

## Azide Activation kit

2 mg

### INSTRUCTIONS

Last revised May 2020

Instructions for product no:

L1-AZ1-200

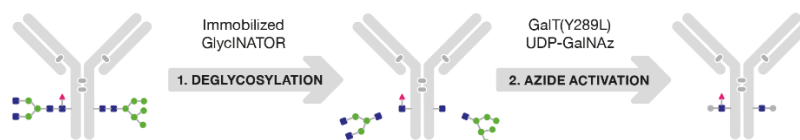
GlyCLICK® Azide activation of up to 2 mg IgG

#### Product description

GlyCLICK Azide activation is for site specific azide activation of up to 2 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 inner GlcNAc. The subsequent azide activation at the GlcNAc enables a following click reaction using e. g. copper free strain promoted azide-alkyne cycloaddition (SPAAC) for selective attachment of a label of choice. 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 inner 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.

## Content and storage

GlyCLICK Azide activation kit, contains enzymes, reagents and material to azide activate up to 2 mg antibody.

GlyCLICK Azide activation kit is shipped cold and components should be stored at 4 °C to 8 °C upon arrival. **Note: Do NOT freeze!**

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

| Name   | Amount       | Store at                           |
|--|--------------|------------------------------------|
| Desalting Spin column, 0.5 ml, 40K                           | 1 piece      | 4 °C to 8 °C                       |
| Antibody concentrator (incl 2 collection tubes), 0.5 ml, 50K | 1 piece      | 4 °C to 25 °C                      |
| Desalting Spin column, 2 ml, 40K                             | 1 piece      | 4 °C to 8 °C                       |
| Immobilized GlycINATOR®, microspin column                    | 1 piece      | 4 °C to 8 °C                       |
| UDP-GalNAz   | 1 vial solid | 4 °C to 8 °C<br>Protect from light |
| 20× TBS pH 7.4 (0.5 M)                                       | 2 x 2 ml     | 4 °C to 8 °C                       |
| Buffer additive  | 1 x 50 µl    | 4 °C to 8 °C<br>Protect from light |
| β-1,4-galactosyltransferase (Y289L) (GalT)                   | 1 x 40 µl    | 4 °C to 8 °C<br>Protect from light |

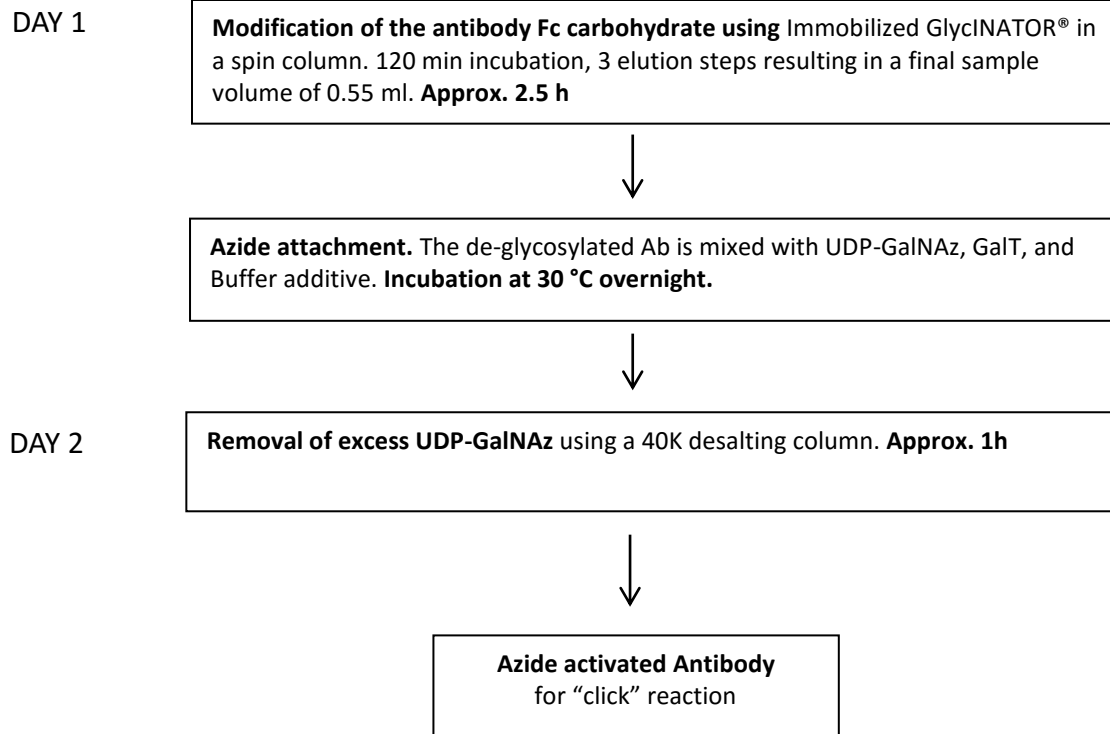
**Before you begin, briefly centrifuge tubes.**

