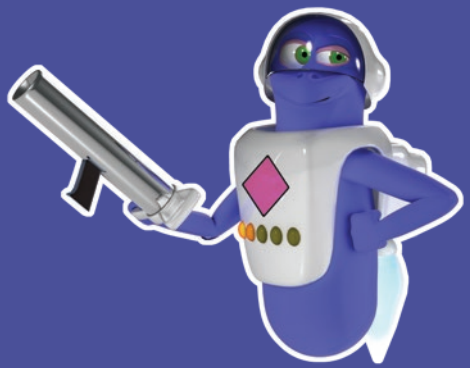
OpeRATOR™

SmartEnzymes™

GENOVIS

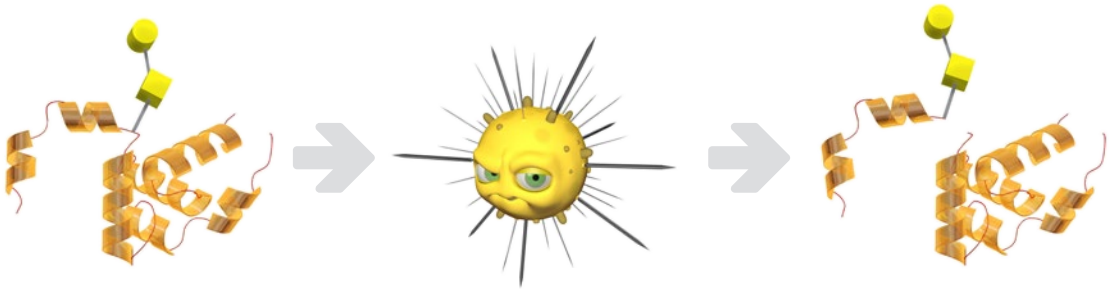
Enzymes for
O-glycans

OglyZOR™



SialEXO™

OpeRATOR™



OpeRATOR™ is an O-glycan specific protease that digests O-glycosylated proteins N-terminally of the S/T glycosylation site.

The OpeRATOR enzyme is a novel tool for analysis of O-glycans on glycoproteins. The endoprotease binds to O-glycans and digests the amino acid backbone N-terminally of the S/T residue. This generates glycopeptides decorated with O-glycans and allows for O-glycan profiling and site-occupancy determination using mass spectrometry.

The OpeRATOR enzyme is active on native glycoproteins and does usually not require denaturation of the glycoprotein. O-glycans are required for OpeRATOR activity and the enzyme will not digest glycoproteins with only N-linked glycans. OpeRATOR is active on O-glycan proteins with or without sialic acids. The activity is higher on O-glycosylated proteins where sialic acids have been removed (*Figure 1*). OpeRATOR can be used in combination with SialEXO™, for removal of sialic acids and subsequent digestion. OpeRATOR is derived from *Akkermansia muciniphila* and is expressed in *E. coli*.

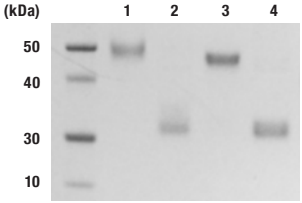
Key Characteristics

- O-glycan specific endoprotease
- Digests N-terminally of O-glycans, at serine or threonine residues
- Requires O-glycans for activity – no activity on glycoproteins with only N-glycans
- Increased performance when pretreated with SialEXO

Applications of OpeRATOR

- O-glycan profiling
- O-glycan site determination
- Middle-down approaches for O-glycosylated proteins
- O-glycopeptide mapping

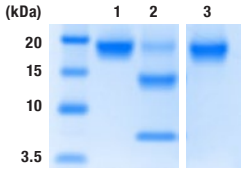
Effect of SialEXO Pretreatment on OpeRATOR Enzymatic Activity



1. Control TNFαR
2. + OpeRATOR
3. + SialEXO
4. + OpeRATOR + SialEXO

Figure 1. OpeRATOR is active on sialylated O-glycoproteins, but the activity is higher when the sialic acids are removed using SialEXO. The native O-glycosylated TNFα receptor (TNFαR) was incubated with OpeRATOR with or without the addition of SialEXO overnight, and the reactions were separated on SDS-PAGE.

