



# GlyCLICK®

Fluorophore 555 250 µg

STORE AT

**+4-8°C**



FOR RESEARCH USE ONLY

## Instructions for Use

GlyCLICK® Fluorophore 555 250 µg (L1-F02-025)  
Process 250 µg IgG



DOWNLOAD INSTRUCTIONS FOR USE  
[www.genovis.com/ifu-L1-F02-025](http://www.genovis.com/ifu-L1-F02-025)

## Site-specific Conjugation of IgG with Alexa Fluor® 555

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 Fluorophore 555 is available for site-specific labeling of 250 µg or 2 mg IgG with Alexa Fluor® 555. The conjugation is performed by combining enzymatic steps and copper-free click chemistry to covalently link the label to the Fc domain of the IgG. 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 Fluorophore 555 250 µg contains all reagents needed to conjugate 250 µg IgG. The conjugation is performed in four 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\*.
3. **Click Reaction:** The azide-activated antibody reacts with a DIBO-alkyne label in a strain-promoted, copper-free click reaction (SPAAC) to form a stable and homogenous antibody conjugate.
4. **Purification:** Excess DIBO-alkyne label is removed by using a MWCO filter.

### YOU MIGHT ALSO BE INTERESTED IN

#### **GlyCLICK® Fluorophore 488**

Site-specific conjugation of IgG with Alexa Fluor® 488

#### **GlyCLICK® Fluorophore 647**

Site-specific conjugation of IgG with Alexa Fluor® 647

#### **GlyCLICK® Azide Activation**

Site-specific conjugation of IgG with azide-alkyne click chemistry

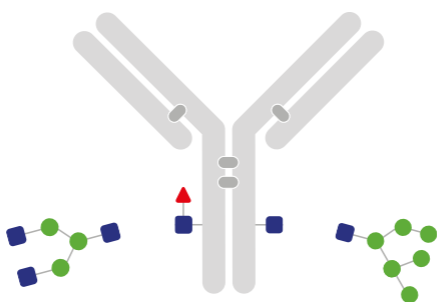
#### **GlyCLICK® Biotin**

Site-specific conjugation of IgG with biotin

#### **GlyCLICK® DFO**

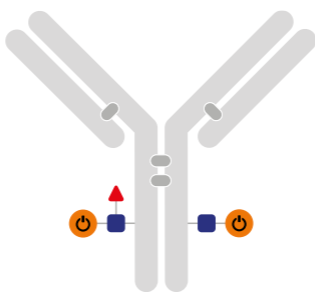
Site-specific conjugation of IgG with DFO

\* 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.



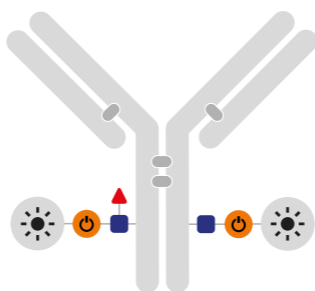
### 1. Deglycosylation

GlycINATOR Immobilized



### 2. Azide Activation

GalT + UDP-GalNAz



### 3. Click Reaction

DIBO-modified label:  
Alexa Fluor® 555

### 4. Purification

Figure 1. Schematic overview of the GlyCLICK technology for fluorophore conjugation.

## 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 column 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).

### Additional Materials Required

- IgG in 1×TBS, pH 7.4, free of carrier proteins and/or azide. 250µg IgG in a maximum volume of 200µl. 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 a concentrator (0.5 ml, 50K) are provided for convenience.
- 1×TBS: 10ml 1×TBS is prepared by adding 0.5 ml of 20×TBS to 9.5 ml of ddH<sub>2</sub>O. Vortex briefly to mix.
- 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 250µg 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”.

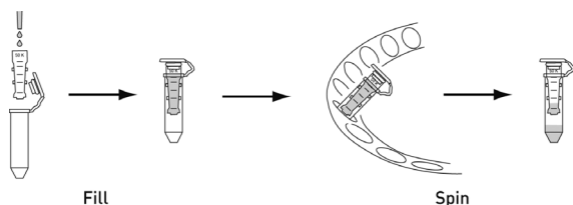
### A. Concentration Step

This step **is required if:**

- The volume of the IgG is more than 200µl.

If the sample volume is 150-200µl but needs a buffer exchange (if it contains phosphate or azide), concentrate the sample to < 150µl and then follow the steps in section “B. Buffer Exchange”.

