% TFA (v/v) in water
Mobile phase B:	0.1% TFA (v/v) in acetonitrile
Flow rate:	0.2 mL/min
Sample temp.:	10 °C
UV detection:	280 nm, 10 Hz
Seal wash and LC autosampler wash:	90% water/10% acetonitrile (v/v)
Reconstitution:	100 µL 0.1% formic acid in water
Injection volume:	4 µL

Gradient:

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0.00	0.2	85.0	15.0	6
20.00	0.2	45.0	55.0	6
20.30	0.2	20.0	80.0	6
21.30	0.2	20.0	80.0	6
21.60	0.2	85.0	15.0	6
25.00	0.2	85.0	15.0	6

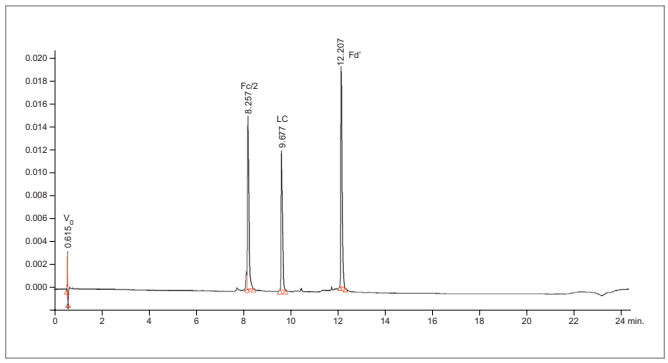

Figure 2. A representative RPLC chromatogram of the mAb Subunit Standard as obtained with an ACQUITY UPLC H-Class Bio System and an ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm, 2.1 × 50 mm Column. These analyses were performed using a 4 µL injection of a sample made from dissolving 25 µg of mAb Subunit Standard in 100 µL of 0.1% formic acid in water.

System:	ACQUITY UPLC H-Class Bio
Column:	BioResolve™ RP mAb Polyphenyl, 450Å, 2.7 µm, 2.1 x 50 mm (p/n: 176004156)
Temp.:	80 °C
Mobile phase A:	0.1% acid additive (v/v) in water
Mobile phase B:	0.1% acid additive (v/v) in acetonitrile
Flow rate:	0.2 mL/min
Sample temp.:	10 °C
UV detection:	280 nm, 10 Hz
Seal wash and LC autosampler wash:	90% water/10% acetonitrile (v/v)
Reconstitution:	100 µL 0.1% formic acid in water
Injection volume:	4 µL

Gradient:

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0.00	0.2	85.0	15.0	6
20.00	0.2	45.0	55.0	6
20.30	0.2	20.0	80.0	6
21.30	0.2	20.0	80.0	6
21.60	0.2	85.0	15.0	6
25.00	0.2	85.0	15.0	6

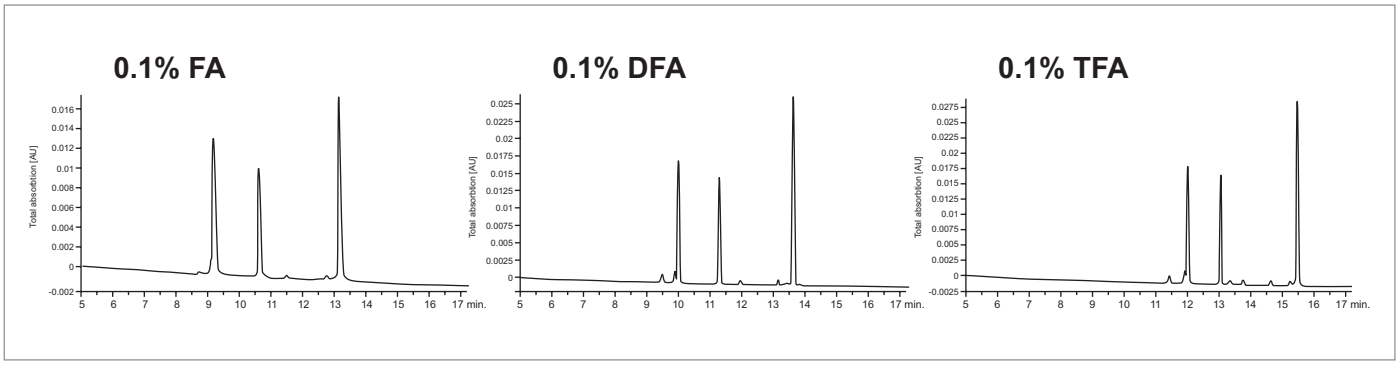

Figure 3. Representative RPLC chromatograms of the mAb Subunit Standard as obtained with an ACQUITY UPLC H-Class Bio System and a BioResolve RP mAb Polyphenyl, 450Å, 2.7 µm, 2.1 × 50 mm Column. This analysis was performed using various acid additives and a 4 µL injection of a sample made from dissolving 25 µg of mAb Subunit Standard in 100 µL of 0.1% formic acid in water (FA, formic acid; DFA, difluoroacetic acid; TFA, trifluoroacetic acid).

