



# GlyCLICK®

Azide Activation 2 mg

STORE AT

**+4-8°C**



FOR RESEARCH USE ONLY

## Instructions for Use

GlyCLICK® Azide Activation 2 mg (L1-AZ1-200)  
Process 2 mg IgG



## 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<sup>1</sup> 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 2 mg contains all reagents needed to azide-activate 2 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)<sup>\*</sup> and UDP-GalNAz<sup>\*</sup>.

### 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**

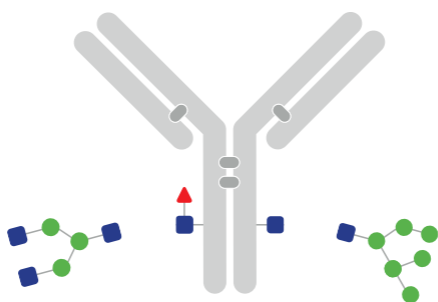
Site-specific conjugation of IgG with Alexa Fluor® 488, 555 or 647

#### **GlyCLICK® DFO**

Site-specific conjugation of IgG with DFO

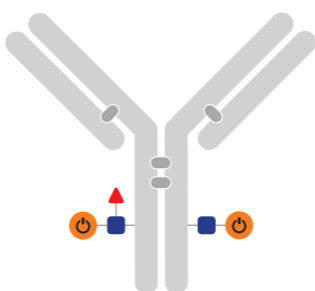
<sup>1</sup> For copper-free click chemistry, the label must be functionalized by a cyclooctyne.

<sup>\*</sup> 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.

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

### Additional Materials Required

- IgG in 1×TBS, pH 7.4, free of carrier proteins and/or azide. 2 mg of IgG in a maximum volume of 250  $\mu$ l. To adjust the antibody solution, please follow “Guidance for Concentration and Buffer Exchange”. 20×TBS, a Desalting Spin column 0.5 ml, 40K for buffer exchange and an Antibody concentrator 0.5 ml, 50K are provided for convenience.
- Centrifuge tubes: 1.5-2 ml and 15 ml.
- ddH<sub>2</sub>O.

## Guidance for Concentration and Buffer Exchange

It is advisable to start with more IgG than 2 mg if concentration and/or buffer exchange of the sample is needed prior to “1. Deglycosylation: Modification of the N-glycan on the Antibody Fc Domain”.

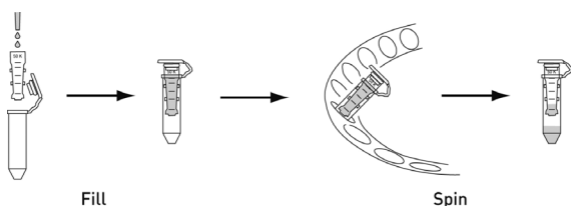
### A. Concentration Step

This step is **required if:**

- The volume of the IgG is more than 250  $\mu$ l.

If the sample volume is 250  $\mu$ l but needs a buffer exchange (if it contains phosphate or azide), concentrate the sample to <200  $\mu$ l and then follow the steps in section B: “Buffer Exchange”.

- A.1 Add 500  $\mu$ l of ddH<sub>2</sub>O to the antibody concentrator (0.5 ml, 50K) and cap the device as shown in Figure 2.



**Figure 2.** Antibody concentration step.

- A.2 Centrifuge at  $5000\times g$  for 6 min. **Make sure that the cap strap and one membrane panel of the concentrator face the center of the rotor** (Fig. 2).
- A.3 Discard the flow-through.
- A.4 Add the IgG solution to the antibody concentrator.
- A.5 Centrifuge at  $5000\times g$  for 2-6 min. **Make sure that the cap strap and one membrane panel of the concentrator face the center of the rotor** (Fig. 2).

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

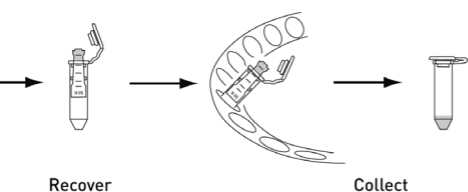
- A.6 Invert the antibody concentrator into the collection tube as shown in Figure 2.
- A.7 Centrifuge at  $1000\times g$  for 3 min to collect the concentrated IgG. After collection, the volume of concentrated IgG should be approximately  $150\text{-}200\mu\text{l}$  in the collection tube.

