Azide activation kit

Last revised Feb 2018

10 mg

INSTRUCTIONS

Intructions for product no:

L1-AZ1-100	GlyCLICK™ Azide activation of up to 10 mg IgG

Product description

GlyCLICK™ Azide activation is for selective azide activation of up to 10 mg of antibody. The conserved N-linked glycosylation site on the CH2 domain of each heavy chain of the Fc region is used by GlyCLICK™ for selective antibody modification. The azide activation kit introduces GalNAz site-specifically on Fc for subsequent click reaction.

Immobilized GlycINATOR® removes all Fc N-glycans, including high-mannose, hybrid-type and bisected glycans to the first GlcNAc. The subsequent azide activation at the GlcNAc can be followed by a "click" reaction using e. g. strain promoted azide-alkyne cycloaddition for selective attachment of a label. The modification primes the antibody for conjugation of the desired molecule at activated sites on the Fc regions for incorporation of two labels per antibody (DOL=2), see Figure 1.

The modification procedure is performed by combining two enzymatic steps at the Fc domain of the IgG. All steps are performed under physiological conditions, thus maintaining the quality of the antibody. The site-specific modification on the Fc domain preserves the affinity of the antigen binding sites.

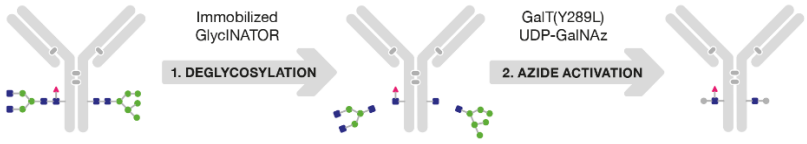


Figure 1. The modification is performed in two steps:

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

*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.

Customer Service USA Phone: 617-444-8421 I info@genovis.com I Genovis AB, 245 First Street, Cambridge, MA 02142, USA
Customer Service Europe Phone: 0046 (0)46 10 12 30 I info@genovis.com I Genovis AB, Box 790, SE-220 07 Lund, Sweden
Support support@genovis.com **Order** order@genovis.com I Fax: 0046 (0)46 12 80 20
www.genovis.com

Content and storage

GlyCLICK™ Azide activation kit contains enzymes, reagents and material to azide activate up to 10 mg antibody.

GlyCLICK™ Azide activation kit is shipped cold and components should be stored at different temperatures upon arrival.

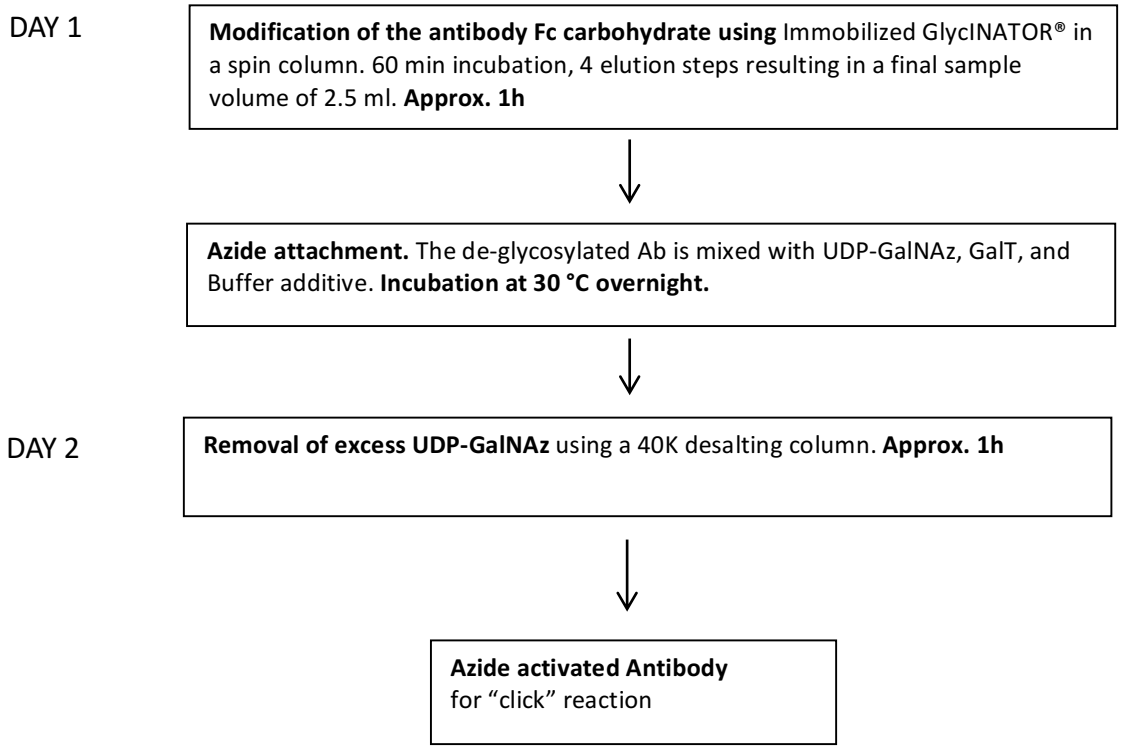
Table 1. Content and storage temperatures of GlyCLICK™ components.