- A.1 Add 500µl 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 150µl and the sample needs a buffer exchange, centrifuge for an additional 2 min at  $5000\times g$ , or until the appropriate volume is achieved.*

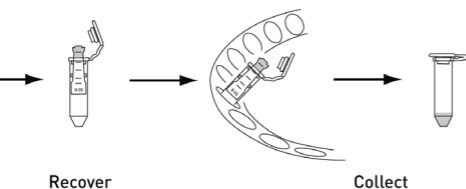
- A.6 Invert the antibody concentrator into a new collection tube as shown in Figure 2.
- A.7 Centrifuge at  $1000\times g$  for 3 min to collect the concentrated IgG. After collection, the volume of concentrated IgG should be approximately 100-150µl in the collection tube.

## B. Buffer Exchange

This step **is required if:**

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

- B.1 Break off the bottom closure of the Desalting Spin column (0.5 ml, 40K). Loosen the lid (do not remove the lid).
- B.2 Place the column in a microcentrifuge tube and centrifuge at  $700\times g$  for 1 min to remove the storage solution.
- B.3 Discard the flow-through and place the column in the microcentrifuge tube.
- B.4 Add 300µl of 1x TBS on top of the resin. Centrifuge at  $700\times g$  for 1 min and discard the flow-through.
- B.5 Perform step B.4 two additional times. The last time, centrifuge for 2 min.
- B.6 Blot the bottom of the column to remove excess liquid. Place the column in a new microcentrifuge tube.
- B.7 Apply the IgG solution (100-150µl) on top of the resin.
- B.8 Centrifuge at  $700\times g$  for 2 min and collect the flow-through containing the antibody in 1x TBS.



Recover

Collect

## Site-specific Conjugation of IgG with Alexa Fluor® 555

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

Make sure that the antibody solution is 250 µg IgG in 100-200 µl of 1× TBS (pH 7.4, without azide).

Time required: 15 min hands-on, 30 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. Break off the bottom cap of the GlycINATOR Immobilized column (save the cap) and place the column in a microcentrifuge tube. Loosen the lid.
  - 1.2 Centrifuge at 200× g for 1 min to remove the storage solution. Discard the flow-through.
  - 1.3 Place the column in the microcentrifuge tube.
  - 1.4 Add 300 µl 1× TBS on top of the resin. Centrifuge at 200× g for 1 min and discard the flow-through.
  - 1.5 Perform step 1.4 two additional times.
  - 1.6 Insert the bottom cap.
  - 1.7 Immediately add the antibody solution (100-200 µl) 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 30 min.
  - 1.11 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.
  - 1.12 Centrifuge at 1000× g for 1 min to collect the processed material.
  - 1.13 Insert the bottom cap. Add 50 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 microcentrifuge tube. Loosen the lid.
  - 1.16 Centrifuge at 1000× g for 1 min to collect the processed material.
  - 1.17 Repeat steps 1.13 to 1.16.
  - 1.18 Pool the collected fractions, including the sample from step 1.12.

## 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 30 µl Buffer additive to the tube containing UDP-GalNAz.
- 2.2 Add the pooled fractions from step 1.18, and add 1× TBS to achieve a total volume of 375 µl.
- 2.3 Mix the solution by carefully pipetting up and down.
- 2.4 Add 25 µl of the GalT enzyme. The final reaction volume should be 400 µl. Mix the 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

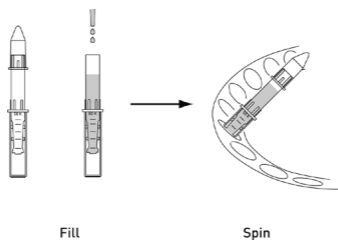
Time required: 1 h

Materials from kit:

- 1× TBS (prepared from 20× TBS)
- Antibody concentrator (2 ml, 50K)

- 3.1 Remove the conical collection tube from the antibody concentrator as shown in Figure 3.
- 3.2 Add 2 ml of 1× TBS to the antibody concentrator and centrifuge at 1200×g for 10 min. **Make sure that one membrane panel of the concentrator faces the center of the rotor.**
- 3.3 Discard the flow-through.
- 3.4 Add 1.6 ml of 1× TBS and 400 µl of the azide-activated antibody from step 2.5 to the antibody concentrator (Fig. 3).
- 3.5 Centrifuge at 1200×g for 6 min. **Make sure that one membrane panel of the concentrator faces the center of the rotor.**
- 3.6 Discard the flow-through.
- 3.7 Add 1× TBS to a total of 2 ml to the antibody concentrator.
- 3.8 Centrifuge at 1200×g for 10 min. **Make sure that one membrane panel of the concentrator faces the center of the rotor.**
- 3.9 Discard the flow-through.
- 3.10 Perform steps 3.7-3.9 two additional times.  
**Note:** *If the antibody volume in the concentrator is more than ~200 µl, the volume in the concentrator can be reduced by additional centrifugation e.g. for an additional 5 min at 1200×g or until the appropriate volume is achieved.*

## Instructions for Use



**Figure 3.** Use of the Antibody concentrator (2 ml, 50K).

- 3.11 Invert the antibody concentrator into the conical collection tube as shown in Figure 4.
- 3.12 Centrifuge at  $1000\times g$  for 3 min to collect the concentrated azide-activated antibody.
- 3.13 Transfer the azide-activated antibody from the conical collection tube to a microcentrifuge tube.
- 3.14 At this stage, the azide-activated antibody can be stored at  $2-8^{\circ}\text{C}$  protected from light for conjugation of a label at a later time.



