

## Application News

# No.**B60**

MALDI-TOF Mass Spectrometry

## Detection of Protein Aggregates: Detection of Multimeric Proteins Using MALDI-TOF MS with a High Mass Detector

The unintended formation for protein aggregates may cause antigenicity and impair protein function in protein drug development and during the various types of research that use proteins. Consequently, monitoring the formation of protein aggregates in different environments is a very important matter both for acquiring consistent biochemical data and for the quality control of drugs.

In this article, we will describe the detection of protein aggregates using MALDI-TOF MS that is capable of detecting high mass molecules.

### Detection of Multimeric Proteins Generated by Freeze-Thaw and Heat Treatment

Mouse antibody samples (1  $\mu$ M, 10  $\mu$ L) either subjected to two cycles of freeze-thaw treatment (-80 °C/room temperature) or held at 50 °C for 3 hours were mixed with K200 stabilizer (CovalX), a protein cross-linking reagent, and reacted for 1 hour under room temperature conditions.

After the reaction, sample solutions were mixed with a MALDI matrix (sinapinic acid, 10 mg/mL, 50 % acetonitrile aqueous solution/0.1 % trifluoroacetic acid aqueous solution) and analyzed using MALDI-TOF MS with a high mass detector. A sample of untreated antibodies reacted with K200 stabilizer at room temperature was also analyzed.

Analysis of the untreated commercially available antibodies by MALDI-TOF MS with a high mass detector revealed a strong antibody-derived signal at around 148 kDa as well as a weak antibody dimer signal (Fig. 1).

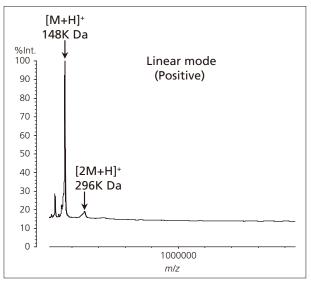


Fig. 1 Mass Spectrum of Untreated Sample

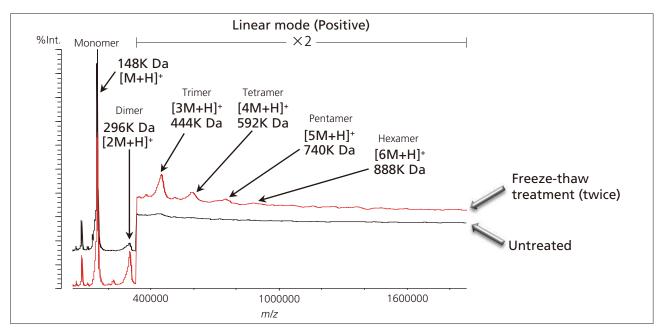


Fig. 2 Comparison of Freeze-Thaw Sample and Untreated Sample Mass Spectra

Compared against the untreated antibody sample, analysis of the sample subjected to repeated freezethaw treatment revealed a stronger signal derived from an antibody dimer, along with signals corresponding to an antibody trimer, tetramer, pentamer, and hexamer (Fig. 2). Similar to the sample subjected to freeze-thaw treatment, analysis of the sample held at high temperature also revealed clear signals derived from multimeric antibodies (Fig. 3).

Analysis of multimer-forming proteins using MALDI-TOF MS capable of detecting high mass molecules while utilizing a linking reagent to create cross-links allows us to understand how specific environments change protein aggregation.

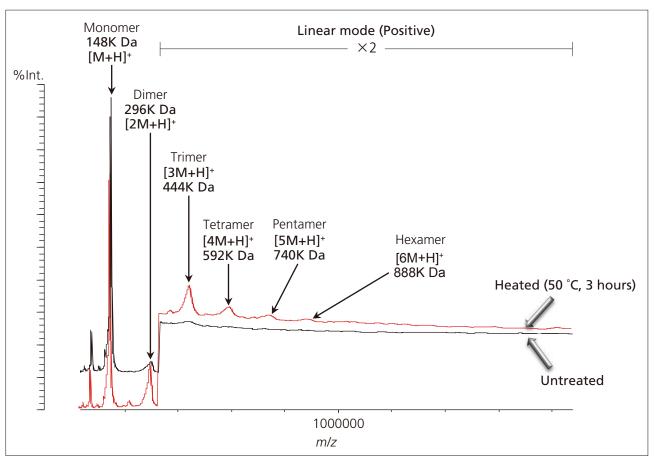


Fig. 3 Comparison of Heated Sample (50 °C, 3 hours) and Untreated Sample Mass Spectra

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