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

GlyCLICK™ Azide activation kit is for R&D use only.

Before you begin, briefly centrifuge tubes.

Overview of the protocol for antibody azide activation using GlyCLICK™



Protocol for modification of up to 10 mg of antibody

Equipment required:

- Centrifuge with swinging bucket rotor that can accommodate 17 mm × 100 mm (15 ml) and 30 mm x 115 mm (50 ml) centrifuge tubes.
- Incubator or water bath for 25 °C and 30 °C.
- End-over-end mixer

Additional Materials required

- Antibody, in 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. 20x TBS is provided.
- Centrifuge tubes, 15 ml and 50 ml.
- ddH_2O**. Note: if a chelating agent will be used as label it is important to use metal free water (trace analysis grade) throughout the protocol.**

Sodium azide must be avoided throughout the protocol! If labeling is performed with conjugation reagent with chelator, the antibody must not be in contact with glass or metal.

Step 1. Modification of the carbohydrate on Antibody Fc domain

Time Required: 15 minutes hands-on, 60 minutes hands-off

Materials from kit: 1× TBS buffer (prepared from 20× TBS),
Spin column with Immobilized GlycINATOR®

1.1. Let the Immobilized GlycINATOR column equilibrate to room temperature before use.

1.2. Break of the bottom plastic closure of the GlycINATOR® column and slightly open the lid. Place the column in a 15 ml collection tube.

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

1.4. Place the column in the collection tube.

1.5. Add 2.5 ml 1× TBS buffer on top of the resin. Centrifuge the column at 100 x g for 1 minute and discard the flow-through.

1.6. Repeat the steps in 1.5 **two times**.

1.7. Put on the bottom cap on the column. Apply parafilm to prevent leakage.

1.8. Adjust the sample volume to the maximum volume, 1 ml, and immediately add the antibody solution to the column.

1.9. Seal the column with the lid. Take care to seal it tightly by applying parafilm to prevent leakage.

1.10. Fully suspend the media manually and make sure it is flowing in the column.

1.11. Incubate the column by end-over-end mixing at room temperature for 60 minutes.

1.12. Centrifuge the column at 100 × g for 1 minute to elute the antibody sample.

1.13. Attach the bottom cap. Add 500 µl 1x 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 clean centrifuge tube. Loosen the lid.

1.16. Centrifuge at 100 × g for 1 minute to elute the antibody sample.

1.17. Repeat steps 1.13 to 1.16 two times.

1.18. Pool the eluates, resulting in a total sample volume of approximately 2.5 ml.

Step 2. Azide attachment

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

Materials from kit: 1× TBS buffer (prepared from 20× TBS),
UDP-GalNAz,
GalT enzyme,
Buffer additive

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

2.2. Reconstitute the UDP-GalNAz with 250 µl TBS and transfer to the pooled eluate.

2.3. Wash the UDP-GalNAz vial with an additional 200 µl TBS and transfer to the pooled eluate.

2.4. Add 200 µl GalT to the pooled eluate.

2.5. Mix the solution by carefully pipetting up and down.

2.6. Incubate overnight protected from light, at 30 °C.

Step 3. Removal of excess UDP-GalNAz

Time required: 1 hour

Materials from kit: 1× TBS buffer (prepared from 20x TBS),
Desalting column

3.1. Break of the bottom plastic closure of the column and slightly open the lid. Place the column in a 50 ml collection tub.

3.2. Centrifuge the column at 1000 × g for 2 minutes to remove the storage solution. Discard the flow-through.

3.3. Place the column in the collection tube.

3.4. Add 5 ml 1× TBS buffer on top of the resin. Centrifuge the column at 1000 x g for 2 minutes and discard the flow-through.

3.5. Repeat the steps in 3.4 **two times.** The last centrifugation should be 6 minutes**.**

3.6. Place the column in a new 50 ml collection tube.

3.7. Apply the sample (from step 2.6) on top of the resin.

3.8. Centrifuge the column at 1000 x g for 4 minutes and retain the flow-through that contains the azide-modified antibody.

3.9. At this stage, the antibody can be stored at 2-8 °C protected from light for conjugation later.

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. Ramakrishnan, B. & Qasba, P.K., 2002. Structure-based design of beta 1,4-galactosyltransferase I (beta 4Gal-T1) with equally efficient N-acetylgalactosaminyltransferase activity: point mutation broadens beta 4Gal-T1 donor specificity. J Biol Chem, 277(23), pp.20833–20839.
