0.5 mg Glycoprotein Desialylated **in 30 min**

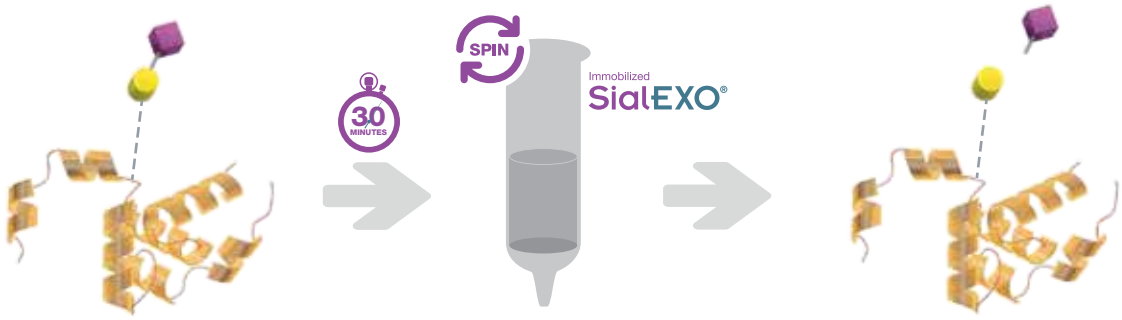
Immobilized SialEXO®

Complete Desialylation of Glycoproteins

SmartEnzymes™

GENOVIS

Immobilized SialEXO®



Immobilized SialEXO® is a microspin column for complete removal of sialic acids from native glycoproteins in 30 minutes.

SialEXO hydrolyzes sialic acids on native glycoproteins, and is active on both O- and N-linked glycans. It is a combination of two sialidases acting on α2-3, α2-6 and α2-8 linkages. Immobilized SialEXO is a resin with the enzymes covalently coupled to agarose beads for complete removal of sialic acids with no enzyme in the final preparation. 0.5 mg glycoprotein is desialylated in 30 min at room temperature.

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

Efficient Desialylation of a Monoclonal Antibody

Antibodies with additional glycans in their Fab regions are often sialylated to a significant extent. Cetuximab carries a Fab glycan which is partially modified with N-Glycolylneuraminic acid (Neu5Gc). These sialic acids can be removed rapidly using Immobilized SialEXO (*Fig. 1*), demonstrating the utility of this enzyme for analysis of monoclonal antibodies and its activity towards non-human sialic acids.

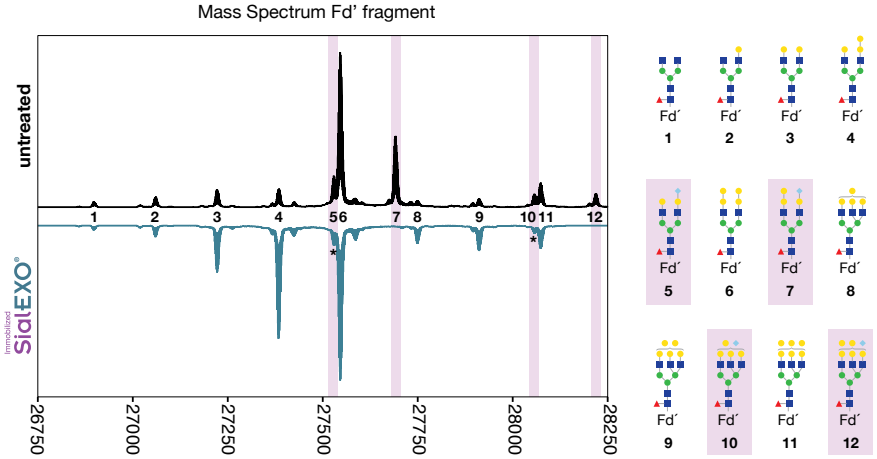


Figure 1. Subunits of cetuximab were obtained using FabRICATOR digestion and reduction. Deconvoluted mass spectra of the Fd' fragment of cetuximab, showing the Fab glycosylation profile. Sialylated structures (Neu5Gc, light blue diamond) are highlighted in purple and are absent in the lower spectrum. The asterisk marks peaks originating through neutral loss during ionization rather than remaining sialylated Fd' fragments.

Efficient Desialylation of a Complex Glycoprotein

The performance of SialEXO and Immobilized SialEXO was tested on the human C1 inhibitor. This glycoprotein is a challenging substrate with 6 N- and up to 26 O-glycans, modified with both α2,3 and α2,6-linked sialic acids. Analysis of released N-glycans demonstrates a complete desialylation by both SialEXO in solution and Immobilized SialEXO (*Fig. 2*) .

