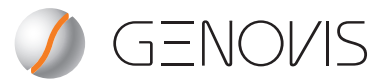




IgGZERO®

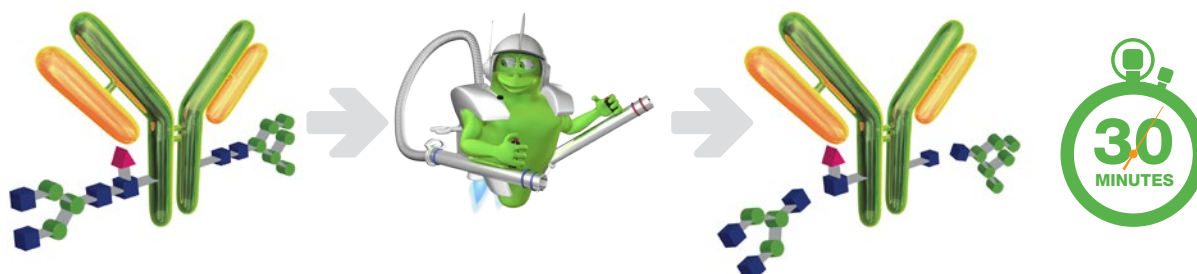
SmartEnzymes™



GlycINATOR®

GlycINATOR®

Antibody Fc Deglycosylation



GlycINATOR (EndoS2) is an endoglycosidase that rapidly hydrolyzes the N-glycan structure of the Fc domain of IgG.

The N-glycan is cleaved off after the first GlcNAc. GlycINATOR effectively removes high-mannose, as well as complex and hybrid, Fc glycans from IgG. The reaction is fast and mild under physiological conditions, and the enzyme works on native IgG without the need for detergents. GlycINATOR can be an effective tool by minimizing side effects in various immunoassays caused by complement binding or cross-reactivity.

- ▶ **Effective on high-mannose and bisected glycans**
- ▶ **Rapid deglycosylation – a 30 minute protocol**


Enzyme	Digestion site	Works on native IgG	pH optimum	Reaction time	IgG specific
GlycINATOR		Yes	7.4	30 minutes	Yes*
IgGZERO		Yes	7.4	30 minutes	Yes
PNGase F		Yes/No	7.5	6-24 h	No
Endo H		Yes/No	5-6	1-18 h	No

* GlycINATOR has one more known substrate, alpha-1-acid glycoprotein.

GlycINATOR®

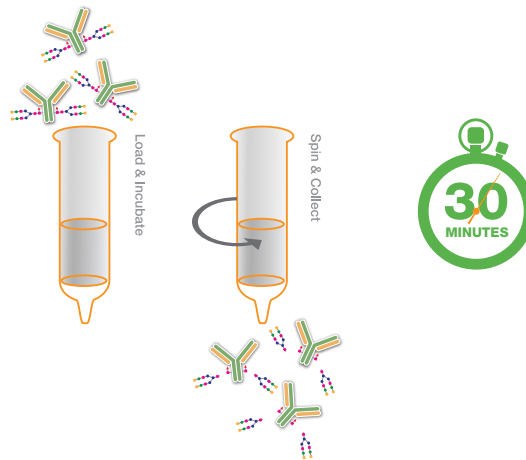
Lyophilized




GlycINATOR hydrolyzes Fc N-glycans, including high-mannose and bisected glycans. The enzyme is provided as a lyophilized preparation for deglycosylation of 2 mg IgG.

	Product ID	Description	Deglycosylation	EUR	USD
	A0-GL1-020	GlycINATOR 2,000 Units	2 mg IgG	410	570
	A0-GL8-020	GlycINATOR LE (low endotoxin) 2,000 Units	2 mg IgG	450	595

Immobilized GlycINATOR®

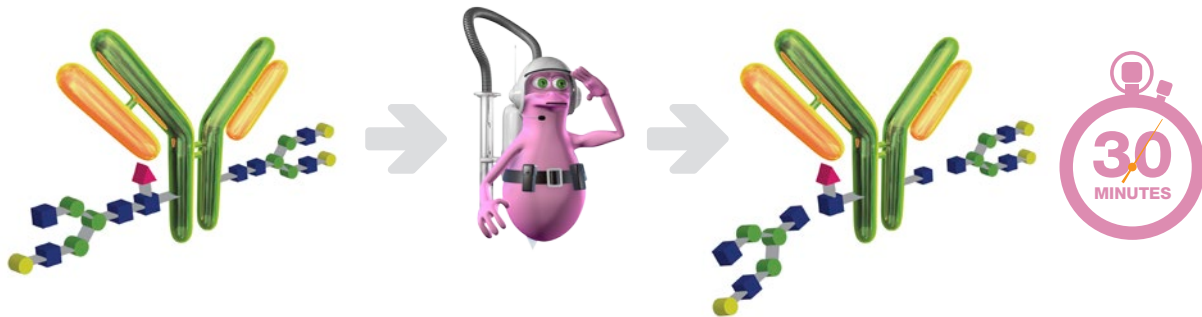
The Immobilized GlycINATOR spin columns contain the GlycINATOR enzyme covalently coupled to agarose beads, for deglycosylation of Fc glycans without contaminating the final preparation with enzyme.



