[product solution]

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Intact mAb Mass Check Standard

IgG1: Critical in Many Therapeutic and Diagnostic Applications

Monoclonal antibodies comprise a significant proportion of biotechnology-driven molecules used for diagnostic and therapeutic applications. The inherent heterogeneity of such products has dictated the need for thorough analytical characterization methodologies so that safe, effective and reproducible products can be produced. In addition, while antibodies can have vastly differing binding selectivity, the overall structure is highly conserved between antibodies of the same class, thus standard analytical methods can often be used as a starting point for developing an optimized analytical strategy for an individual molecule¹. LC and High Resolution MS have become a powerful tool as part of the standard analysis package to:

- Characterize these important biomolecules
- Assess batch-to-batch variation
- Study antibody structure



Figure 1. Structure of the Intact mAb Mass Check Standard. This particular IgG was found to contain N-linked biantennary carbohydrates linked to each of the heavy chains. The major product variants that were observed correspond to terminal pyroglutamic acids and glycoform heterogeneity (galactose additions to the fucosylated biantennary core).







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INTACT mAb MASS CHECK STANDARD

Intact mass analysis of mAbs by LC/MS is a routine analytical task performed by many different biopharmaceutical laboratories, and provides a rapid approach for confirming antibody mass and glycosylation profile. Low molecular weight protein standards that are commonly available for instrument tuning or performance checks (e.g. myoglobin) are not well suited to check mAb instrument performance, as optimal instrument settings may differ for the smaller nonglycosylated protein.

The Intact mAb Mass Check Standard is a LC/MS standard can be used as a qualitative tool for confirming LCMS system operation.

- Known molecular weight for multiple glycoforms, ideal for higher molecular weight intact mass measurements.
- Provided in convenient Waters Max Recovery Vial (P/N 18600327c) for direct solubilization and injection.

Molecular Weight Information

Light Chain Formula:	Avg. Mw =
$C_{1066} \; H_{1649} \; N_{287} \; O_{343} \; S_{7}$	24197.7
Heavy Chain Formula:	Avg. Mw =
$C_{_{2170}}H_{_{3338}}N_{_{562}}O_{_{661}}S_{_{19}}$	48484.3 Da
Intact Protein Formula:	Average Mw =
$C_{_{6472}}H_{_{9940}}N_{_{1698}}O_{_{2008}}S_{_{52}}$	145,329.7 Da

Total number of Disulfide Bond = 17

Fixed Modification on 2 x Pyroglutamic Acid Q

For sequence information to cut/paste please go to:http://www.waters.com/ waters/nav.htm?cid=134634380 and Click on Intact mAb Mass Check Standard

Protein Sequence

The figure below contains the protein structure information. For Waters Mass Spectrometry users who wish to process the intact protein LC/MS data using Waters Informatics software such as BiopharmaLynx or UNIFI, the sequence can be incorporated into data processing methods (this is also located on the website for easy cut/paste).

Modifications on the Protein:

- 17 Disulfide Bonds
- N-terminus of each heavy chain has fixed Pyroglutamic acid from Q
- One N-linked glycosylation on each heavy chain

