Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

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 molecule[1]. 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

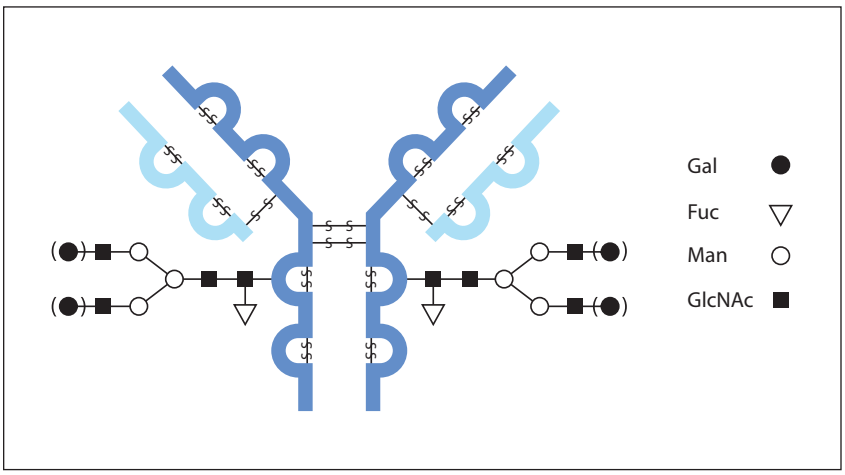
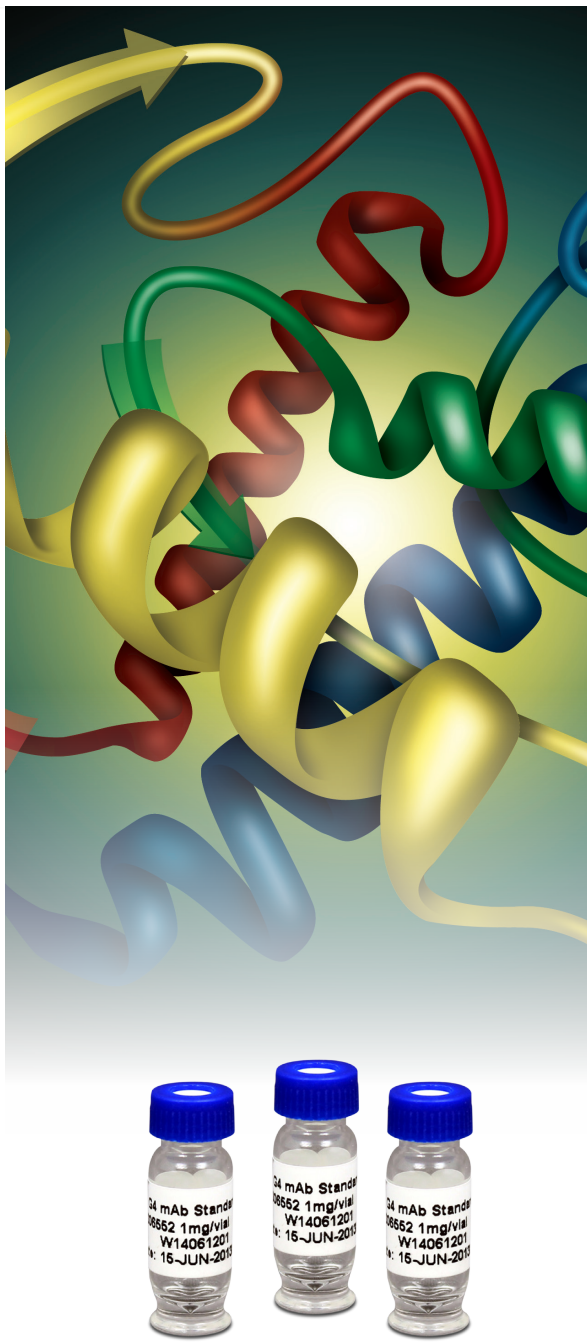


Figure 1. Structure of the Intact mAb Mass Check Standard. This particular IgG was found to contain N-linked biantennary carbohydrates linked to each of the heavy chains. The major product variants that were observed correspond to terminal pyroglutamic acids and glycoform heterogeneity (galactose additions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



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: $C_{1066}H_{1649}N_{287}O_{343}S_7$ **Avg. Mw =** 24197.7

Heavy Chain Formula: $C_{2170}H_{3338}N_{562}O_{661}S_{19}$ **Avg. Mw =** 48484.3 Da

