GENOVIS

SialEXO™

Hydrolysis of Sialic Acids

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The SialEXO products are sialidases for the removal and analysis of sialic acids. The enzymes are active on both N- and O-linked glycans present on native glycoproteins or released glycan structures. SialEXO is used to pretreat O-glycosylated proteins prior to digestion with OpeRATOR or OglyZOR. Other applications include reduction of sample complexity, charge variant analyses and exoglycosidase arrays.

- Sialic acids on N- and O-linked glycans
- α2-3, α2-6 and α2-8-linked sialic acids
- 30 min – 2 h reaction
- Requires no co-factors

Desialylation Workflow

The inherent negative charge of sialic acids might complicate analytical workflows and mask other important modifications. The removal of sialic acids therefore facilitates the study of underlying variants in the protein.

SialEXO hydrolyzes glycoproteins under native conditions and displays a high activity in a broad pH range, 6.5 to 9.

Product Formats

SialEXO™ Lyophilized
Lyophilized enzyme mix for desialylation of glycoproteins

SialEXO™ 2-3 Lyophilized
Lyophilized enzyme for hydrolysis of α2-3-linked sialic acids

SialEXO™ Immobilized
Immobilized enzymes for desialylation of glycoproteins in spin columns

SialEXO™ Lyophilized

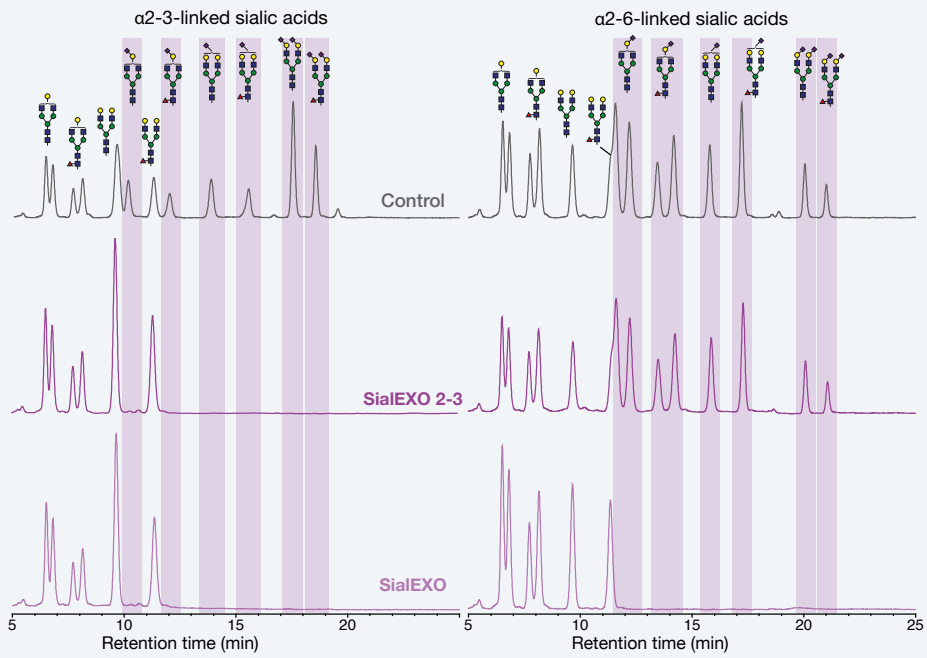
PRODUCT	DESCRIPTION	ID
SialEXO Lyophilized 2000 units	Process 2 mg glycoprotein	G1-SM1-020

SialEXO™ 2-3 Lyophilized

PRODUCT	DESCRIPTION	ID
SialEXO 2-3 Lyophilized 500 units	Process 0.5 mg glycoprotein	G1-SD2-005

Analysis of Released Sialic Acids using SialEXO™ and SialEXO™ 2-3

Sialic acids present on biopharmaceuticals are primarily attached in α2-3 or α2-6-linkages. The broad acting sialidase mix SialEXO hydrolyzes all sialic acid linkages, including α2-3, α2-6, and α2-8. SialEXO 2-3 is an enzyme that display high specificity for α2-3-linked sialic acids. Here, hydrophilic interaction liquid chromatography (HILIC) was used to analyze two glycan libraries. One library contained released glycans with α2-3-linked sialic acids and the other contained α2-6-linked sialic acids. The shift in retention times clearly showed the release of α2-3 and α2-6-linked sialic acids from the glycans by SialEXO 2-3 and SialEXO respectively.

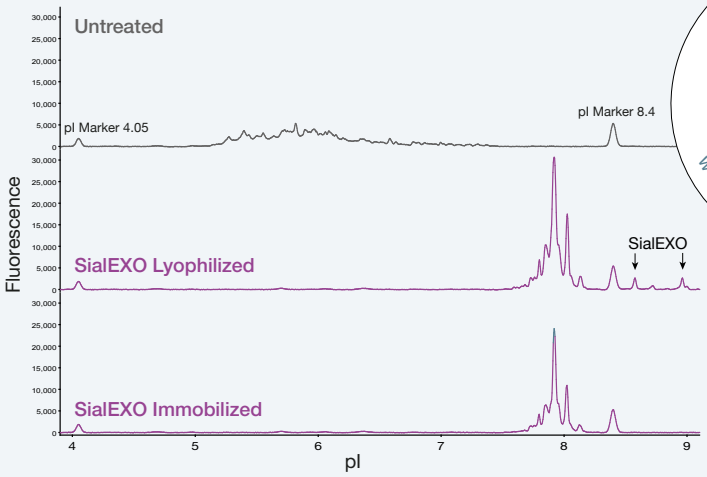


Analysis of released sialic acids. HILIC analysis of released glycans with α2-3-linked (left) or α2-6-linked (right) sialic acids after incubation with SialEXO 2-3 or SialEXO respectively. Sialylated glycan structures are shaded in purple.

Charge Variant Analysis using SialEXO™ Immobilized

Capillary isoelectric focusing is commonly used to determine charge variants during characterization and quality control of biologics. Etanercept is a highly sialylated Fc-fusion protein which makes the analysis of its charge variants difficult.

SialEXO Immobilized microspin columns contain SialEXO covalently coupled to agarose beads for desialylation of native glycoproteins in 30 minutes without any residual enzyme in the final preparation. To study the underlying charge variants on etanercept, the protein was here desialylated using SialEXO Immobilized and analyzed by imaged capillary isoelectric focusing using Maurice from ProteinSimple. The desialylation removes the charge heterogeneity originating from the sialic acids and allows for the analysis of the underlying charge variants of the protein.



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Unmasking charge variants. The analytical workflow improved the separation and enabled charge variant analysis of glycoproteins. Etanercept is difficult to analyze without first removing the sialic acids, but using SialEXO Immobilized, separated peaks were obtained. Data was obtained in collaboration with ProteinSimple (San Jose, CA, USA).

SialEXO™ Immobilized

PRODUCT	DESCRIPTION	ID
SialEXO Immobilized Microspin 2 × 0.5 mg	Process 2 × 0.5 mg glycoprotein	G1-SM6-010
SialEXO Immobilized Microspin 5 × 0.5 mg	Process 5 × 0.5 mg glycoprotein	G1-SM6-025
SialEXO Immobilized Microspin 10 × 0.5 mg	Process 10 × 0.5 mg glycoprotein	G1-SM6-050

SmartEnzymes™

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