**Figure 4.** Collection of processed antibody material.

## 4. Conjugation with Alexa Fluor<sup>®</sup> 555

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

### Materials from kit:

- DIBO-modified label: Alexa Fluor<sup>®</sup> 555

- 4.1 Adjust the volume of the azide activated antibody from step 3.14 with 1xTBS buffer to 225  $\mu\text{l}$ .
- 4.2 Add 25  $\mu\text{l}$  of DIBO-Alexa Fluor<sup>®</sup> 555 to 225  $\mu\text{l}$  of azide activated antibody in 1xTBS (from step 4.1). Mix by carefully pipetting up and down.
- 4.3 Seal the tube with Parafilm<sup>®</sup> or similar.
- 4.4 Incubate overnight, protected from light at  $25^{\circ}\text{C}$ .
- 4.5 After the incubation, the antibody conjugate can be stored at  $+4-8^{\circ}\text{C}$ , protected from light, until needed.  
**DO NOT FREEZE!** If preferred, sodium azide or thimerosal can be added to a final concentration of 0.02% (w/v) for long time storage.

## 5. Removal of Excess Alexa Fluor® 555<sup>1</sup>

Time required: 1 h.

Materials from kit:

- Antibody concentrator (2 ml, 50K)
  - 1× TBS buffer (prepared from 20× TBS) or other buffer of choice, for example PBS
- 5.1 Remove the conical collection tube from a new antibody concentrator as shown in Figure 3.
  - 5.2 Add 2 ml of 1× TBS or PBS to the antibody concentrator and centrifuge at 1200× g for 10 min. **Make sure that one membrane panel of the concentrator faces the center of the rotor** (Fig. 3).
  - 5.3 Discard the flow-through.
  - 5.4 Add 1.6 ml of 1× TBS or PBS and the conjugated antibody from step 4.5 to the antibody concentrator.
  - 5.5 Centrifuge at 1200× g for 10 min. **Make sure that one membrane panel of the concentrator faces the center of the rotor** (Fig. 3).
  - 5.6 Discard the flow-through.
  - 5.7 Add 1× TBS or PBS to a total volume of 2 ml to the antibody concentrator and centrifuge at 1200× g for 10 min. **Make sure that one membrane panel of the concentrator faces the center of the rotor** (Fig. 3).
  - 5.8 Discard the flow-through.
  - 5.9 Perform steps 5.7-5.8 at least two additional times.
- Note:** If an antibody concentration of more than 2 mg/ml is desired, the volume in the concentrator can be reduced by prolonged centrifugation e.g. for an additional 5 min at 1200× g or until the appropriate volume is achieved.
- 5.10 Invert the antibody concentrator into the conical collection tube as shown in Figure 4.
  - 5.11 Centrifuge at 1000× g for 3 min to collect the antibody conjugate.
  - 5.12 Transfer the antibody conjugate from the conical collection tube to a 1.5 ml microcentrifuge tube.
  - 5.13 The antibody conjugate can now be stored protected from light at +4-8 °C. **DO NOT FREEZE!** If preferred, sodium azide or thimerosal can be added to a final concentration of 0.02% (w/v) for long time storage.

1. This step is optional and dependent on your application.



**CONTENT AND STORAGE**

GlyCLICK Fluorophore 555 250 µg contains several components. The product is shipped cold, and the components should be stored refrigerated upon arrival (see Table 1).

GlyCLICK Fluorophore 555 is for R&D use only.

*Table 1. Content and Storage Temperatures of GlyCLICK Components*

<b>Name</b>	<b>Amount</b>	<b>Store at</b>
Antibody concentrator, 0.5ml, 50K	1 piece	4-25 °C
Collection tube for antibody concentrator, 0.5ml, 50K	2 pieces	4-25 °C
Desalting Spin column, 0.5ml, 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 (0.5M)	1 × 1.8 ml	4-8 °C
Buffer additive	1 × 30 µl	4-8 °C Protect from light
β-1,4-galactosyltransferase GalT (Y289L)	1 × 25 µl	4-8 °C Protect from light
Antibody concentrator, 2ml, 50K	2 pieces	4- 25 °C
DIBO-modified label: Alexa Fluor® 555	1 × 25 µl	4-8 °C Protect from light

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