O-glycan Site-specific Digestion of EPO using OpeRATOR



1. EPO + SialEXO
2. EPO + SialEXO + OpeRATOR
3. EPO + SialEXO + OglyZOR, followed by incubation with OpeRATOR

Figure 2. Erythropoietin (EPO) was incubated with SialEXO to remove sialic acids (lane 1), and with OpeRATOR to digest the protein N-terminally of the O-glycan (lane 2). To demonstrate the O-glycan-dependent activity of OpeRATOR, EPO was incubated with OglyZOR™ to remove the O-glycan. After the incubation, OpeRATOR was added to the reaction. As seen in lane 3, there is no digestion of EPO by OpeRATOR, indicating an O-glycan-dependent mode of action.

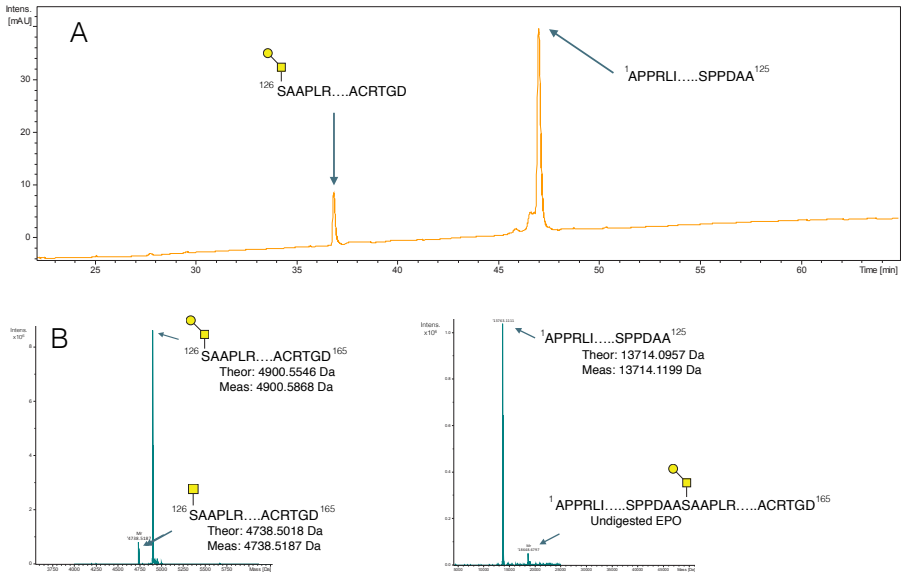


Figure 3. EPO is a ~30 kDa glycoprotein with one O-glycan site. The protein was used as a substrate to demonstrate the specific activity of the OpeRATOR protease. N-glycans were removed from EPO with PNGaseF and sialic acids were removed using SialEXO. In parallel, OpeRATOR hydrolyzed the protein N-terminally to the serine O-glycan site. After reduction of disulfide bridges with DTT, the resulting two fragments were separated on a RP C4 column and intact mass was analyzed with a Bruker Impact II ESI QTOF MS. UV trace **(A)** and QTOF MS **(B)**.

LC/MS Analysis of Etanercept using OpeRATOR Reveals O-glycosylation Heterogeneity

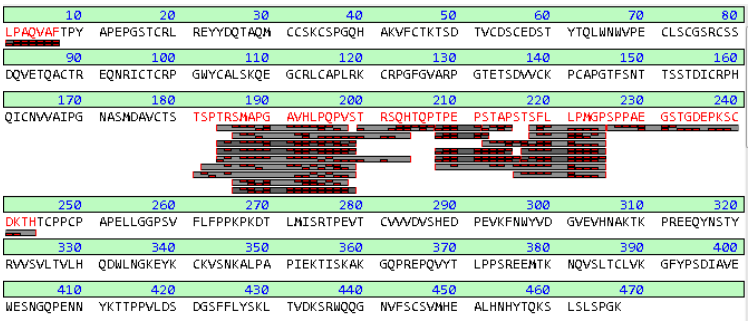


Figure 4. Etanercept (Enbrel®), a fusion protein with a highly O-glycosylated linker region was used to demonstrate the applicability of the OpeRATOR enzyme. Etanercept was treated with PNGaseF, SialEXO and OpeRATOR in a native, overnight digestion reaction. One part of the sample was further treated with OglyZOR for a few hours to remove O-glycans from the created peptides. The resulting peptides and glycopeptides were separated in a RP-UPLC gradient on a C18 column connected to a Bruker Impact II ESI QTOF MS generating MSMS data. Due to the heterogeneity in the O-glycan pattern of the protein and the OpeRATOR protease specificity for O-glycan structures, overlapping peptides were formed, making it possible to acquire a complete map of the O-glycan sites.