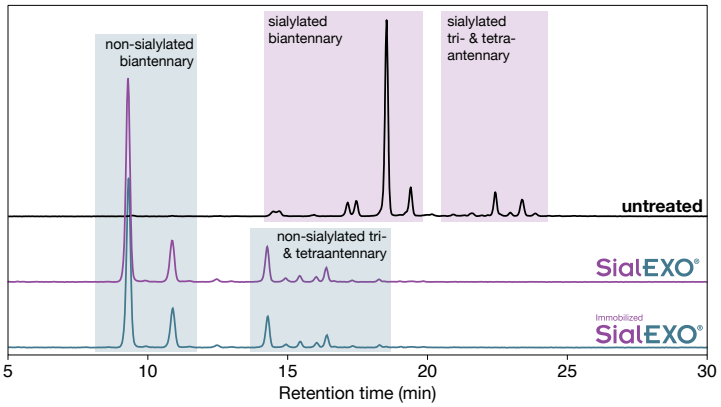


Figure 2. The C1 inhibitor was either treated with SialEXO in solution for 2 hours at 37°C, or with Immobilized SialEXO for 30 min at room temperature. N-glycans were released from the resulting desialylated protein using PNGase F and the resulting free glycans were labeled with 2-AB and analyzed by HILIC-FLD HPLC.

Simplified Charge Variant Analysis of Biologics

Capillary electrophoresis is commonly used to determine charge variants during characterization and quality control of biologics. The inherent charge of sialic acids might complicate charge variant profiles, masking other important modifications. The removal of sialic acids therefore facilitates the study of underlying charge variants in the protein. Cetuximab and etanercept were desialylated using Immobilized SialEXO which improved the separation in imaged isoelectric focusing (icIEF; *Fig. 3*).

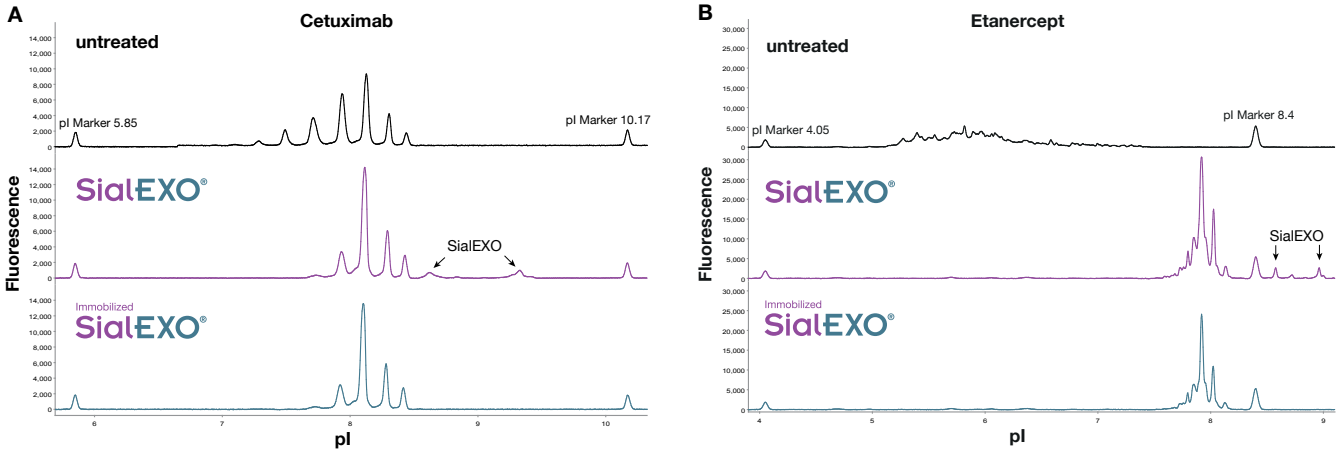


Figure 3. Desialylation of cetuximab **(a)** and etanercept **(b)** using Immobilized SialEXO followed by imaged isoelectric focusing. Data obtained in collaboration with ProteinSimple.

Immobilized
SialEXO®

The Immobilized SialEXO Microspin columns contain SialEXO covalently coupled to agarose beads, for desialylation of up to 0.5 mg native glycoprotein in 30 minutes.

	Product ID	Description	Desialylation	EUR	USD
	G1-SM6-025	Immobilized SialEXO Microspin	5 x 0.5 mg	775	995
	G1-SM6-050	Immobilized SialEXO Microspin	10 x 0.5 mg	1,295	1,745

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