

**Glycosylation Profile of IgGs Using a Linear Benchtop MALDI-TOFMS and Affinity Purification of Fc**

Glycosylation on protein plays wide-range vital roles in biological processes from the stabilization of protein conformation to the expression of binding specificity. In this view, a characterization of the *N*-*O*-linked glycan is rather significant, especially in the development of biopharmaceuticals. To date, whereas intensive efforts have been conducted on the precise characterization of glycans with high-end mass spectrometers, inexpensive easy-to-use commercial instruments have been anticipated for batch analysis assuming screening or quality assurance/quality control (QA/QC). A newly developed bench-top MALDI-TOFMS, MALDI-8020, is expected to be a suitable instrument in terms of sufficient specification, through-put, and cost effectiveness. We attempted to characterize the glycosylation of IgG using the MALDI-8020 without a release of glycan. To this end, we examined a quick preparation using affinity beads and enzymatic cleavage.

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■ Preparation of Fc

A work-flow of Fc affinity separation is shown in Fig. 1. NIST mAb, IgG from mouse serum, and myeloma IgG were subject to MS analysis. IdeZ and Protein-A magnetic beads (Mag Sepharose™) were purchased from Promega and GE Healthcare, respectively. The IgGs dissolved in tris-NaCl buffer were incubated with IdeZ at 37 °C for two hours. Resultant solutions including Fc and F(ab')<sub>2</sub> were applied to the magnetic beads equilibrated with the tris-buffer, then washed with the same buffer several times. The Fc associated with Protein-A was eluted with acidic solution, then desalted with ZipTip® C18.

■ MALDI-TOFMS

All MS analysis in positive ion mode was performed with a bench-top linear MALDI-TOFMS (MALDI-8020). Sinapinic acid (SA) and Ferulic acid (FA) were applied to MS analysis as matrix at the same condition as follows; 20 mg/mL in 50 % acetonitrile/milliQ water with 0.1 % TFA.

■ Statistical Data Analysis

eMSTAT Solution™ (Shimadzu Corp., Japan) was applied to perform classification/differentiation of glycosylated-Fc regions.

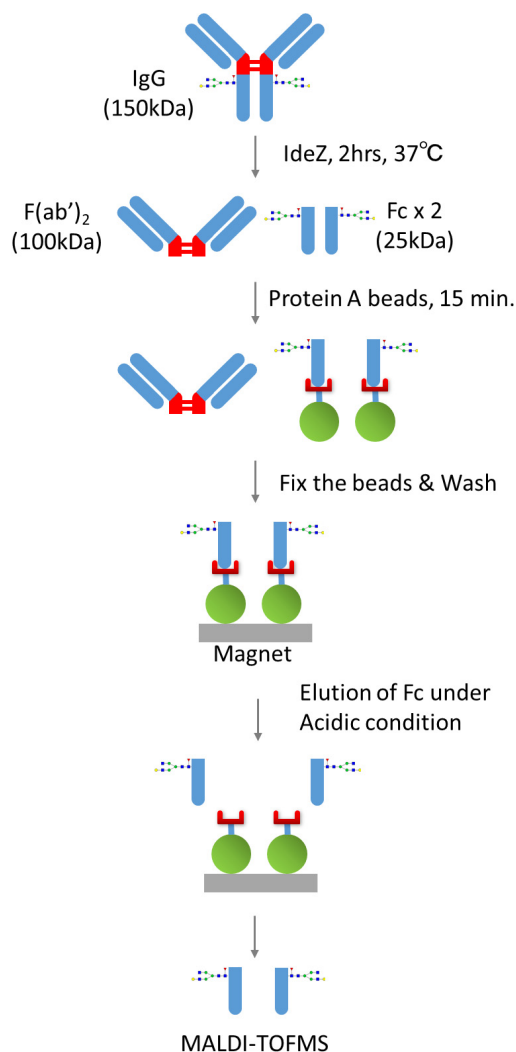


Fig. 1 Work-Flow of Fc Affinity Separation/Detection

■ Results

Fig. 2 illustrates the mass spectra of whole IgGs. At nearly 150 kDa, their molecular weights could be easily observed. However, almost no information relating to glycosylation was obtained due to insufficient mass resolving power in this high *m/z* range .

Subsequently, all IgGs were subjected to the work-flow shown in Fig. 1 to purify Fc regions that retain varieties of glycosylation. Molecular weights of the purified Fc were nearly 25 kDa, in which the MS resolving power of MALDI-8020 is sufficient to recognize the varieties of glycosylation.

### Results (Continued)

Mass spectra of three Fc purified with the work-flow are shown in Fig. 3. Heterogeneities due to glycan modifications were observed in each mass spectrum, in which mass gaps between peaks indicate a sugar unit. As seen in Fig. 3, absolutely distinctive patterns between the three Fc mainly due to varieties of glycosylation were recognized using the work-flow.