**Always wear suitable laboratory protective clothing and gloves when handling these reagents.**

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

## Overview of the protocol for antibody azide activation using GlyCLICK®

For azide activation of 2 mg IgG



### Equipment required

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

### Additional Materials required

- Antibody in 1x TBS, pH 7.4, free of carrier proteins and/or azide, in a maximum volume of 250 µl, max 2 mg IgG. 20x TBS buffer, a desalting spin column (40K) for buffer exchange and a concentrator (50K) is provided for convenience. For adjusting the antibody solution, please follow "Guidance for concentration and buffer exchange" below.
- Centrifuge tubes: 1.5- 2 ml and 15 ml.
- ddH<sub>2</sub>O. **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.**

## Guidance for concentration and buffer exchange

The antibody concentration step **is required if:**

- The volume is more than 250  $\mu\text{l}$

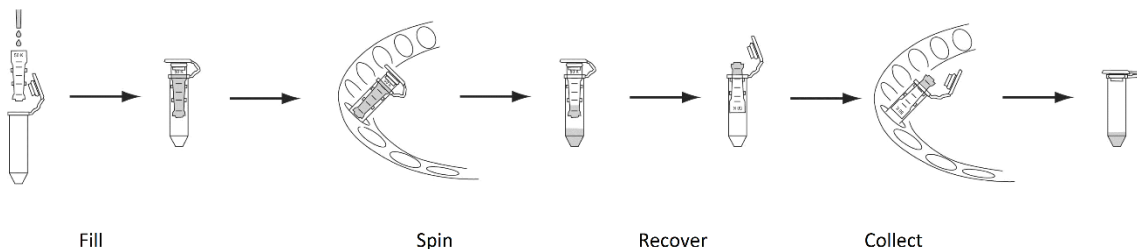
If the sample volume is 250  $\mu\text{l}$  but needs a buffer exchange (if it contains phosphate or azide), concentrate the sample to <200  $\mu\text{l}$  and then follow the instruction in section “Buffer exchange with Desalting column, 0.5 ml”. It is advisable to start with more than 2 mg of antibody if you need to concentrate or buffer exchange the sample prior to “Step 1. Modification of the carbohydrate on antibody Fc domain, deglycosylation”.

### Concentration step

1. Add 500  $\mu\text{l}$  of ddH<sub>2</sub>O to the small antibody concentrator and cap the device as shown in Figure 2.
2. Centrifuge at 5000  $\times$  g for 6 minutes. Make sure that **the cap strap and one membrane panel of the concentrator face the center of the rotor** (Fig. 2).
3. Discard the flow-through.
4. Add the antibody solution to the small antibody concentrator.
5. Centrifuge at 5000  $\times$  g for 2-6 minutes. Make sure that **the cap strap and one membrane panel of the concentrator face the center of the rotor** (Fig. 2).
6. Discard the flow-through.

**Note:** If the antibody volume in the concentrator is more than 200  $\mu\text{l}$  and the sample needs a buffer exchange, centrifuge for an additional 2 minutes at 5000  $\times$  g, or until the appropriate volume is achieved.

7. Invert the small antibody concentrator into the collection tube as shown in Figure 2.
8. Centrifuge at 1000  $\times$  g for 3 minutes to collect the concentrated antibody. After collection, the amount of concentrated Ab should be approximately 150-200  $\mu\text{l}$  in the collection tube.



**Figure 2.** Antibody concentration step

**If buffer exchange is needed, follow the steps below:**

### Buffer exchange with Desalting Spin column, 0.5 ml

1. Prepare 10 ml 1x TBS buffer by adding 500  $\mu\text{l}$  20 $\times$  TBS to 9.5 ml ddH<sub>2</sub>O in a 15 ml tube. Vortex briefly to mix.
2. Break off the bottom closure of the Desalting Spin column. Loosen the lid (**do not** remove the lid).
3. Place the column in a collection tube (1.5-2 ml) and centrifuge at 1500  $\times$  g for 1 min to remove the storage solution.
4. Discard the flow-through and place the column in the collection tube.
5. Add 300  $\mu\text{l}$  1x TBS buffer on top of the resin. Centrifuge the column at 1500  $\times$  g for 1 min and discard the flow through.
6. Repeat step 5 **two more times**. Last spin for 2 minutes.
7. Blot the bottom of the column to remove excess liquid. Place the column in a new collection tube (1.5-2 ml).
8. Apply the antibody solution on top of the resin (100-200  $\mu\text{l}$ ).
9. Centrifuge at 1500  $\times$  g for 2 min and collect the flow-through containing the antibody in 1x TBS buffer.

