IgGZERO®

FOR RESEARCH
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STORE AT
-20°C

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

IgGZERO® 1000 units (A0-IZ1-010)
Deglycosylation of up to 1 mg IgG

IgGZERO® 5000 units (A0-IZ1-050)
Deglycosylation of up to 5 mg IgG

IgGZERO® LE 2000 units (A0-IZ8-020)
Low endotoxin (<0.2 EU/vial)
Deglycosylation of up to 2 mg IgG

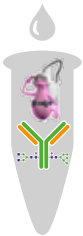
Last revised Jan 2020

1 Prepare IgGZERO®

Reconstitute IgGZERO in ddH_2O according to Table 1, to a concentration of 20 units/µl.

2 Add IgGZERO®

Add 1 unit IgGZERO / 1 µg IgG.



3 Deglycosylation

Incubate for 30 min at 37°C.

PRODUCT DESCRIPTION

IgGZERO (EndoS) is an endoglycosidase for deglycosylation of IgG Fc glycan moieties. IgGZERO hydrolyzes Fc glycans on IgG of all human IgG subclasses and IgG from the following species: mouse, rat, monkey, sheep, goat, cow and horse. In contrast to GlycINATOR®, IgGZERO has limited activity on high-mannose and hybrid-type glycans (1).
IgGZERO hydrolyzes the β1,4 linkage between the core GlcNAc residues in the Fc glycan, leaving the innermost GlcNAc intact on the Fc.

Physiological reaction conditions at pH 7.4 and 37°C yields optimal enzyme activity. Other buffers and pH (6-8) are compatible with enzyme activity but the reaction conditions need to be tested to ensure efficient deglycosylation.

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

IgGZERO is cloned from *Streptococcus pyogenes* and expressed in *E. coli*. The enzyme contains a His-tag and the molecular weight is 112 kDa.

Unit Definition

One unit IgGZERO 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

IgGZERO is supplied lyophilized in 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, with no preservatives added.

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

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

IgGZERO 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 IgGZERO LE, use endotoxin-free materials and solutions.

Sample Preparation

- Prepare IgG in reaction buffer[1] at a concentration of 0.5-10 mg/ml.

Deglycosylation of IgG

1. **Prepare IgGZERO®**
   Reconstitute IgGZERO in ddH_2O according to Table 1[2,3].

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

3. **Deglycosylation**
   Incubate for 30 min[4] at 37°C.

Table 1. *Volumes for reconstitution of IgGZERO®.*

Product	Product size	Reconstitution volume
A0-IZ1-010	1000 units	50 µl
A0-IZ1-050	5000 units	250 µl
A0-IZ8-020	2000 units	100 µl (LE)

Notes

1. *Optimal enzymatic activity is obtained at physiological reaction conditions (i.e pH 7.4 and 37°C). Many buffers at pH between 6-8 can be used but the reaction conditions need to be optimized.*
2. *For IgGZERO LE, use endotoxin-free ddH_2O.*
3. *To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).*
4. *An increased incubation time may improve deglycosylation of IgG from other species than human.*

Quality Control

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

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

Product Reference

1. 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

deGlycIT™
Immobilized IgGZERO, deglycosylation of IgG Fc domain

GlycINATOR®
Deglycosylation of IgG Fc domain

Legal and Disclaimers

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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.

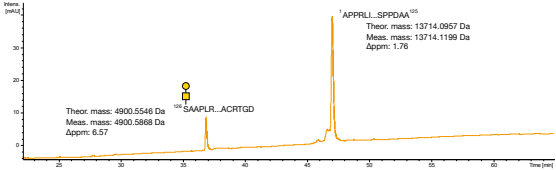
** SiteClick™ is provided under an intellectual property license from Life Technologies Corporation. The trademark SiteClick™ is the property of Life Technologies Corporation.*

OpeRATOR®

O-glycan-specific Endoprotease

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

- O-glycan-specific, mucin-type
- 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 core 1 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 by Q-TOF MS using ESI.

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