Intact Protein Formula: $C_{6472}H_{9940}N_{1698}O_{2008}S_{52}$ **Average Mw =** 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

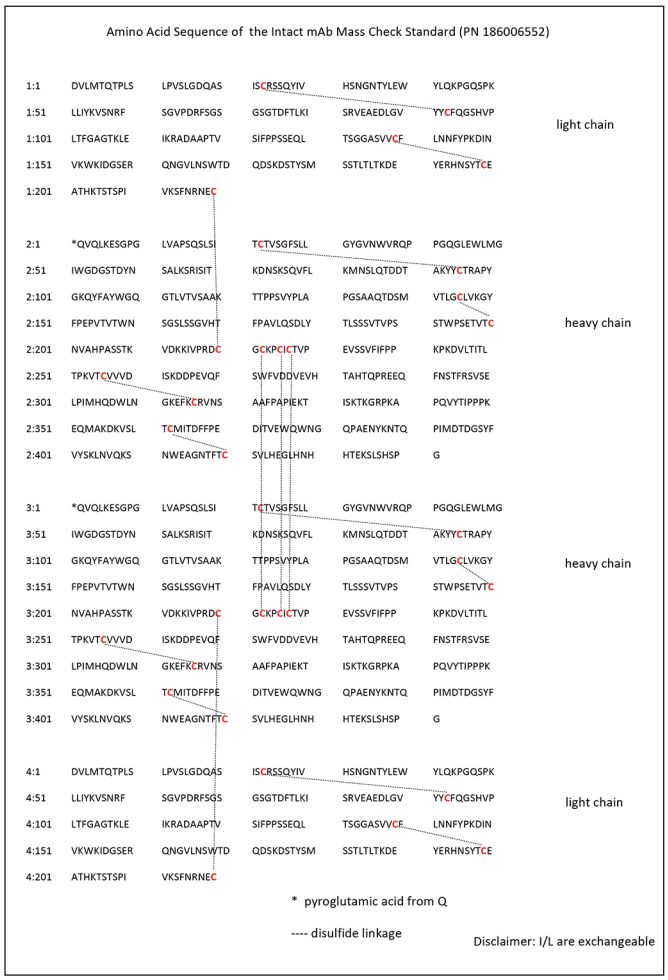


Figure 2. Protein Sequence Information

Chain 1: Light Chain

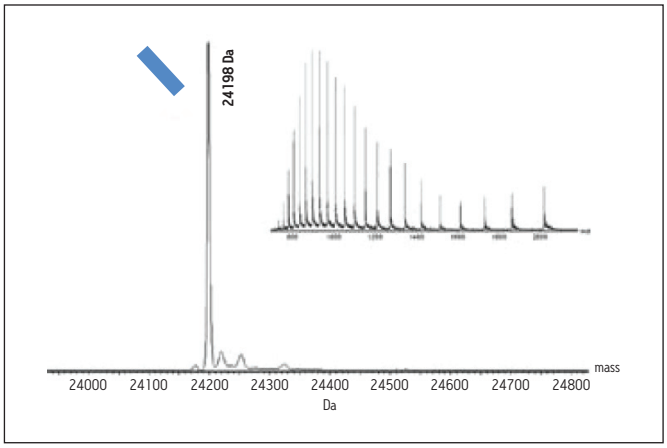


Figure 3. Combined mass spectrum (inset) and deconvoluted mass spectrum of the light chain.

Light Chain Formula: $C_{1066}H_{1649}N_{287}O_{343}S_7$

Avg. MW = 24197.7

Chain 2: Heavy Chain

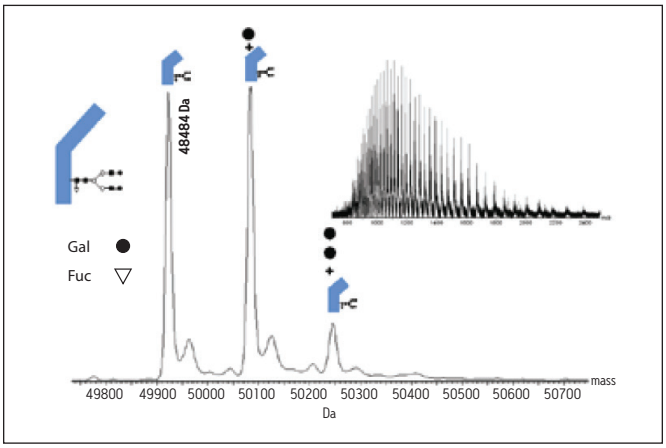


Figure 4. Combined mass spectrum (inset) and deconvoluted mass spectrum of the glycosylated heavy chain.