OpeRATOR™

The OpeRATOR enzyme consists of 2000 units for digestion of 2 mg glycoprotein. The enzyme is provided together with 2000 units of SialEXO for optional sialic acid removal. Both enzymes are provided as lyophilized powders.

	Product ID	Description	Digestion	EUR	USD
	G2-OP1-020	OpeRATOR, 2000 units	2 mg	795	895

OglyZOR™

OglyZOR™ is an endoglycosidase that specifically hydrolyzes core 1 and core 3 O-glycans on native glycoproteins. It requires removal of sialic aids for activity, and is provided together with SialEXO™ (sialidase).

OglyZOR is an endoglycosidase that catalyzes the removal of core 1 and core 3 O-linked disaccharides from native glycoproteins. The enzyme acts on native glycoproteins, and does usually not require denaturation of the substrate.

The sialic acids of the glycans should be removed for OglyZOR activity. For this reason, the OglyZOR enzyme is provided together with SialEXO – a novel sialidase mix for efficient and complete removal of sialic acids.

OglyZOR is derived from *Streptococcus oralis* and is expressed in *E. coli*.

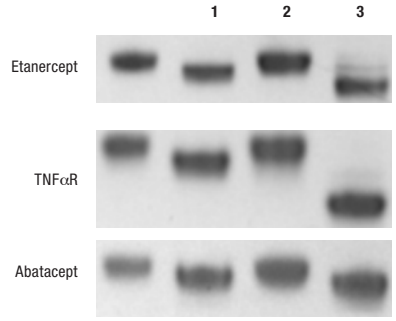
Key Characteristics

- Endoglycosidase acting on O-glycans
- Active on native glycoproteins
- Specific for core 1 and core 3 O-glycan disaccharides
- Requires pretreatment with SialEXO

Applications of OglyZOR

- Removal of O-glycans for glycan analysis
- Confirmation of O-glycan presence
- Reduction of sample heterogeneity

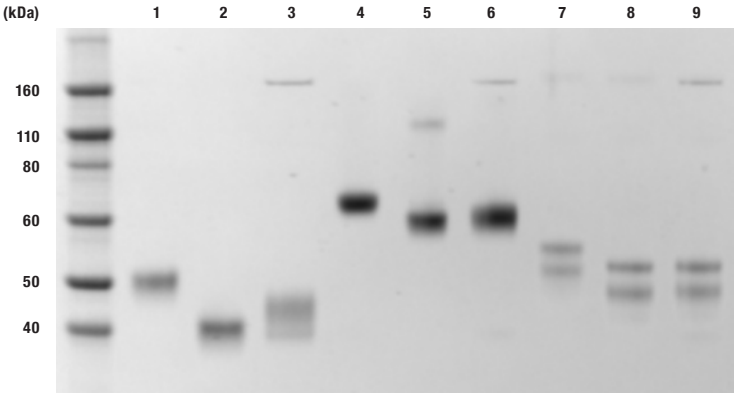
OglyZOR and SialEXO Remove O-glycans from Native Glycoproteins



1. + SialEXO
2. + OglyZOR
3. + SialEXO + OglyZOR

Figure 1. To investigate OglyZOR activity on native glycoproteins, the enzyme was incubated with etanercept (Enbrel®), TNFα receptor (TNFαR) and abatacept (Orencia®) for 1 h at 37°C with or without SialEXO. The endoglycosidase activity was verified on SDS-PAGE. The results demonstrate that it is necessary to remove the sialic acids with SialEXO. Also, the combination of OglyZOR and SialEXO results in the loss of O-glycans from the glycoproteins.