VI. EXAMPLE HILIC CONDITIONS AND REPRESENTATIVE DATA

System:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 150 mm (p/n: 176003702)
Temp.:	60 °C
Mobile phase A:	0.1% TFA (v/v) in water
Mobile phase B:	0.1% TFA (v/v) in acetonitrile
Flow rate:	0.4 mL/min
Sample temp.:	10 °C
Seal wash and LC autosampler wash:	30% water/70% acetonitrile (v/v)
Reconstitution volume:	50 µL water
Injection volume:	0.5 µL
FLR detection:	Em 280 nm, Ex 360 nm, 5 Hz

Gradient:

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0.0	0.4	15.0	85.0	6
0.2	0.4	18.0	72.0	6
10.2	0.4	36.0	64.0	6
10.3	0.4	15.0	85.0	6
13.3	0.4	15.0	85.0	6

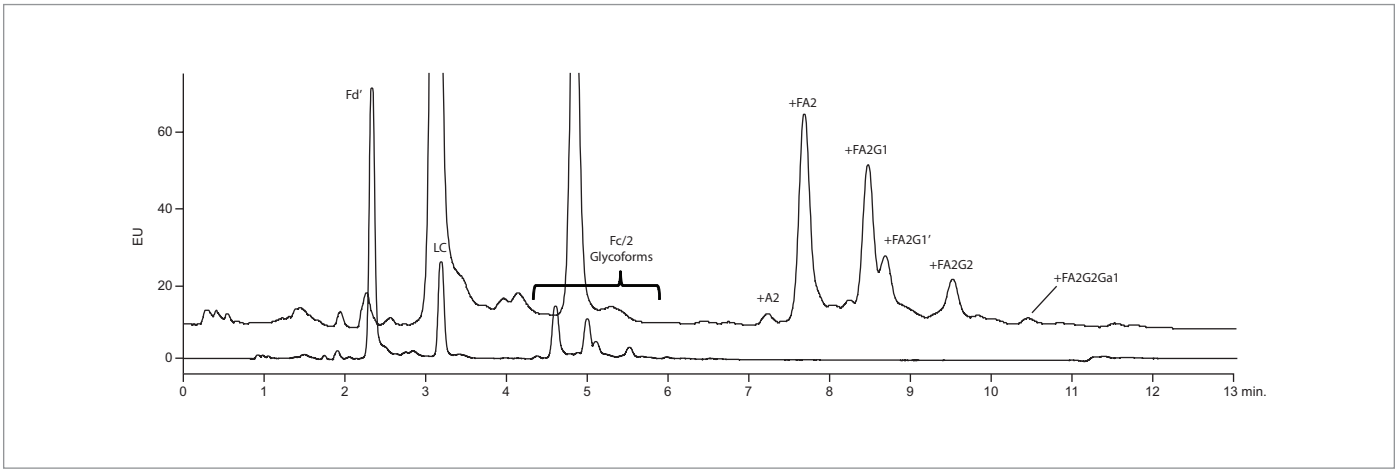

Figure 4. A representative HILIC chromatogram of the mAb Subunit Standard as obtained with an ACQUITY UPLC I-Class System and an ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 × 150 mm Column. This analysis was performed using a 0.5 µL injection of a sample made from dissolving 25 µg of mAb Subunit Standard in 50 µL of water.

VII. REPRESENTATIVE MS DATA

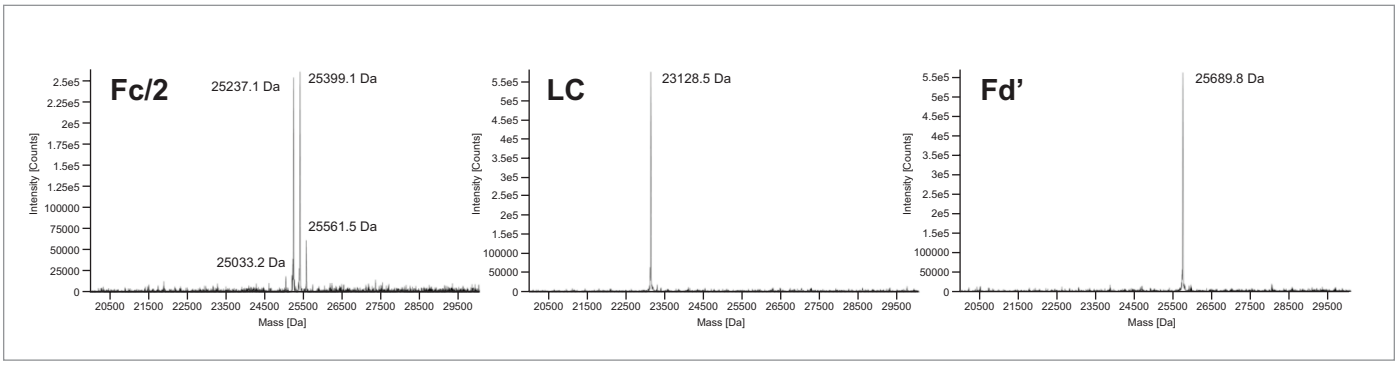

Figure 5. Deconvoluted ESI mass spectra of the mAb Subunit Standard as obtained with a Xevo™ G2 QTof Mass Spectrometer.

Table 1. Molecular Weights for the mAb Subunit Standard

Subunit	$MW_{Avg,Theo}$ (Da)
LC	23127.8
Fd'	25689.2
Fc/2 + A2	25033.1
Fc/2 + FA2	25236.3
Fc/2+ FA2G1	25398.5
Fc/2 + FA2G2	25560.6
Fc/2 + FA2G2Ga1	25722.8

VIII. ORDERING INFORMATION

Product description	P/N
mAb Subunit Standard	186008927
ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm, 2.1 x 50 mm Column	186004495
BioResolve RP mAb Polyphenyl, 450Å, 2.7 µm, 2.1 x 50 mm Column	176004156
ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 150 mm Column	176003702

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