Heavy Chain Formula: $C_{2170}H_{3338}N_{5620661}S_{19}$

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* + G0F + G0F	148,220.4
2	M* + G0F + 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.

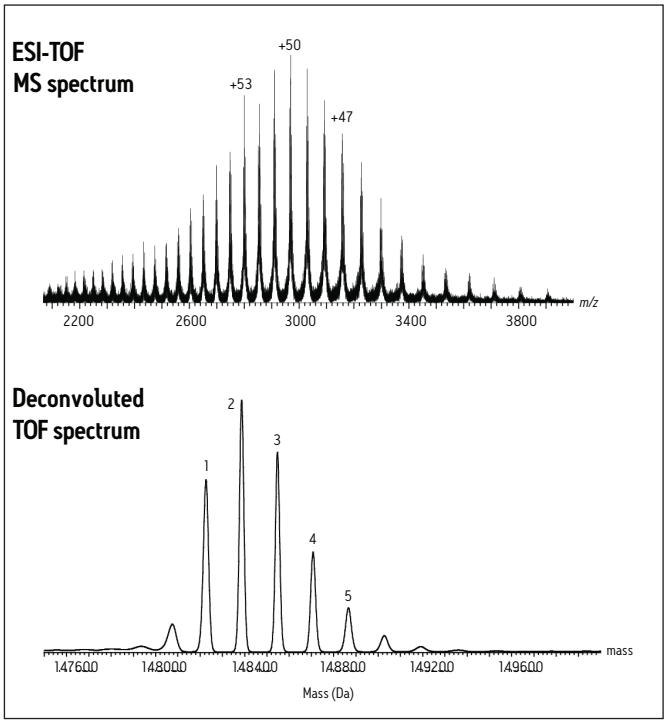


Figure 5. Example data obtained with ESI-TOF instrumentation.

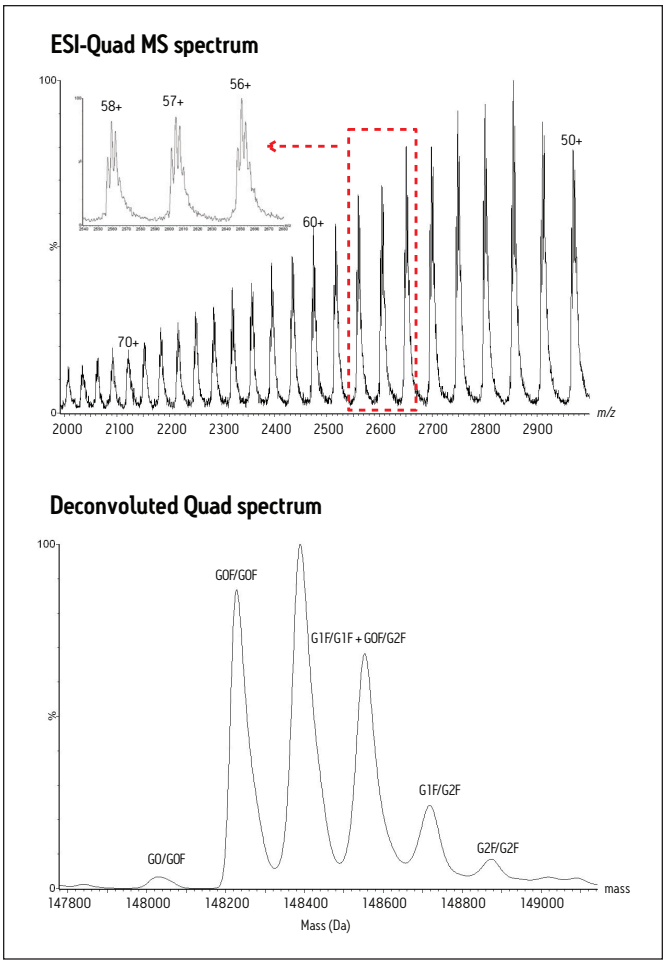


Figure 6. Example data obtained with ESI-Quad instrumentation.

References:

1. 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

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com