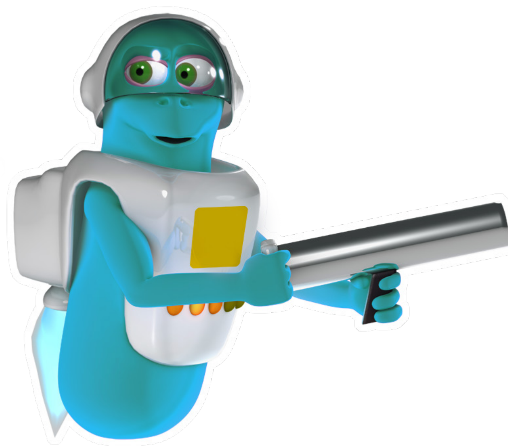
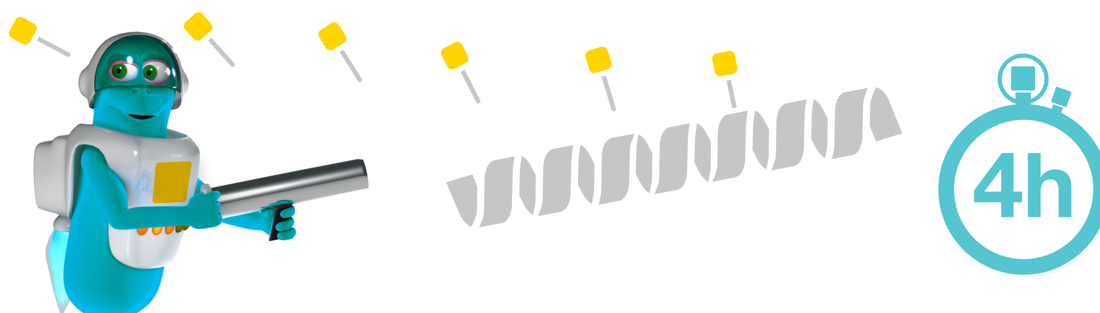
GalNAcEXO™



Hydrolysis of α-linked GalNAcs

SmartEnzymes™

GalNAcEXO™



GalNAcEXO™ is an efficient α-GalNAcase for hydrolysis of α-linked GalNAcs.

GalNAcEXO is an α-GalNAcase that hydrolyses the reducing *N*-acetylgalactosamines (GalNAcs), known as Tn antigens, on native *O*-glycoproteins. The enzyme has been derived from *Akkermansia muciniphila* and recombinantly expressed for the hydrolysis of α-GalNAcs on *O*-glycoproteins. The enzyme is active in neutral pH without the need for any co-factors. The exoglycosidase acitivty of GalNAcEXO allows for the removal of α-linked GalNAcs on up to 2 mg of native *O*-glycosylated protein. The GalNAcEXO enzyme is a valuable tool that reduces sample heterogeneity for the analysis of complex *O*-glycoproteins that carry α-linked GalNAc resudies as immature truncated *O*-glycoforms.

- Hydrolyzes α-linked GalNAc residues on native *O*-glycoproteins
- α-linked GalNAcs on Ser or Thr sites
- 4 h incubation
- No co-factors required

Key Characteristics

- Efficient hydrolysis of α-linked GalNAcs
- Exoglycosidase for native *O*-glycoproteins
- Acitivty without need for any co-factors

Performance on Complex Substrates

Etanercept is a model biopharmaceutical carrying 13 *O*-glycan sites of which 9 to 10 are occupied on average. After *N*-glycan removal by PNGaseF and truncation of *O*-glycans with SialEXO and GalactEXO, GalNAc related peaks were observed in the mass spectra (Fig. 1A). After incubation with the GalNAcEXO enzyme, over 95% of the remaining α-GalNAcs were removed (Fig. 1B).

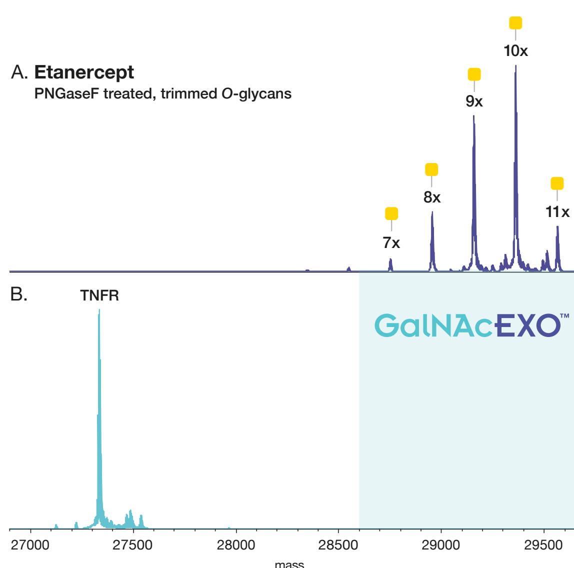


Figure 1. Deconvoluted mass spectra of partially deglycosylated etanercept (A), and after treatment with GalNAcEXO (B, O/N at 37 °C). Samples were FabRICATOR digested, separated by RP-HPLC (Waters BioResolve RP mAb, 2.7 µm, 2.1 x 100 mm) and analyzed by ESI-Q-TOF MS (Bruker Impact II).

