GENOVIS

OmniGLYZOR™

Sugar-free Proteins in a Few Hours

OmniGLYZOR™

Hydrolysis of N- and Mucin-type O-glycans

OmniGLYZOR contains a mixture of immobilized enzymes for the removal of N- and simple mucin-type O-glycans on antibodies, fusion proteins and other glycosylated proteins. Removal of glycans is widely used to reduce heterogeneity to facilitate analysis of the protein by for example mass spectrometry. Deglycosylation can also be used to study the functional role of the glycans.

- N- and simple mucin-type O-glycans on glycoproteins
- Hydrolysis of N- and O-glycans from glycoproteins
- 1-4 hour reaction
- *Rapi*Gest™* and PNGase F included

Deglycosylation Workflow

OmniGLYZOR contains enzymes required for the removal of N-glycans and the most commonly occurring mucin-type O-glycans, namely mono- and disialyl core 1 and Tn antigen (α-GalNAc). PNGase F, O-glycosidase, sialidase and α-GalNAcase activities are included in OmniGLYZOR.

The glycoprotein sample is incubated with the OmniGLYZOR resin in a microspin column for 1-4 h under native reaction conditions. The deglycosylated glycoprotein is then easily collected by a centrifugation step. Some N-glycosylation sites are poorly or not accessible to PNGase F unless the substrate protein is denatured. Remaining N-glycans can therefore be removed by an additional deglycosylation step using lyophilized PNGase F under denaturing conditions. Lyohilized PNGase F and *Rapi*Gest SF is included in the OmniGLYZOR kit.

Product Formats

OmniGLYZOR™

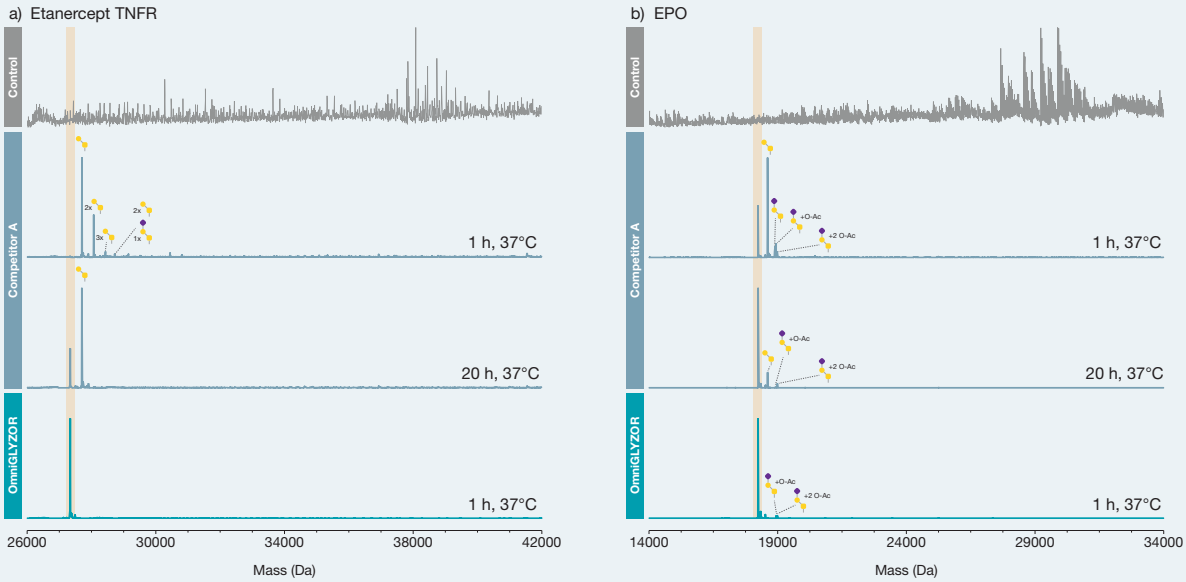
A mix of immobilized enzymes in spin columns for deglycosylation of glycoproteins carrying N- and simple O-glycans

