SmartEnzymes™

GENOVIS

GlyCLICK®

DFO 2 mg

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DIFFERENT
TEMPERATURES

FOR RESEARCH USE ONLY

Instructions for Use

GlyCLICK® DFO 2 mg (L1-C01-200)
Process 2 mg IgG

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www.genovis.com/ifu-L1-C01-200

www.genovis.com

Site-specific Conjugation of IgG wih DFO

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 DFO is available for site-specific labeling of 250 µg or 2 mg IgG with deferoxamine (DFO), a chelator used for metal-tagging or radiolabeling of IgG for *in vivo* and *in vitro* imaging. 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 DFO 2 mg contains all reagents needed to conjugate 2 mg 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 desalting column.

YOU MIGHT ALSO BE INTERESTED IN

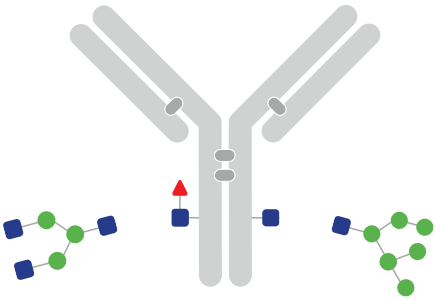
GlycINATOR® Immobilized
Immobilized enzyme for deglycosylation of IgG in spin columns

GlyCLICK® Azide Activation
Site-specific conjugation of IgG with azide-alkyne click chemistry

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

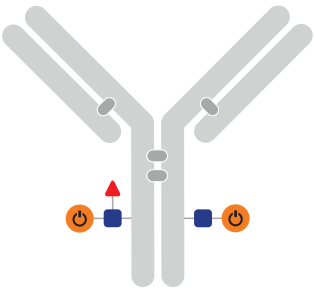
GlyCLICK® Biotin
Site-specific conjugation of IgG with biotin

* 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.



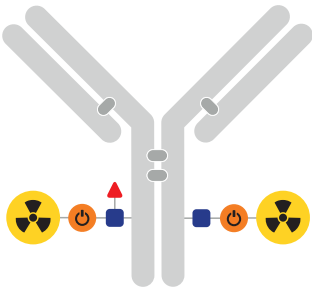
1. Deglycosylation

GlycINATOR Immobilized



2. Azide Activation

GalT + UDP-GalNAz



3. Click Reaction

DIBO-modified label: DFO

4. Purification

Figure 1. Schematic overview of the GlyCLICK technology for DFO 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.

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do *not* remove the lid).
- Let the GlycINATOR Immobilized column equilibrate to room temperature before use.
- Since 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. 2 mg of IgG in a maximum volume of 250 µl. 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.
- Dimethyl sulfoxide (DMSO) for reconstitution of DFO.
- ddH_2O.

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 µl.

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

A.1 Add 500 µl of ddH_2O to the antibody concentrator (0.5 ml, 50K) and cap the device as shown in Figure 2.

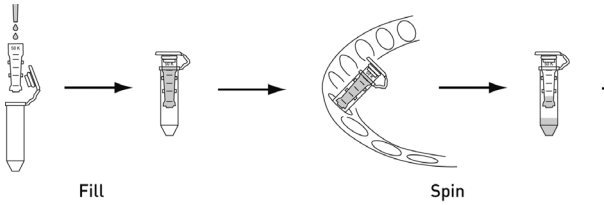


Figure 2. Antibody concentration step.

A.2 Centrifuge at 5000 × 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 × 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 µl and the sample needs a buffer exchange, centrifuge for an additional 2 min at 5000 × 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 × g for 3 min to collect the concentrated IgG. After collection, the volume of concentrated IgG should be approximately 150-200 µl 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 Prepare 10 ml of 1× TBS buffer by adding 0.5 ml of 20× TBS to 9.5 ml of ddH_2O 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 × g for 1 min to remove the storage solution.

B.4 Discard the flow-through and place the column in the collection tube.

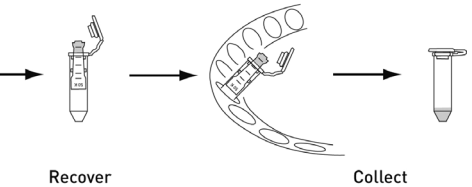
B.5 Add 300 µl of 1× TBS buffer on top of the resin. Centrifuge the column at 1500 × g for 1 min and discard the flow-through.

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 (100-200 µl).

B.9 Centrifuge at 1500 × g for 2 min and collect the flow-through containing the antibody in 1× TBS buffer.



Site-specific Conjugation of IgG wih DFO

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 20x 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.

4. Conjugation with DFO

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

Materials from kit:

- DIBO-modified label: DFO

4.1 Reconstitute the DIBO-DFO in 26 µl DMSO per vial.

4.2 Transfer the azide-activated antibody in 1× TBS (from step 3.9) to a 1.5 ml centrifuge tube and add all of the DIBO-modified label from step 4.1. Mix the sample solution by carefully pipetting up and down.

4.3 Seal the tube with the lid and wrap the lid with Parafilm® or similar.

4.4 Incubate overnight protected from light at 25°C.

4.5 After the incubation, the antibody conjugate can be stored at +4-8°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 DFO[1]

Time required: 1 h.

Materials from kit:

- 1× TBS buffer (prepared from 20× TBS) or other buffer of choice, for example PBS.
- Desalting Spin column (2 ml, 40K)

5.1 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.

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

5.3 Place the column in the collection tube.

5.4 Add 1 ml buffer on top of the resin. Centrifuge the column at 1000 × g for 2 min and discard the flow-through.

5.5 Repeat step 5.4 two times. The last time, centrifuge for 3 min.

5.6 Place the column in a new 15 ml collection tube.

5.7 Apply the antibody conjugate sample (from step 4.4) on top of the resin.

5.8 Centrifuge at 1000 × g for 3 min and collect the flow-through that contains the antibody conjugate.

5.9 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 DFO 2 mg contains several components. The product is shipped cold, and the components should be stored at different temperatures upon arrival (see Table 1).

GlyCLICK DFO is for R&D use only.

Table 1. Content and Storage Temperatures of GlyCLICK Components

Name	Amount	Store at
Desalting Spin column, 0.5 ml, 40 K	1 piece	4-8°C
Antibody concentrator (incl 2 collection tubes), 0.5 ml, 50 K	1 piece	4-25 °C
Desalting Spin column, 2 ml, 40 K	2 pieces	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
DIBO-modified label: DFO	2 vials (solid)	(-25)-(-5)°C Protect from light

USA & Canada
Genovis Inc.
245 First Street, Suite 1800, Cambridge, MA 02142, USA
Phone: 1-855-782-0084 (toll free)
Fax: 1-858-524-3006

EMEA & Asia
Genovis AB
Box 4, SE-244 21 Kävlinge, Sweden
Phone: +46 46 10 12 30
Fax: +46 46 12 80 20

support@genovis.com
www.genovis.com

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