	Product ID	Description	Deglycosylation	EUR	USD
	A0-GL6-010	Immobilized GlycINATOR Microspin	2 x 0.5 mg	305	425
	A0-GL6-025	Immobilized GlycINATOR Microspin	5 x 0.5 mg	695	960
	A0-GL6-050	Immobilized GlycINATOR Microspin	10 x 0.5 mg	1,150	1,605
	A0-GL6-100	Immobilized GlycINATOR Midispin	1-10 mg	925	1,285
	A0-GL6-1000	Immobilized GlycINATOR Maxispin	10-100 mg	2,760	3,860

IgGZERO®

Antibody Fc Deglycosylation



IgGZERO (EndoS) is an endoglycosidase with a unique specificity for native IgG. It is specific for N-glycans on the Fc domain which are cleaved off after the first GlcNAc.

The enzyme only hydrolyzes N-glycans on IgG, even if other glycosylated proteins are present. The reaction is fast and mild under physiological conditions, with no need for detergents. The enzyme can be an effective tool by minimizing side effects in various immunoassays caused by complement binding or cross-reactivity.

- ▶ **Fc glycan specific**
- ▶ **Rapid deglycosylation – a 30 minute protocol**
- ▶ **Works in serum**


Enzyme	Cleavage site	Works on native IgG	pH optimum	Reaction time	IgG specific
IgGZERO		Yes	7.4	30 minutes	Yes
GlycINATOR		Yes	7.4	30 minutes	Yes*
PNGase F		Yes/No	7.5	6-24 h	No
Endo H		Yes/No	5-6	1-18 h	No

* GlycINATOR has one more known substrate, alpha-1-acid glycoprotein.

IgGZERO®

Lyophilized

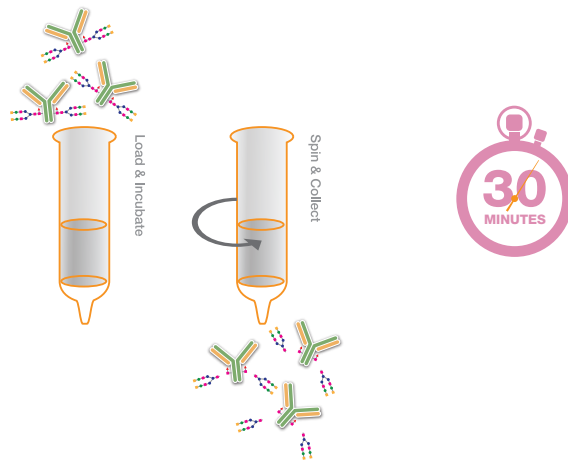
IgGZERO specifically hydrolyzes N-glycans on Fc of IgG. The enzyme is provided as a lyophilized powder.




	Product ID	Description	Deglycosylation	EUR	USD
	A0-IZ1-010	IgGZERO 1,000 Units	1 mg IgG	205	290
	A0-IZ1-050	IgGZERO 5,000 Units	5 mg IgG	830	1,150
	A0-IZ8-020	IgGZERO LE (low endotoxin) 2,000 Units	2 mg IgG	450	595

deGlycIT™

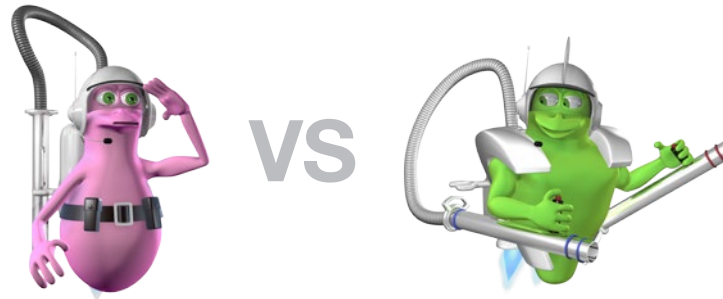
Immobilized IgGZERO

deGlycIT is IgGZERO immobilized on agarose, and it allows for rapid and selective removal of N-linked Fc glycans. The spin columns are provided prefilled with immobilized IgGZERO for deglycosylation of small amounts up to hundreds of mg of antibody.



