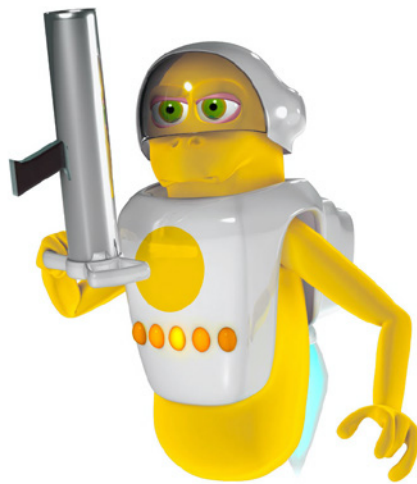




# GalactEXO™



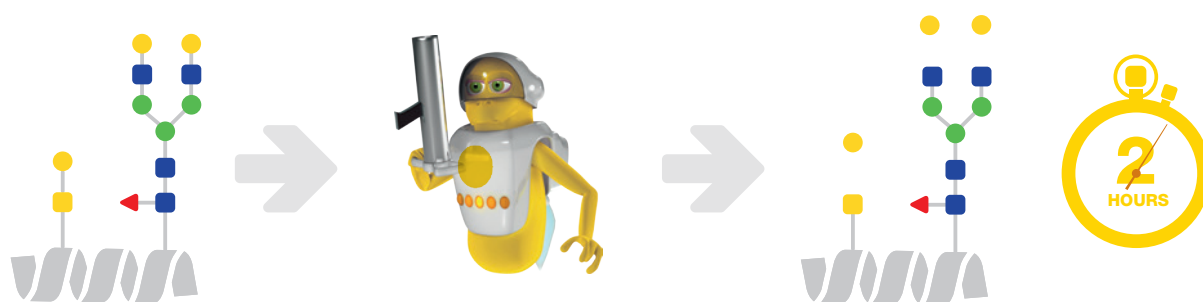
Efficient Hydrolysis of  $\beta$ 1-3,4 linked Galactose

SmartEnzymes™

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# GalactEXO™



GalactEXO™ is a  $\beta$ -galactosidase for efficient hydrolysis of galactose from glycoproteins and released glycans

GalactEXO is a  $\beta$ -galactosidase mix for complete hydrolysis of galactose residues on *N*- and *O*-linked glycans on native glycoproteins. The enzymes were discovered and characterized from *Akkermansia muciniphila* for efficient hydrolysis of  $\beta$ 1-3 and  $\beta$ 1-4 linked galactoses. The combined enzymatic activities will completely hydrolyze all galactoses on 2 mg of native glycoprotein. GalactEXO can be used for trimming of the released glycans in exo-glycosidase array sequencing experiments where  $\beta$ 1-3 and  $\beta$ 1-4 linked galactose are removed within 1 h of incubation. GalactEXO can be applied to obtain antibodies with homogenous G0 glycosylation profiles.

-  Hydrolyzes galactose residues on *N*- and *O*-glycosylated proteins
-   $\beta$ 1-3 and  $\beta$ 1-4 linked galactose
-  2 h incubation
-  No co-factors required

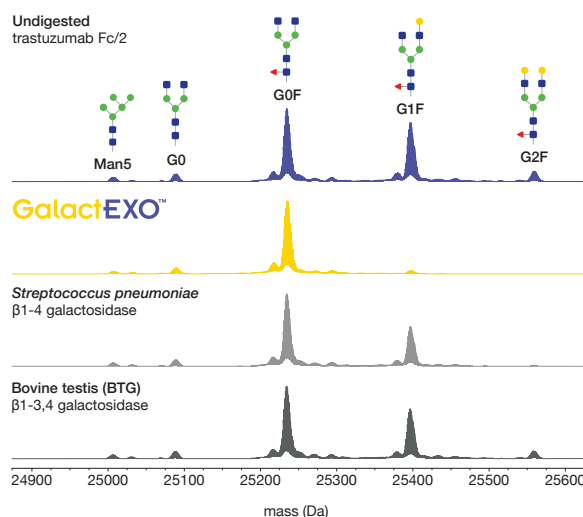
## Key Characteristics

- ▶ Efficient removal of  $\beta$ 1-3,4 linked galactose
- ▶ Activity on both *N*- and *O*-glycan structures
- ▶ For native glycoproteins and free glycans

## Hydrolysis of $\beta$ 1-4 Galactose on a mAb

The  $\beta$ -galactosidase activity of GalactEXO was demonstrated on trastuzumab carrying galactose residues in the Fc domain of the antibody. After a 2 h incubation with GalactEXO or with competing enzymes from other sources, the antibodies were digested into subunits using FabRICATOR® and analyzed using LC-MS (Fig. 1). The shift to G0F can be seen using GalactEXO whereas enzymes from other sources results in incomplete digestion.