Deglycosylation of the C1 Inhibitor

During production of *O*-glycosylated biopharmaceuticals, Tn antigens may appear as a result of incomplete processing of the core GalNAc. This appears as repeating mass shifts with a 203 Da HexNAc unit difference. To confirm the presence of Tn antigens, the following workflow can be applied, demonstrated on the heavily (26 sites) *O*-glycosylated C1 inhibitor (Fig 1A). At first, the glycoprotein was trimmed of the *N*-glycans by PNGaseF and core 1 *O*-glycans using OglyZOR and SialEXO. A pattern of repeating peaks was observed in the mass spectra (Fig. 2B). Secondly, the GalNAcEXO enzyme completely removed all remaining GalNAcs leaving a single peak (Fig. 2C). This workflow is further beneficial for analysis of PTMs not related to protein *O*-glycosylation.

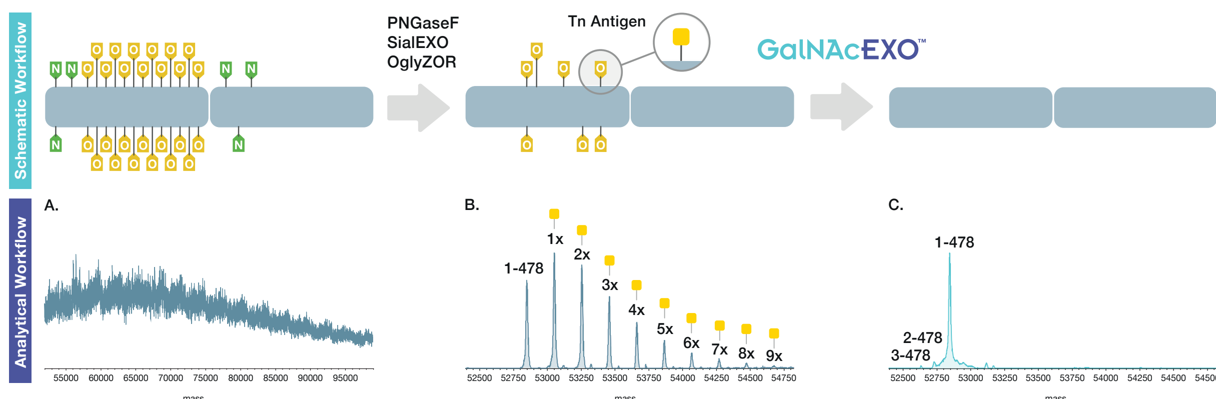


Figure 2. Total deglycosylation of recombinant C1 inhibitor. The C1 inhibitor was analyzed by LC (Waters ACQUITY UPLC Protein BEH C4 column, 1.7 µm, 2.1 x 100 mm) and ESI-Q-TOF mass spectrometry (Bruker Impact II). In its intact form (A) the protein is too complex and deconvolution of the mass spectra only yielded noise. After removal of *N*- and core 1 *O*-glycans the Tn antigens remain (B) and can efficiently be removed by incubation with GalNAcEXO (C, over night at 37 °C).

Efficient Hydrolysis without Co-factors

GalNAcEXO is active in physiological buffers in a neutral pH range, making it compatible with most samples. The activity is not dependent on co-factors, such as BSA, that can impair LC-MS analysis (Table 1). Depending on the substrate, the reaction for *O*-glycoproteins is complete in 4 hours while more complex samples may require incubation over night. GalNAcEXO is available immobilized in spin columns for easy processing without residual enzyme in the final sample.

Table 1. Key characteristic features of the GalNAcEXO enzyme.

Enzyme Feature	GalNAcEXO
Incubation time	2 to 18 hours
pH range	6.0 - 7.6
MS compatibility	Yes
Special buffers	No
Co-factors	No
Additives or BSA	No

GalNAcEXO™

Product ID	Description	EUR	USD
G1-NA1-020	GalNAcEXO, 2000 units	750	845
G1-NA6-025	Immobilized GalNAcEXO, 5 x 0.5 mg	795	995
G1-NA6-050	Immobilized GalNAcEXO, 10 x 0.5 mg	1,295	1,745

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