SmartEnzymes™

GENOVIS

GlyCLICK®

Azide Activation 10 mg

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TEMPERATURES

FOR RESEARCH USE ONLY

Instructions for Use

GlyCLICK® Azide Activation 10 mg (L1-AZ1-100)
Process 10 mg IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-L1-AZ1-100

www.genovis.com

Site-specific Conjugation of IgG with Azide-alkyne Click Chemistry

GlyCLICK is a site-specific conjugation technology for IgG using Fc N-glycan remodeling and click chemistry. The technology generates stable and homogenous antibody conjugates from several species and subclasses. Fc N-glycan remodeling by deglycosylation of the antibody allows for site-specific conjugation using robust click chemistry, resulting in a degree of labeling (DOL) or drug-antibody ratio (DAR) of 2.

GlyCLICK Azide Activation is available for azide activation of 250 µg, 2 mg or 10 mg IgG, for site-specific custom conjugation using an alkyne-modified label[1] of choice. All steps are performed under physiological conditions, thus maintaining the quality of the antibody. The site-specific conjugation on the Fc domain preserves the affinity of the antigen-binding sites.

GlyCLICK Azide Activation 10 mg contains all reagents needed to azide-activate 10 mg IgG. The azide activation is performed in two steps:

1. **Deglycosylation:** GlycINATOR Immobilized hydrolyzes the N-glycans on the Fc-part of the IgG to the inner GlcNAc.
2. **Azide Activation:** Azide attachment on the GlcNAc using GalT (Y289L)* and UDP-GalNAz*.

YOU MIGHT ALSO BE INTERESTED IN

GlycINATOR™ Immobilized
Immobilized enzyme for deglycosylation of IgG in spin columns

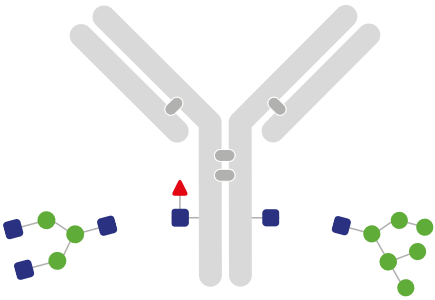
GlyCLICK® Biotin
Site-specific conjugation of IgG with biotin

GlyCLICK® Fluorophore
Site-specific conjugation of IgG with Alexa Fluor® 488, 555 or 647

GlyCLICK® DFO
Site-specific conjugation of IgG with DFO

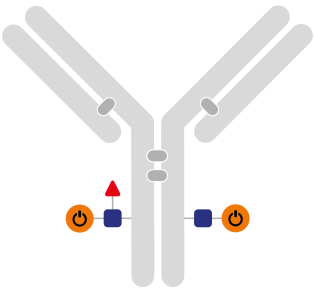
1 For copper-free click chemistry, the label must be functionalized by a cyclooctyne.

* GalT (Y289L) and UDP-GalNAz are components of SiteClick™ and are provided under an intellectual property license from Life Technologies Corporation. The trademark SiteClick™ is the property of Life Technologies Corporation.



1. Deglycosylation

GlycINATOR Immobilized



2. Azide Activation

GalT + UDP-GalNAz

Figure 1. Schematic overview of the GlyCLICK technology for azide activation.

Preparations

Important Information
Before you begin, briefly centrifuge tubes. Always wear suitable laboratory protective clothing and gloves when handling the reagents. **Keep in mind:** Sodium azide must be avoided throughout the protocol.

- Let the GlycINATOR Immobilized and the Desalting spin columns equilibrate to room temperature before use.
- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do *not* remove the lid).
- If a chelating agent will be used as a label, it is important to use metal-free water (trace analysis grade) throughout the protocol. The antibody must not be in contact with glass or metal.

Additional Materials Required

- IgG in 1×TBS pH 7.4, free of carrier proteins and/or azide, in a maximum volume of 1 ml and with maximum concentration of 10 mg/ml.
- 1×TBS: 10 ml 1×TBS is prepared by adding 0.5 ml of 20×TBS to 9.5 ml of ddH_2O. Vortex briefly to mix.
- Centrifuge tubes: 15 ml and 50 ml.
- ddH_2O.

Site-specific Azide Activation of IgG

1. Deglycosylation: Modification of the N-glycan on the Antibody Fc Domain

The antibody solution should be in 1× TBS pH 7.4, with no azide. Max 10 mg IgG in 1 ml.

Time required: 15 min hands-on, 60 min hands-off.