	Product ID	Description	Deglycosylation	EUR	USD
	A0-IZ6-010	deGlycIT Microspin	2 x 0.5 mg	305	425
	A0-IZ6-025	deGlycIT Microspin	5 x 0.5 mg	695	960
	A0-IZ6-050	deGlycIT Microspin	10 x 0.5 mg	1,150	1,605
	A0-IZ6-100	deGlycIT Midispin	1-10 mg	925	1,285
	A0-IZ6-1000	deGlycIT Maxispin	10-100 mg	2,760	3,860

IgGZERO vs GlycINATOR

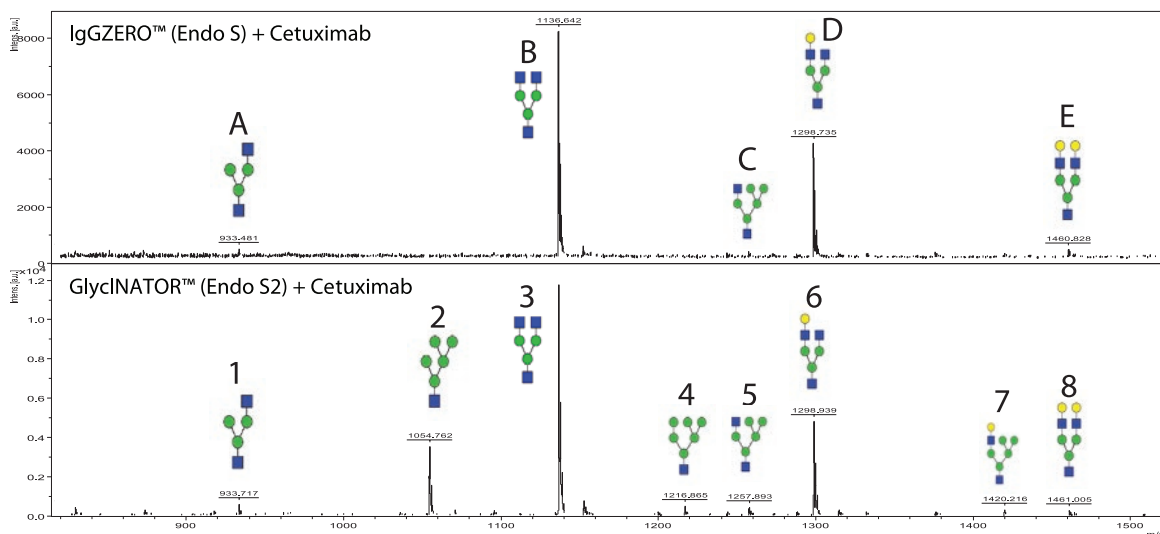


IgGZERO (EndoS) and GlycINATOR (EndoS2) rapidly hydrolyze N-glycans on the Fc domain of IgG. GlycINATOR removes all high-mannose glycans on human IgG, and IgGZERO does not. Both enzymes remove complex glycans whereas GlycINATOR is more effective on hybrid glycans.

Deglycosylation of Cetuximab

To compare the N-glycan specificity of IgGZERO and GlycINATOR, cetuximab was deglycosylated using IgGZERO and GlycINATOR. After deglycosylation, the antibody was digested

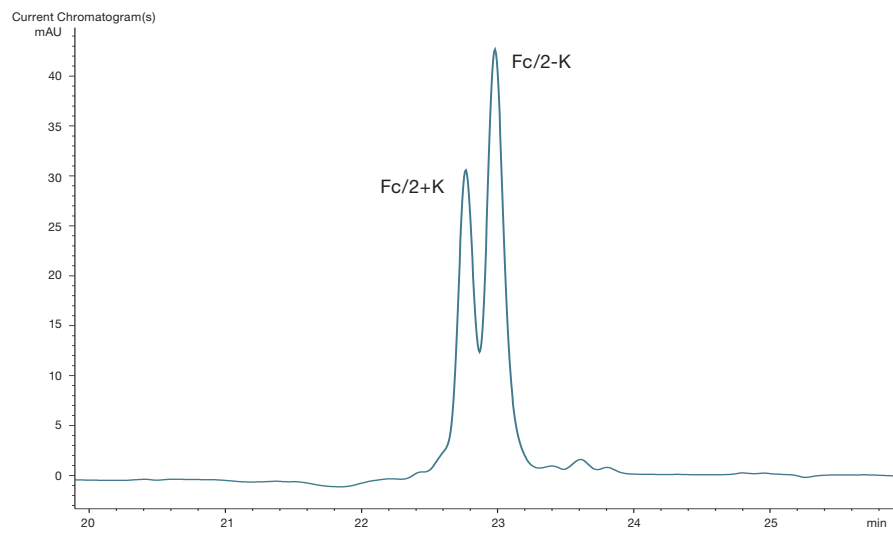
with FabRICATOR to generate F(ab')₂ and Fc fragments. Cetuximab contains high-mannose glycans, which are cleaved off by GlycINATOR but not by IgGZERO after 30 minutes reaction time.