Amino Acid Sequence of the Intact mAb Mass Check Standard (PN 186006552)						
1:1	DVLMTQTPLS	LPVSLGDQAS	ISCRSSQYIV	HSNGNTYLEW	YLQKPGQSPK	
1:51	LLIYKVSNRF	SGVPDRFSGS	GSGTDFTLKI	SRVEAEDLGV	YYCFQGSHVP	
1:101	LTFGAGTKLE	IKRADAAPTV	SIFPPSSEQL	TSGGASVVCF	LNNFYPKDIN	light chain
1:151	VKWKIDGSER	QNGVLNSWTD	QDSKDSTYSM	SSTLTLTKDE	YERHNSYTCE	
1:201	ATHKTSTSPI	VKSFNRNEC				
2:1	*QVQLKESGPG	LVAPSQSLS	TCTVSGFSLL	GYGVNWVRQP	PGQGLEWLMG	
2:51	IWGDGSTDYN	SALKSRISIT	KDNSKSQVFL	KMNSLQTDDT	AKYYCTRAPY	
2:101	GKQYFAYWGQ	GTLVTVSAAK	TTPPSVYPLA	PGSAAQTDSM		
2:151	FPEPVTVTWN	SGSLSSGVHT	FPAVLQSDLY	TLSSSVTVPS	STWPSETVTC	heavy chain
2:201	NVAHPASSTK	VDKKIVPRDC	GCKPCICTVP	EVSSVFIFPP	KPKDVLTITL	
2:251	TPKVTCVVVD	ISKDDPEVQF	SWFVDDVEVH	TAHTQPREEQ	FNSTFRSVSE	
2:301	LPIMHQDWLN	GKEFKCRVNS	AAFPAPIEKT	ISKTKGRPKA	PQVYTIPPPK	
2:351	EQMAKDKVSL	TCMITDFFPE	DITVEWOWNG	QPAENYKNTQ	PIMDTDGSYF	
2:401	VYSKLNVQKS	NWEAGNTFTC	SVLHEGLHNH	HTEKSLSHSP	G	
3:1	*QVQLKESGPG	LVAPSQSLSI	T <mark>¢TVS</mark> GFSLL	GYGVNWVRQP	PGQGLEWLMG	
3:51	IWGDGSTDYN	SALKSRISIT	KØNSKSQVFL	KMNSLQTDDT	AKYYCTRAPY	
3:101	GKQYFAYWGQ	GTLVTVSAAK	TTPPSVYPLA	PGSAAQTDSM	VTLG <mark>Ç</mark> LVKGY	heavy chain
3:151	FPEPVTVTWN	SGSLSSGVHT	FPAVLQSDLY	TLSSSVTVPS	STWPSETVTC	
3:201	NVAHPASSTK		GCKPCICTVP	EVSSVFIFPP	KPKDVLTITL	
3:251	TPKVTCVVVD	ISKDDPEVQF	SWFVDDVEVH	TAHTQPREEQ	FNSTFRSVSE	
3:301	LPIMHQDWLN	GKEFKCRVNS	AAFPAPIEKT	ISKTKGRPKA	PQVYTIPPPK	
3:351	EQMAKDKVSL	TCMITDFFPE	DITVEWQWNG	QPAENYKNTQ	PIMDTDGSYF	
3:401	VYSKLNVQKS	NWEAGNTETC	SVLHEGLHNH	HTEKSLSHSP	G	
4:1	DVLMTQTPLS	LPVSLGDQAS	ISCRSSQYIV	HSNGNTYLEW	YLQKPGQSPK	
4:51	LLIYKVSNRF	SGVPDRFSGS	GSGTDFTLKI	SRVEAEDLGV	YYCFQGSHVP	light chain
4:101	LTFGAGTKLE	IKRADAAPTV	SIFPPSSEQL	TSGGASVVCE	LNNFYPKDIN	
4:151	VKWKIDGSER	QNGVLNSWTD	QDSKDSTYSM	SSTLTLTKDE	YERHNSYTCE	
4:201	ATHKTSTSPI	VKSFNRNEC				
* pyroglutamic acid from Q						
			disu	ulfide linkage	Disclaimer: I/	'L are exchangeable

Figure 2. Protein Sequence Information

Chain 1: Light Chain



Figure 3. Combined mass spectrum (inset) and deconvoluted mass spectrum of the light chain.

Light Chain Formula: C₁₀₆₆H₁₆₄₉N₂₈₇O₃₄₃S₇

Avg. MW = 24197.7

Chain 2: Heavy Chain



Figure 4. Combined mass spectrum (inset) and deconvoluted mass spectrum of the glycosylated heavy chain.

Heavy Chain Formula: C₂₁₇₀H₃₃₃₈N₅₆₂₀₆₆₁S₁₉

Avg. MW = 48484.3 Da

Because this mAb sample has a similar MW range to conventional therapeutic monoclonal antibodies, the Waters Intact mAb Mass Check Standard serves as a convenient reference sample for mass analysis of large biopolymers. Figures 4 and 5 show examples on various mass spectrometry systems.

Examples of Using the Intact mAb Mass Check Standard for MS Optimization with Q-TOF, TOF and SQD

Peak Number	mAb Glycoform	Expected MW
1	$M^* + GOF + GOF$	148,220.4
2	M* + GOF + G1F	148,382.5
3	M* + G1F + G1F	148,544.6
4	M* + G1F + G2F	148,706.7
5	M* + G2F + G2F	148,868.8

*aglycosylated mAb

Table 1: Deconvoluted MW of the Major Glycoform of the mAb.



Figure 5. Example data obtained with ESI-TOF instrumentation.



Figure 6. Example data obtained with ESI-Quad instrumentation.

References:

- Chakraborty, A., Berger S., Gebler J., Characetrization of an IgG1 Monoclonal Antobody and Related Sub-Strcutures by LC/ESI-TOF/MS. Waters Application Note, March 2007 PN 720002107EN.
- 2. Rapid Commun. Mass Spectrom. 2008; 22: 29-40

ORDERING INFORMATION

Description	Part No.
Intact mAb Mass Check Standard	186006552



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