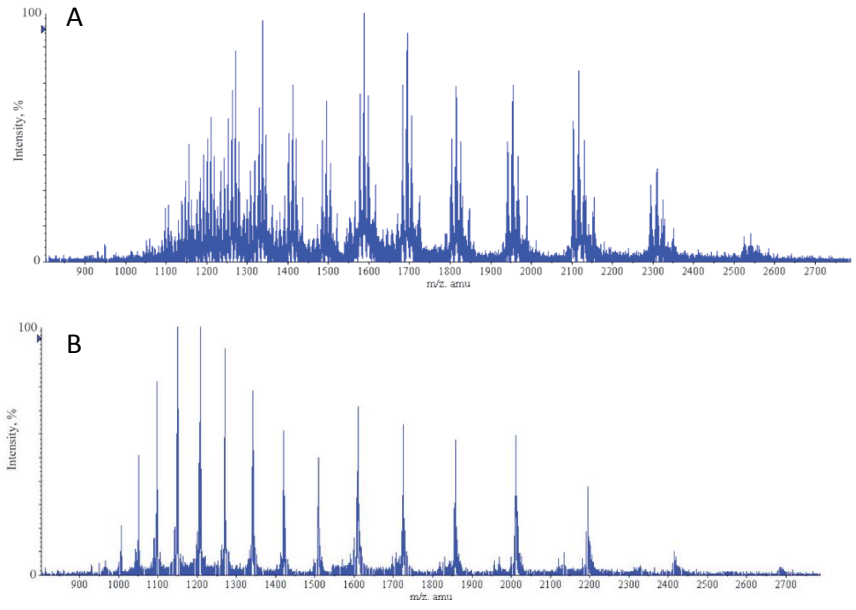
GENOVIS PRODUCT NEWS 2010-06-30

# Unique enzymes improve mass spectrometric analysis of IgG

**Genovis launches a new kit for mass spectrometry preparation of IgG - Mass Spec Kit - that contains both the FabRICATOR® ( recombinant IdeS ) and the IgGZERO™ (recombinant EndoS) enzymes. Using this kit makes it possible to perform both deglycosylation and fragmentation of IgG. The kit processes 1 mg IgG in one hour and the sample can be analyzed directly without further purification. By combining IgGZERO™ with FabRICATOR®, individual fragments of IgG can be studied in more detail with higher accuracy. In addition, the amino acid sequencing for quality control of monoclonal antibodies is facilitated. These unique enzymes have potential to reduce time and cost of quality control during screening, preparation and production of monoclonal antibodies.**

When performing mass spectrometry of large proteins, such as IgG molecules, there is often a need to remove the glycans because of poor quality of the data otherwise obtained. Additionally, fragmentation of IgG can visualize individual parts of IgG and allow for studies in more detail. The obvious solution is then to remove the glycans attached to a Asp297 on the Fc part of IgG molecules and we have evaluated a novel endoglycosidase, IgGZERO™, that selectively deglycosylates IgG molecules, for analysis in mass spectrometric applications. Due to its high specificity and activity, complete deglycosylation of native IgG can be done in less than 30 minutes. Deglycosylation followed by fragmentation with FabRICATOR®, which is very specific and effective, generates $F(ab')_2$ and Fc fragment in less than 30 minutes. This reduces time of preparation significantly and dramatically increase the quality of data obtained when IgG is analyzed by mass spectrometry.

Effect of deglycosylating IgG Fc:




*Mass spectrometry of Fc-fragments from human polyclonal IgG purifed from plasma. (**A**) 1 mg of IgG was incubated with 1000 U of* FabRICATOR® *for 30 minutes and analyzed by mass spectrometry. (**B**) 1 mg of human IgG was incubated with 1000 Units of* FabRICATOR® *for 30 minutes followed by incubation with 1000 Units of* IgGZERO™ *for 45 minutes and finally analyzed by mass spectrometry. Utilization of RPC18 LC-ESI-MS generated a well-defined protein envelope with a deconvoluted mass of 24137 Da corresponding to the deglycosylated IgG1 heavy chain cleaved at position Gly236.*

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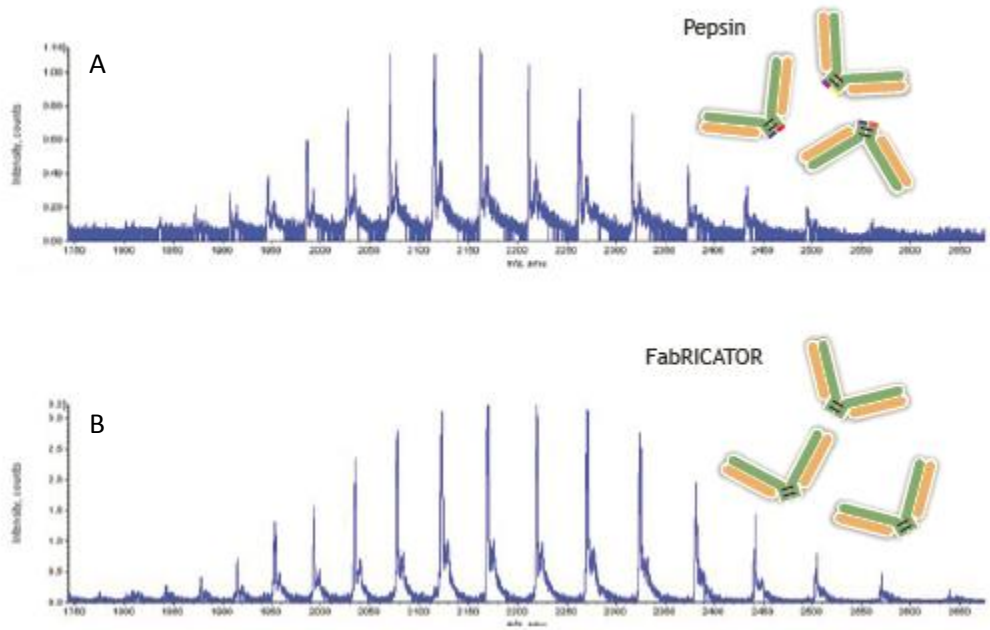
Homogeneity of F(ab')$_2$ fragments Pepsin vs FabRICATOR:




*Mass spectrometry (LS/MS) of F(ab')2-fragments from humanized monoclonal IgG (Hercptiny). (**A**) 1 mg of IgG was digested using Pepsin (Sigma) for 2 hours pH 3.5. F(ab')2 fragments was isolated by size exclusion chromatography before analyzis by mass spectrometry. (**B**) 1 mg of IgG was digested using* FabRICATOR® *for 30 minutes and F(ab')$_2$ fragments was isolated using size exclusion chromatography before analyzis by mass spectrometry.* FabRICATOR® *has one unique cleavage site and generates identical fragments, whereas pepsin generates several different fragments due to multiple cleavage sites resulting in a heterogeneous mixture of fragments.*

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