OmniGLYZOR™

PRODUCT	DESCRIPTION	ID
OmniGLYZOR, Microspin 5 x 50-100 µg	Deglycosylates 5 x 50-100 µg glycoprotein	G3-OM6-005
OmniGLYZOR, Microspin 10 x 50-100 µg	Deglycosylates 10 x 50-100 µg glycoprotein	G3-OM6-010

Efficient Removal of N- and Mucin-type O-glycans

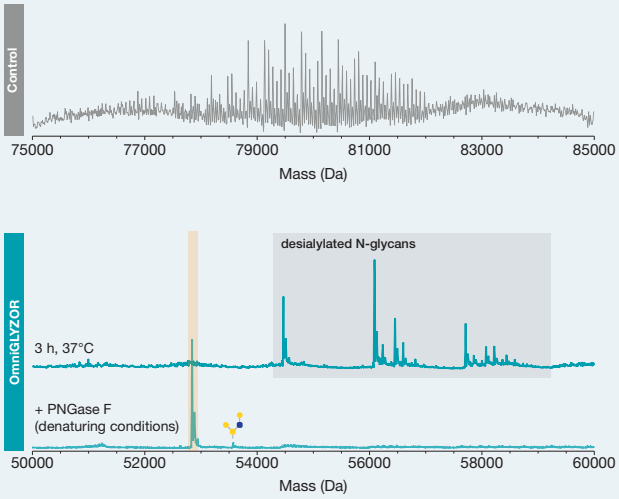
We demonstrate the performance of OmniGLYZOR using two therapeutic proteins as substrates, the heavily glycosylated Fc-fusion protein etanercept and erythropoietin (EPO). Due to the glycan heterogeneity, intact analyses resulted in complex mass spectra. By incubating the samples with OmniGLYZOR for 1 hour at 37°C, the N-and O-glycans were efficiently removed as indicated by single peaks corresponding to the unmodified protein. For EPO, a minor amount of O-glycans modified with acetylated sialic acids were left on the protein since those structures were inefficiently hydrolyzed by OmniGLYZOR. Both substrate proteins were also treated with another commercially available deglycosylation product according to the manufacturer's recommendation (O/N incubation at 37°C), and the data are shown for comparison.



Complete hydrolysis of N- and O-glycans by OmniGLYZOR. Deglycosylation of a) etanercept and b) EPO. The substrate proteins were incubated on OmniGLYZOR Microspin columns for 1 h at 37°C, or with another commercially available deglycosylation product for 1 h and 20 h at 37°C. To simplify the analysis of etanercept, the deglycosylated protein was digested with FabRICATOR to separate the O-glycosylated TNFR domain from the Fc fragment. The resulting subunits were analyzed by reversed-phase LC-MS using a Waters™ BioAccord™ LC-MS system. EPO was analyzed in the same way in its intact state. The peaks corresponding to the fully deglycosylated substrate proteins are shaded in orange.

Deglycosylation under Denaturing Reaction Conditions

The C1-inhibitor is a human plasma-derived biotherapeutic that is modified with 6 N-glycans and up to 28 O-glycans consisting of mostly sialyl core1 structures. Without pretreatment of this highly heterogeneous protein, reversed-phase LC-MS analysis yielded a complex mass spectrum impossible to interpret in detail. Using OmniGLYZOR Microspin columns under native conditions, all O-glycans were efficiently removed within 3 hours. However, between 1 and 3 N-glycans remained on the protein. These inaccessible N-glycans were removed by an additional deglycosylation step under denaturing conditions using the lyophilized PNGase F and MS-compatible *Rapi*Gest™ SF surfactant included in the OmniGLYZOR kit. A complete removal of all glycans was observed, with the exception of the minor amount of core 2 O-glycans present on the molecule.



Complete deglycosylation of plasma-derived human C1 inhibitor. The protein was analyzed by reversed-phase LC-MS using a Waters™ BioAccord™ LC-MS system in its untreated state (top), after deglycosylation on an OmniGLYZOR Microspin column under native conditions (middle) and after additional deglycosylation using PNGase F under denaturing conditions (bottom). The peak corresponding to the deglycosylated substrate protein is shaded in orange.

* *Rapi*Gest™ SF Surfactant from Waters Corporation is included in OmniGLYZOR™. *Rapi*Gest™ is a trademark of Waters Corporation.

SmartEnzymes™

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