## B. Buffer Exchange

This step is required if:

- The IgG is in another buffer than  $1\times$  TBS, and/or
- The IgG is in a buffer containing azide

- B.1 Prepare 10 ml of  $1\times$  TBS buffer by adding 0.5 ml of  $20\times$  TBS to 9.5 ml of ddH<sub>2</sub>O in a 15 ml tube. Vortex briefly to mix.
- B.2 Break off the bottom closure of the Desalting Spin column (0.5 ml, 40K). Loosen the lid (do not remove the lid).
- B.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.
- B.4 Discard the flow-through and place the column in the collection tube.
- B.5 Add  $300\mu\text{l}$  of  $1\times$  TBS buffer on top of the resin. Centrifuge the column at  $1500\times g$  for 1 min and discard the flow-through.
- B.6 Repeat step B.5 two times. The last time, centrifuge for 2 min.
- B.7 Blot the bottom of the column to remove excess liquid. Place the column in a new collection tube (1.5-2 ml).
- B.8 Apply the IgG solution on top of the resin ( $100\text{-}200\mu\text{l}$ ).
- B.9 Centrifuge at  $1500\times g$  for 2 min and collect the flow-through containing the antibody in  $1\times$  TBS buffer.



## 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 buffer pH 7.4, with no azide. Max 2 mg IgG in 250 μl.

Time required: 15 min hands-on, 120 min hands-off.

Materials from kit:

- 1×TBS buffer (prepared from 20×TBS)
  - Spin column with GlycINATOR Immobilized
- 1.1 Let the GlycINATOR Immobilized column equilibrate to room temperature before use. Break off the bottom plastic cap of the GlycINATOR Immobilized column (save the cap) and slightly open the lid. Place the column in a microcentrifuge collection tube.
  - 1.2 Centrifuge the column at 200 × g for 1 min to remove the storage solution.
  - 1.3 Discard the flow-through.
  - 1.4 Place the column in the collection tube.
  - 1.5 Add 300 μl 1×TBS buffer on top of the resin. Centrifuge the column at 200 × g for 1 min and discard the flow-through.
  - 1.6 Repeat step 1.5 two 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 1×TBS and immediately add the antibody solution to the column.
  - 1.9 Seal the column with the lid.
  - 1.10 Fully resuspend the media, mix it by inversion and make sure there is a flow in the column.
  - 1.11 Incubate the column by end-over-end mixing at room temperature for 2 h.
  - 1.12 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.
  - 1.13 Centrifuge the column at 1000 × g for 1 min to collect the deglycosylated antibody sample.
  - 1.14 Seal the column with the bottom cap. Add 100 μl 1×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 new microcentrifuge tube. Loosen the lid.
  - 1.17 Centrifuge at 1000 × g for 1 min to collect the deglycosylated antibody sample.
  - 1.18 Repeat steps 1.14 to 1.17 one time.
  - 1.19 Pool the collected deglycosylated antibody material and adjust the sample volume to 550 μl with 1×TBS buffer.

## 2. Azide Activation

Time required: 5 min 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 7 µl Buffer additive to the pooled deglycosylated antibody from step 1.19.
- 2.2 Add the deglycosylated antibody-solution from step 2.1 to the GalT vial.
- 2.3 Reconstitute the UDP-GalNAz in 40 µl of 1× TBS and transfer the solution to the GalT vial.
- 2.4 Mix the sample solution by carefully pipetting up and down. Seal the vial with the lid and wrap the lid with Parafilm® or similar.
- 2.5 Incubate overnight protected from light at 30°C.

## 3. Removal of Excess UDP-GalNAz

Time required: 1 h.

Materials from kit:

- 1× TBS buffer (prepared from 20× TBS)
- Desalting Spin column (2 ml, 40K)

- 3.1 Break off the bottom plastic cap of the Desalting Spin column (2 ml, 40K) and slightly open the lid. Place the column in a 15 ml collection tube.
- 3.2 Centrifuge the column at 1000 × g for 2 min 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 × g for 2 min and discard the flow-through.
- 3.5 Repeat step 3.4 two times. The last time, centrifuge for 3 min.
- 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 at 1000 × g for 3 min 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.





## CONTENT AND STORAGE

GlyCLICK Azide Activation 2 mg contains several components. The product box is shipped cold, and the components should be stored at +4-8°C upon arrival.

**Do not freeze any of the components.**

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, 0.5ml, 40K	1 piece	4-8°C
Antibody concentrator (incl. 2 collection tubes), 0.5ml, 50K	1 piece	4-25°C
Desalting Spin column, 2ml, 40K	1 piece	4-8°C
GlycINATOR Immobilized Microspin column	1 piece	4-8°C
UDP-GalNAz	1 vial solid	4-8°C Protect from light
20× TBS pH 7.4 (0.5 M)	2 × 2 ml	4-8°C
Buffer additive	1 × 50 µl	4-8°C Protect from light
β-1,4-galactosyltransferase (Y289L) (GalT)	1 × 40 µl	4-8°C Protect from light