OglyZOR and SialEXO Compared to Enzymes from Other Bacteria



1. TNFα receptor
2. + SialEXO and OglyZOR
3. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)
4. Etanercept
5. + SialEXO and OglyZOR
6. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)
7. Fetuin
8. + SialEXO and OglyZOR
9. + Endoglycosidase (*E. faecalis*) and sialidase (*C. perfringens*)

Figure 2. Comparison of the enzymatic activity of OglyZOR and SialEXO to commercially available endoglycosidase and sialidase from *E. faecalis* and *C. perfringens*. All incubations (4 h) were performed according to the manufacturers instructions, and the TNFα receptor, etanercept and fetuin were all separated on SDS-PAGE.

OglyZOR™

The OglyZOR enzyme consists of 2000 units for hydrolysis of O-glycans on 2 mg glycoprotein. The enzyme is provided together with 2000 units of SialEXO for sialic acid removal. Both enzymes are provided as lyophilized powders.

	Product ID	Description	Digestion	EUR	USD
	G2-OG1-020	OglyZOR, 2000 units	2 mg	695	795

SialEXO™

SialEXO™ is a sialidase mix for complete removal of sialic acids from native glycoproteins.

SialEXO is used for removal of sialic acids on native glycoproteins, and works on both O- and N-linked glycans. It is a combination of two sialidases acting on α2-3, α2-6 and α2-8 linkages.

The SialEXO can be used to pretreat an O-glycosylated protein prior to digestion with OpeRATOR™, or prior to deglycosylation with OglyZOR™. By using SialEXO in combination with the above-mentioned enzymes, the activity of the enzymes is enhanced.

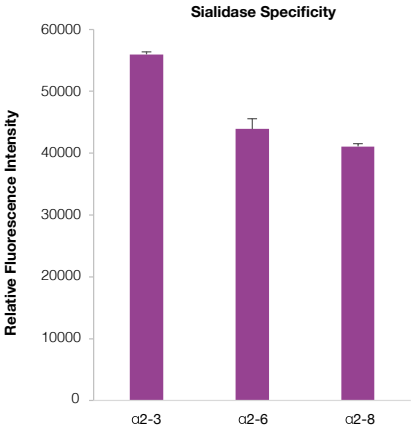
The SialEXO enzymes are derived from *Akkermansia muciniphila* and expressed in *E. coli*.

Key Characteristics

- Acts on native glycoproteins
- Cleaves α2-3, α2-6 and α2-8-linked sialic acids
- Acts on both N- and O-linked glycans
- Hydrolyzes sialic acids within 2 h
- Compatible with OpeRATOR and OglyZOR

Applications of SialEXO

- Removal of all sialic acids
- Pretreatment prior to OpeRATOR incubation
- Pretreatment prior to OglyZOR deglycosylation
- Exoglycosidase array



SialEXO Activity on α2-3, α2-6 and α2-8 Sialic Acid Linkages as Determined on Synthetic Substrates

Figure 1. The substrate specificity of SialEXO was investigated through quantification of liberated free sialic acids from the substrates 3'-sialyllactose (α2-3 bonds), 6'-sialyllactose (α2-6 bonds), and colominic acid (α2-8 bonds).

Effect of SialEXO Pretreatment on OpeRATOR Enzymatic Activity

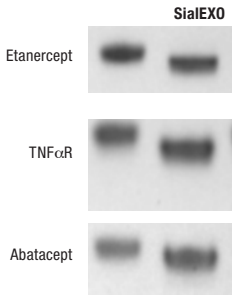


Figure 2. To investigate SialEXO activity on native glycoproteins, the enzyme was incubated with etanercept (Enbrel®), TNFα receptor and abatacept (Orencia®) for 1 h at 37°C, after which sialic acid hydrolysis was verified on SDS-PAGE.

SialEXO™

The SialEXO enzymes consist of 2000 units for hydrolysis of sialic acids on 2 mg glycoprotein. The enzymes are provided as lyophilized powders.

	Product ID	Description	Digestion	EUR	USD
	G1-SM1-020	SialEXO, 2000 units	2 mg	495	595

LÄNGE LEVE 2017 OG1706-1

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