NEW
Efficient Removal of α1-2,3,4 Fucose

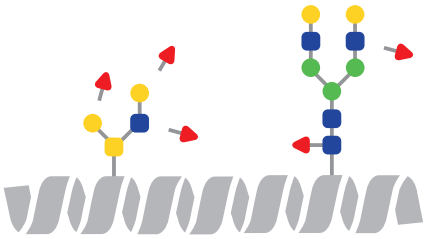
# FucosEXO™

Hydrolysis of α1-2,3,4 linked Fucose

SmartEnzymes™

GENOVIS

# FucosEXO™




Hydrolysis of α1-2,3,4 linked fucose on glycoproteins or oligosaccharides.

FucosEXO is a mix of α-fucosidases for efficient hydrolysis of α1-2, α1-3 and α1-4 linked fucose residues on native *N*- and *O*-glycosylated proteins or free oligosaccharides. The two enzymes are modified with His-tags and expressed in E. *coli.* The molecular weight of the enzymes are 87 kDa and 64 kDa, respectively. FucosEXO is active in neutral pH and does not require any co-factors or special buffers. The exoglycosidase activity of FucosEXO allows for the complete hydrolysis of α1-2,3,4 linked fucose on up to 2 mg of glycoprotein. FucosEXO is a valuable tool for glycan structure analysis on *N*- and *O*-glycoproteins or in exoglycosidase arrays on oligosaccharides.

- Hydrolyzes α1-2,3,4 linked Fucose on native glycoproteins
- α1-2,3,4 linked Fucose
- 1-2 h incubation
- No co-factors required

## Key Characteristics

- Efficient hydrolysis of α1-2,3,4 linked fucose
- For native glycoproteins or oligosaccharides
- Activity without the need for co-factors

## Specific Hydrolysis of Fucose Linkages

Fucose can be attached to both *N*- and *O*-glycans. The α1-2, α1-3 and α1-4 linkages most commonly occur in *O*-glycans or as antenna fucosylation of *N*-glycans, whereas α1-6 linked fucose is found as a modification of the *N*-glycan core. We measured the release of fucose using FucosEXO from a panel of oligosaccharide substrates representing different linkages. FucosEXO efficiently releases α1-2, α1-3 and α1-4 linked fucose, without activity on α1-6 linked fucose residues (Fig. 1).

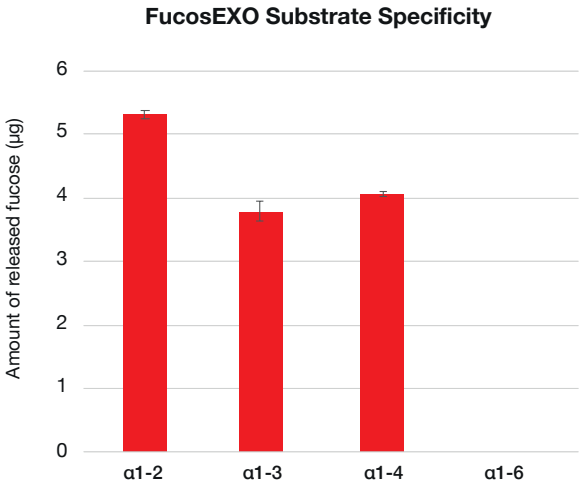



Figure 1. The substrate specificity of FucosEXO was analyzed on equal molar amounts of synthetic oligosaccharides; α1-2 (2'- fucosyllactose,) α1-3 (3-fucosyllactose), α1-4 (Lewis a) and α1-6 (α1-6 fucosylated chitobiose). Substrates were incubated with FucosEXO for 30 min at 37°C and the amount of released fucose measured spectrometrically using an L-Fucose Assay Kit (Megazymes).

## Defucosylation of Native Glycoproteins

Fucosylation of *O*-glycans is involved in synthesis of functionally important glycan epitopes such as blood group antigens and the Lewis structures. The analysis of glycoproteins modified with such complex glycans can be challenging and requires specific and efficient enzymatic tools. We tested FucosEXO on a glycoengineered TNFR protein carrying up to 11 *O*-glycans decorated with α1-2 and α1-3 linked fucose and compared the activity to a commercially available α-fucosidase. Within 1 hour, complete removal of fucose on the TNFR protein was achieved with FucosEXO, while treatment with the other fucosidase only had a minor impact on fucoses (Fig. 2). Hydrolysis of all fucose residues from the TNFR protein was also achieved using Immobilized FucosEXO.

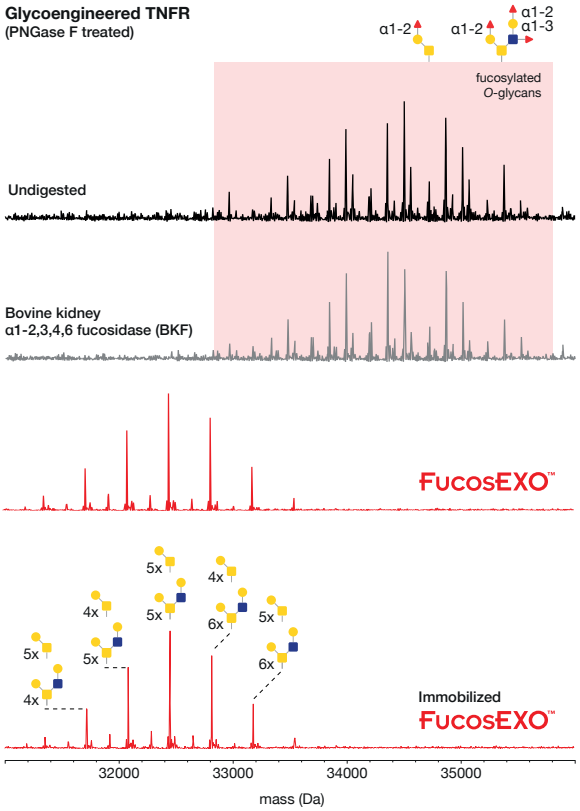



Figure 2. Glycoengineered TNFR with core 1 and core 2 *O*-glycans decorated with both α1-2 and α1-3 fucose was incubated with bovine kidney fucosidase (1h, 37°C), FucosEXO (1h, 37°C) or Immobilized FucoseEXO (1h, RT). The resulting protein was analyzed by reverse phase LC-MS on a Waters BioAccord system equipped with a Waters BioResolve RP mAb column (2.1 x 50 mm).

## No Additives or Co-factors Required

FucosEXO is active in physiological buffers at a neutral pH and the activity is not dependent on any co-factors, making FucosEXO compatible with most samples without the risk of impairing LC-MS analysis (Table 1). Depending on the nature of the substrate, FucosEXO hydrolyzes terminal α1-2,3,4 linked fucose residues on glycoproteins within 1 to 2 hours, while longer incubation may be required for very complex samples. FucosEXO is also available immobilized on agarose beads in a spin column format for convenient processing without residual enzyme in the final preparation.

Table 1. Key characteristics of FucosEXO.

| Enzyme Feature | FucosEXO |
|---|---|
| Incubation time | 1-2 hours |
| pH range | 6.0 - 8.0 |
| MS compatibility | Yes |
| Special buffers | No |
| Co-factors | No |
| Additives or BSA | No |

# FucosEXO™

| Product ID | Description | EUR | USD |
|---|---|---|---|
| G1-FM1-020 | FucosEXO, 2000 units | 750 | 845 |
| G1-FM6-025 | Immobilized FucosEXO, 5 x 0.5 mg | 795 | 995 |
| G1-FM6-050 | Immobilized FucosEXO, 10 x 0.5 mg | 1,295 | 1,745 |

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