Materials from kit:

- 1× TBS (prepared from 20× TBS)
- Spin column with GlycINATOR Immobilized

1.1 Let the GlycINATOR Immobilized column equilibrate to room temperature before use. Twist off the bottom cap of the GlycINATOR Immobilized column (save the cap for later use) and place the column in a 15 ml centrifuge tube. Loosen the lid.

1.2 Centrifuge at 100 × g for 1 min to remove the storage solution. Discard the flow-through.

1.3 Place the column in the centrifuge tube.

1.4 Add 2.5 ml 1× TBS on top of the resin. Centrifuge at 100 × g for 1 min and discard the flow-through.

1.5 Perform step 1.4 two additional times.

1.6 Attach the bottom cap by inverting the cap and firmly pressing it onto the bottom of the column.

1.7 Adjust the antibody sample volume to 1 ml and immediately add the antibody solution to the column.

1.8 Seal the column with the lid.

1.9 Fully suspend the media, mix by inversion and make sure there is a flow in the column.

1.10 Incubate the column with end-over-end mixing at room temperature for 60 min.

1.11 Remove the bottom cap and place the column in a new 15 ml centrifuge tube. Loosen the lid.

1.12 Centrifuge at 100 × g for 1 min to collect the processed material.

1.13 Attach the bottom cap. Add 500 µl 1× TBS and seal the column with the lid.

1.14 Invert the column a couple of times.

1.15 Remove the bottom cap and place the column in a new 15 ml centrifuge tube. Loosen the lid.

1.16 Centrifuge at 100 × g for 1 min to collect the processed material.

1.17 Perform steps 1.13 to 1.16 two additional times.

1.18 Pool the collected fractions, including the sample from 1.12, resulting in a total sample volume of ~ 2.5 ml.

2. Azide Activation

Time required: 5 min hands-on, followed by overnight incubation.

Materials from kit:

- 1× TBS (prepared from 20× TBS)
- UDP-GalNAz
- GalT enzyme
- Buffer additive

2.1 Add 35 µl Buffer additive to the pooled fractions from step 1.18.

2.2 Reconstitute the UDP-GalNAz in 250 µl 1× TBS and transfer to the pooled fractions.

2.3 Rinse the UDP-GalNAz vial with an additional 200 µl 1× TBS and transfer to the pooled fractions.

2.4 Add 200 µl GalT to the pooled fractions.

2.5 Mix the solution by carefully pipetting up and down.

2.6 Incubate overnight protected from light at 30°C.

3. Removal of Excess UDP-GalNAz

Time required: 1 h

Materials from kit:

- 1× TBS (prepared from 20× TBS)
- Desalting Spin column (10 ml, 40 K)

3.1 Let the Desalting Spin column equlibrate to room temperature before use. Twist off the bottom cap of the Desalting Spin column (10 ml, 40K) place the column in a 50 ml centrifuge tube. Loosen the lid.

3.2 Centrifuge at 700 × g for 2 min to remove the storage solution. Discard the flow-through.

3.3 Place the column in the centrifuge tube.

3.4 Add 5 ml 1× TBS on top of the resin. Centrifuge at 700 × g for 2 min and discard the flow-through.

3.5 Add 5 ml 1× TBS on top of the resin. Centrifuge at 700 × g for 3 min and discard the flow-through.

3.6 Add 5 ml 1× TBS on top of the resin. Centrifuge at 700 × g for 5 min and discard the flow-through.

3.7 Place the column in a new 50 ml centrifuge tube.

3.8 Apply the azide-activated antibody sample from step 2.6 on top of the resin.

3.9 Centrifuge at 700 × g for 4 min and collect the flow-through that contains the azide-activated antibody.

3.10 At this stage, the azide-activated antibody can be stored at 2-8°C protected from light for conjugation of a label at a later time.

CONTENT AND STORAGE

GlyCLICK Azide Activation 10 mg contains several components. The product is shipped cold, and the components should be stored at different temperatures upon arrival (see Table 1).

GlyCLICK Azide Activation is for R&D use only.

Table 1. Content and Storage Temperatures of GlyCLICK Components

Name	Amount	Store at
Desalting Spin column, 10 ml, 40 K	1 piece	4-8 °C
GlycINATOR Immobilized spin column (1 ml)	1 piece	4-8 °C
UDP-GalNAz	1 vial solid	(-25)-(-5) °C Protect from light
20× TBS pH 7.4 (0.5 M)	2 × 1.8 ml	4-8 °C
Buffer additive	1 × 100 µl	4-8 °C Protect from light
β-1,4-galactosyltransferase GalT (Y289L)	1 × 200 µl	4-8 °C Protect from light

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