GlycINATOR®

FOR RESEARCH
USE ONLY

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STORE AT
-20°C

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

GlycINATOR® 2000 units (A0-GL1-020)
Deglycosylation of up to 2 mg IgG

GlycINATOR® LE 2000 units (A0-GL8-020)
Low endotoxin (<0.2 EU/vial)
Deglycosylation of up to 2 mg IgG

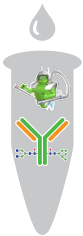
Last revised Jan 2021

1 Prepare GlycINATOR®

Reconstitute GlycINATOR in 50 µl ddH_2O to a concentration of 40 units/µl.

2 Add GlycINATOR®

Add 1 unit GlycINATOR / 1 µg IgG.



3 Deglycosylation

Incubate for 30 min at 37°C.

PRODUCT DESCRIPTION

GlycINATOR (EndoS2) is an endoglycosidase for deglycosylation of the Fc N-glycan moieties of IgG (1). All IgG glycoforms are hydrolyzed, including high-mannose, hybrid-type and bisected glycans (2). GlycINATOR hydrolyzes the 1,4 linkage between the core GlcNAc residues in the Fc glycan, leaving the innermost GlcNAc intact on the Fc. GlycINATOR deglycosylates all human IgG subclasses and IgG from the following species: mouse, rat, monkey, sheep, goat, cow and horse.

Physiological reaction conditions at pH 7.4 and 37°C yield optimal enzyme activity. Other buffers and pH may be compatible, but the reaction conditions need to be tested to ensure efficient deglycosylation.

GlycINATOR LE is a low endotoxin product. Therefore, use endotoxin-free materials and solutions.

GlycINATOR is derived from *Streptococcus pyogenes* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 92 kDa.

Unit Definition

One unit GlycINATOR deglycosylates ≥ 95% of 1 µg human IgG when incubated in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4 at 37°C for 30 min.

Content and Storage

GlycINATOR is supplied lyophilized in 10 mM Tris, 150 mM NaCl, pH 7.6, with no preservatives added.

GlycINATOR is shipped at ambient temperature and should be stored at -20°C upon arrival.

After reconstitution, GlycINATOR is stable for 1 month at +4-8°C.

GlycINATOR is for R&D use only.

Additional Materials Required

- Reaction buffer[1]: 10 mM sodium phosphate or 10 mM Tris, 150 mM NaCl, pH 7.4 or similar physiological buffer.
- For GlycINATOR LE, use endotoxin-free materials and solutions.

Sample Preparation

- Prepare IgG in reaction buffer at a concentration of 0.5-10 mg/ml.

Deglycosylation of IgG

1. **Prepare GlycINATOR®**
   Reconstitute GlycINATOR in 50 µl ddH_2O to 40 units/µl[2].

2. **Add GlycINATOR®**
   Add 1 unit GlycINATOR / 1 µg IgG.

3. **Deglycosylation**
   Incubate for 30 min[3] at 37°C.

Notes

1. *GlycINATOR hydrolyzes the IgG glycans at physiological conditions. Other buffers or pH may also be compatible with enzyme activity.*
2. *For GlycINATOR LE, use endotoxin-free ddH_2O.*
3. *An increased incubation time may improve deglycosylation of IgG from other species than human.*

Quality Control

GlycINATOR is tested to meet the specifications and lot-to-lot consistency.

GlycINATOR is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

Product References

1. Sjögren, J. et al., 2013. EndoS2 is a unique and conserved enzyme of serotype M49 group A Streptococcus that hydrolyses N-linked glycans on IgG and α1-acid glycoprotein. *The Biochemical Journal*, 455(1), pp.107–118.

2. Sjögren, J. et al., 2015. EndoS and EndoS2 hydrolyze Fc-glycans on therapeutic antibodies with different glycoform selectivity and can be used for rapid quantification of high-mannose glycans. *Glycobiology*, 25(10), pp.1053–1063.

Related Products

IgGZERO®
Deglycosylation of IgG Fc domain

deGlycIT™
Immobilized IgGZERO
Deglycosylation of IgG Fc domain

Immobilized GlycINATOR®
Deglycosylation of IgG Fc domain

GlycINATOR®
Legal and Disclaimers

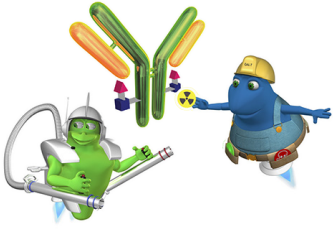
Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

GlyCLICK®

Site-specific Labeling of Antibodies

GlyCLICK is a site-specific conjugation technology for antibodies based on enzymatic remodeling of the N-linked Fc glycans and click chemistry*.

- Degree of label (DOL) = 2
- Intact immunoreactivity
- A variety of labels can be conjugated to the antibody, including drugs, chelators, biotin and fluorophores.



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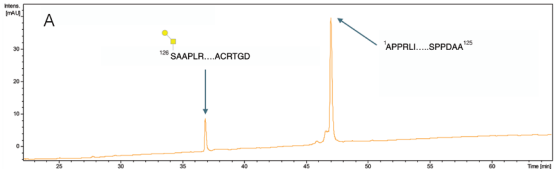
OpeRATOR®

O-glycan-Specific Endoprotease

OpeRATOR is a novel tool for analysis of O-glycans on glycoproteins. The protein binds to O-glycans and digests the amino acid backbone N-terminally of the S/T glycosylation site.

- O-glycan specific
- Requires O-glycans for activity
- Generates glycopeptides with O-glycans and allows for O-glycan profiling and site-occupancy determination using mass spectrometry.



Erythropoietin (EPO) is a ~30 kDa glycoprotein with one O-glycan site. The protein was used here as a substrate to demonstrate the specific activity of the OpeRATOR protease. OpeRATOR hydrolyzed the protein N- terminally of the serine O-glycan site, and after reduction of disulfide bridges, the resulting two fragments were separated and intact mass was analyzed using a Bruker Impact II ESI QTOF MS.

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