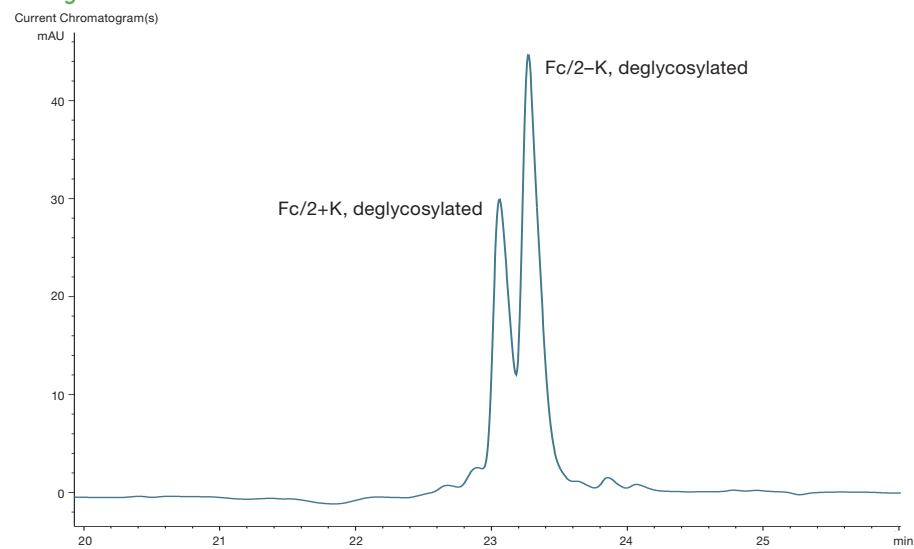
The N-glycans released by IgGZERO and GlycINATOR were analyzed by MALDI-TOF. High-mannose structures were readily cleaved off by GlycINATOR but not by IgGZERO.

Analysis of Deglycosylation with RP-HPLC

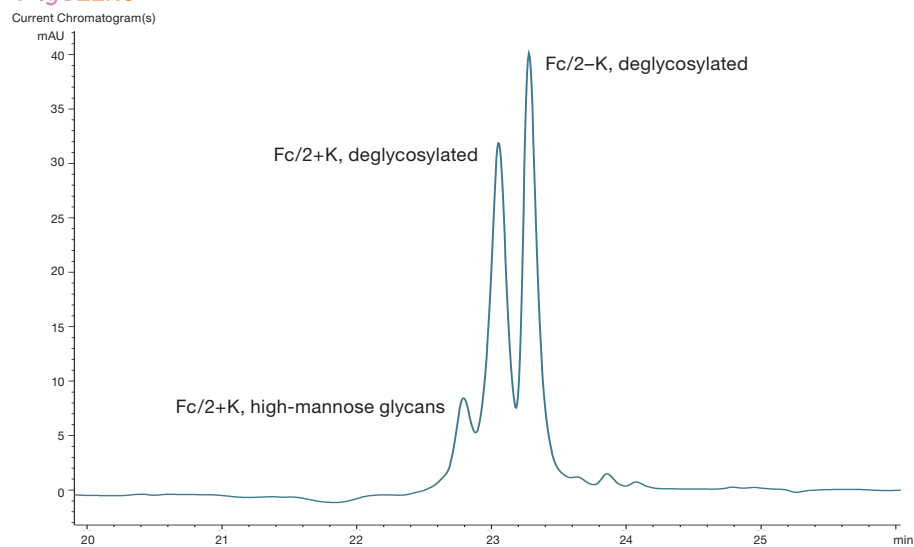
A: Non-deglycosylated



B: GlycINATOR™



C: IgGZERO®



The removal of glycans on Fc is seen as a shift in retention time in reversed phase chromatography. Chromatogram **A**: Fc with intact N-glycans, Chromatogram **B**: IgG deglycosylated with GlycINATOR, Chromatogram **C**: IgG deglycosylated with IgGZERO.

US & Canada

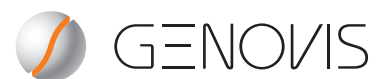
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