MS measurement for each Fc were repeated three times to conduct a statistical analysis using eMSTAT Solution. Exported peak lists of the mass spectra were saved as text files in unique data folders titled "myelo", "nist", and "serum" nested under the "IgG" folder, as shown in Fig. 4. It is necessary only to open the "IgG" folder to conduct a statistical analysis using the eMSTAT Solution. After loading a data set of Fc, a score plot shown in Fig. 4 was obtained quickly with only minimum adjustment of some parameters.

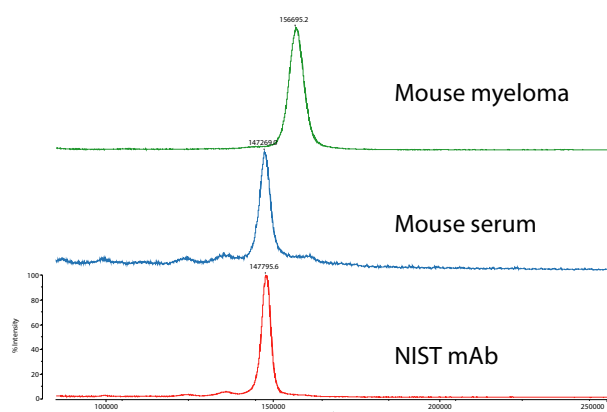


Fig. 2 MS of Whole IgGs

### Conclusion

Purification of enzymatically fragmented IgGs by affinity-beads enables batch analysis for the glycosylation using a bench-top MALDI-TOFMS, MALDI-8020. The MS resolution of MALDI-8020 is sufficient to recognize three Fc that mainly differ in glycosylation. Furthermore, a statistical analysis by eMSTAT Solution enables a classification of three glycosylated Fc smoothly and quickly, which could be applicable to QA/QC.

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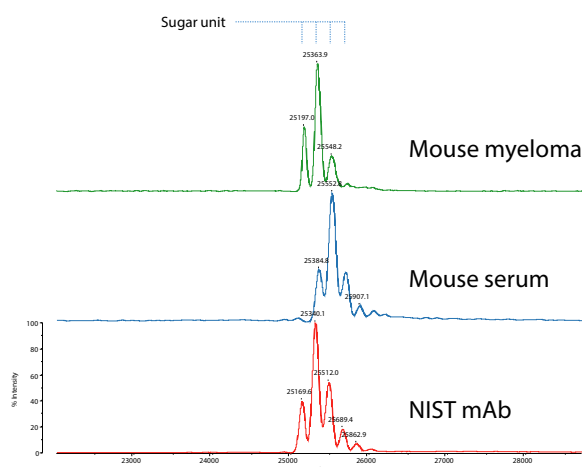


Fig. 3 A Comparison of Three Fc Purified with the Work-Flow

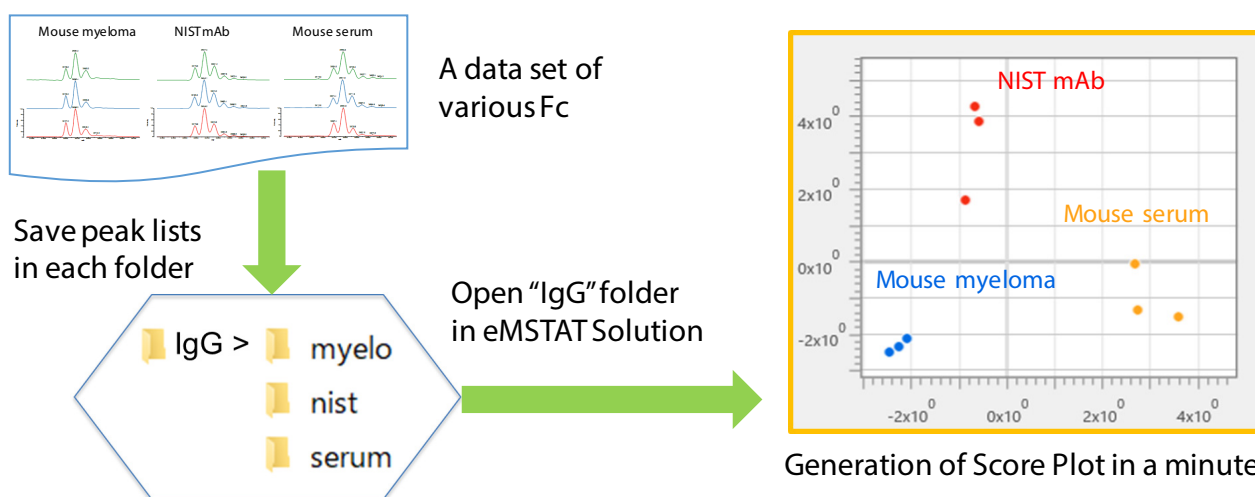


Fig. 4 Classification of Three Fc Using eMSTAT Solution