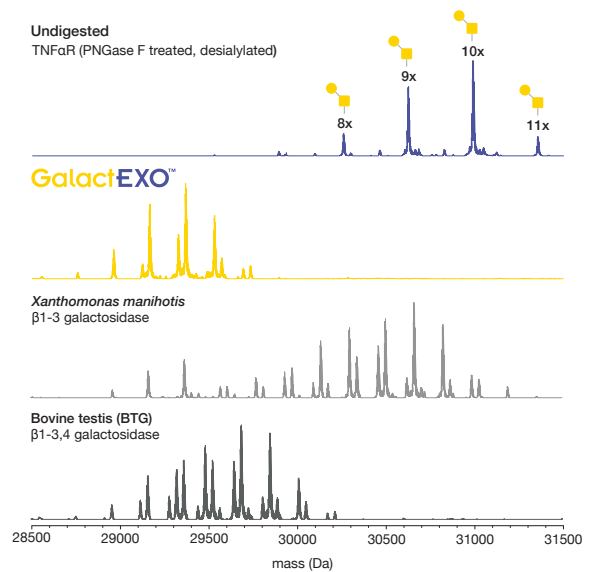
Figure 1. Deconvoluted mass spectra of the Fc/2 of trastuzumab treated with three different  $\beta$ -galactosidases. The antibody was digested with FabRICATOR, separated by reverse phase HPLC (Waters BioResolve RP, 2.1x100 mm) and analyzed by ESI-Q-TOF mass spectrometry (Bruker Impact II).



## Activity on $\beta$ 1-3 Gal from Etanercept

GalactEXO also shows exogalactosidase activity for  $\beta$ 1-3 galactose, present on O-glycosylated biopharmaceuticals. Etanercept was incubated with GalactEXO or galactosidases from other sources and the TNFaR fragment was analyzed using LC-MS after digestion with FabRICATOR to remove the Fc fragment (Fig. 2). The results show efficient galactosidase activity from GalactEXO compared to the enzymes from other sources.

Figure 2. Deconvoluted mass spectra of the TNFaR fragment of etanercept treated with three different beta-galactosidases. The protein was digested with FabRICATOR, separated by reverse phase HPLC (Waters BioResolve RP, 2.1x100 mm) and analyzed by ESI-Q-TOF mass spectrometry (Bruker Impact II).



## GalactEXO for Released Glycan Trimming

When analyzing released glycan structures using exoglycosidases it is crucial to obtain complete hydrolysis to minimize errors in data interpretation. A labeled N-glycan library was incubated with the SialEXO<sup>®</sup> sialidase and GalactEXO galactosidase for 1 h and analyzed for exoglycosidase activities (Fig. 3). The HILIC-FLD separations show complete

hydrolysis of both the sialylated and galactosylated structures and the remaining peaks can easily be identified as G0, G0F or G0F with bisecting GlcNAc.

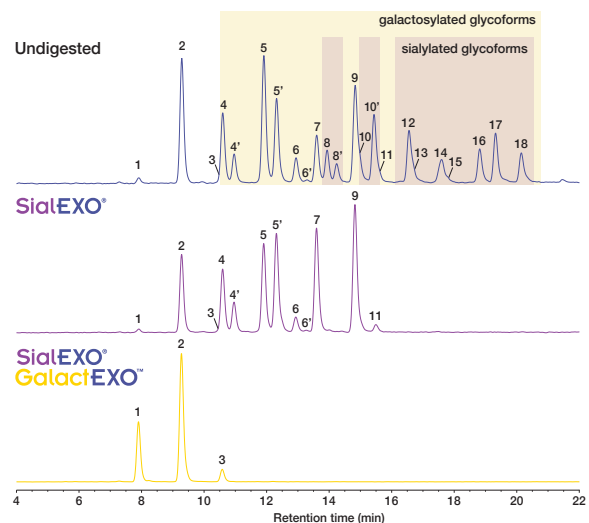
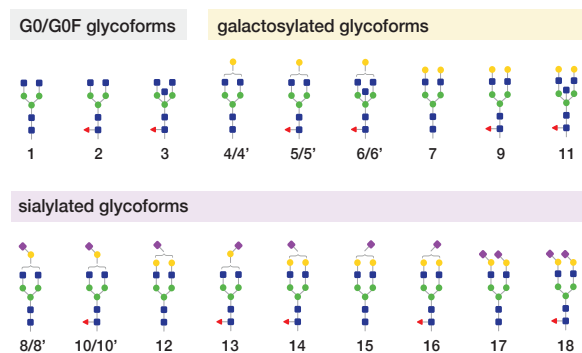


Figure 3. HILIC-FLD UHPLC chromatograms of a 2-AB labeled glycan library analyzed undigested (top), after treatment with SialEXO (middle) or both SialEXO and GalactEXO (bottom). Analysis was performed on a Thermo Scientific Vanquish Duo UHPLC system equipped with a Thermo Scientific Accurcore 150 Amide HILIC column (2.1 x 150 mm).

# GalactEXO™



Product ID	Description	EUR	USD
G1-GM1-020	GalactEXO, 2000 units	750	845
G1-GM6-025	Immobilized GalactEXO Microspin, 5 x 0.5 mg	795	995
G1-GM6-050	Immobilized GalactEXO Microspin, 10 x 0.5 mg	1,295	1,745

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