## Protocol for azide activation of up to 2 mg antibody

### Step 1. Modification of the carbohydrate on Antibody Fc domain, deglycosylation

The antibody solution should be in 1x TBS buffer pH 7.4, with no azide. Max 2 mg in 250 µl.

**Time Required:** 15 minutes hands-on, 120 minutes hands-off

- Lids and bottom caps are used during the incubation
- Before centrifugation remove the bottom cap and loosen the lid (do not remove it). Save the bottom cap.

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

- 1.1. Let the Immobilized GlycINATOR column equilibrate to room temperature before use.
- 1.2. Break off the bottom plastic cap of the GlycINATOR column and slightly open the lid. Place the column in a microcentrifuge collection tube. **Note:** Save the bottom cap.
- 1.3. Centrifuge the column at 200 × 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 300 µl 1x TBS buffer on top of the resin. Centrifuge the column at 200 × g for 1 minute and discard the flow-through.
- 1.6. Repeat the steps in 1.5 **two more times**.
- 1.7. Re-insert the bottom cap into the bottom of the spin column.
- 1.8. Adjust the antibody sample volume (**containing 2 mg antibody**) to 250 µl using 1x TBS and immediately add the antibody solution to the column.
- 1.9. Seal the column with the lid.
- 1.10. Fully suspend the resin manually and make sure the resin is flowing in the column.
- 1.11. Incubate the column by end-over-end mixing at room temperature for 120 minutes.
- 1.12. Remove the bottom cap and place the column in a clean microcentrifuge tube. Loosen the top lid.
- 1.13. Centrifuge the column at 1000 × g for 1 minute to collect the deglycosylated antibody sample.
- 1.14. Attach the bottom cap. Add 100 µl 1x TBS and seal the column with the lid.
- 1.15. Invert the column a couple of times.
- 1.16. Remove the bottom cap and place the column in a clean microcentrifuge tube. Loosen the lid.
- 1.17. Centrifuge at 1000 × g for 1 minute to collect the deglycosylated antibody sample.
- 1.18. Repeat steps 1.14 to 1.17 one more time.
- 1.19. Pool the collected deglycosylated antibody material and adjust the sample volume to 550 µl with 1x TBS buffer.

### Step 2. Azide attachment

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

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

- 2.1. Add 7 µl Buffer additive to the pooled deglycosylated antibody from step 1.19.
- 2.2. Add the deglycosylated antibody-solution to the GalT vial.

- 2.3. Reconstitute the UDP-GalNAz in 40 µl 1x TBS and transfer the solution to the GalT vial.
- 2.4. Mix the sample solution by carefully pipetting up and down.
- 2.5. 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 Spin column, 2 ml

- 3.1. Break off the bottom plastic cap of the column and slightly open the lid. Place the column in a 15 ml collection tube.
- 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 1 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 more times**. The last centrifugation should be 3 minutes.
- 3.6. Place the column in a new 15 ml collection tube.
- 3.7. Apply the azide activated antibody sample (from step 2.5) on top of the resin.
- 3.8. Centrifuge the column at 1000 x g for 3 minutes and collect the flow-through that contains the azide activated antibody.
- 3.9. At this stage, the azide activated antibody can be stored at 2-8 °C protected from light for conjugation at a later time.

## 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.

## GlyCLICK®

---

### Legal and Disclaimers

All rights reserved. Genovis products are covered by one or more patents, trademarks and/or copyrights owned or controlled by Genovis AB. For more information about commercial rights, please contact Genovis team at [info@genovis.com](mailto:info@genovis.com). Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals. All goods and services are sold subject to Genovis' General Terms and Conditions of Sale.

### SiteClick™

This product is provided under an intellectual property license from Life Technologies Corporation. The transfer of this product is conditioned on the buyer using the purchased product solely in research conducted by the buyer, excluding contract research or any fee for service research, and the buyer must not (1) use this product or its components for (a) diagnostic, therapeutic or prophylactic purposes; (b) testing, analysis or screening services, or information in return for compensation on a per-test basis; or (c) manufacturing or quality assurance or quality control, and/or (2) sell or transfer this product or its components for resale, whether or not resold for use in research. For information on purchasing a license to this product for purposes other than as described above, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 USA or [outlicensing@lifetech.com](mailto:outlicensing@lifetech.com).

The trademark SiteClick™ is the property of Life Technologies Corporation.

©2020 Genovis AB.