



GlyCLICK®

ADC

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TEMPERATURES



FOR RESEARCH USE ONLY

Instructions for Use

GlyCLICK® ADC MMAE 2mg (L1-T02-200)

Process 2 mg of IgG

GlyCLICK® ADC PNU 2mg (L1-T01-200)

Process 2 mg of IgG

DOWNLOAD INSTRUCTIONS FOR USE



www.genovis.com/ifu-L1-T0

Site-specific Conjugation of IgG with MMAE or PNU

GlyCLICK is a site-specific conjugation technology for IgG using Fc 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 ADC is available for site-specific labeling of 2 mg IgG with MMAE (monomethyl auristatin E) or PNU (PNU anthracycline payload), and is suitable for the development of antibody-drug conjugates (ADCs).

The conjugation is performed by combining enzymatic steps and copper-free click chemistry to covalently link the toxin 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 ADC 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 DBCO-toxin in a strain-promoted, copper-free click reaction (SPAAC) to form a stable and homogenous antibody-drug conjugate.
4. **Purification:** Excess toxin reagent is removed from the antibody-drug conjugate by affinity chromatography.

YOU MIGHT ALSO BE INTERESTED IN

GlycINATOR™ Immobilized

Immobilized enzyme for deglycosylation of IgG in spin columns

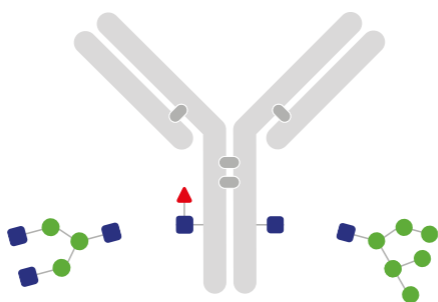
GlyCLICK® Azide Activation

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

GlyCLICK® Fluorophore

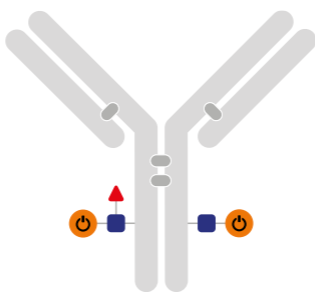
Site-specific conjugation of IgG with Alexa Fluor® 488, 555 or 647

* 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.



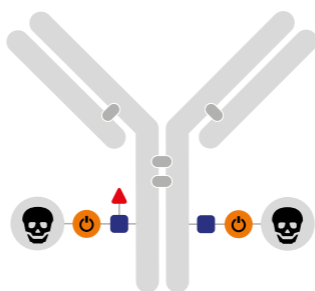
1. Deglycosylation

GlycINATOR Immobilized



2. Azide Activation

GalT + UDP-GalNAz



3. Click Reaction

DBCO-toxin

4. Purification

Figure 1. Schematic overview of the GlyCLICK technology for ADC generation.

Preparations

Important Information

Before you begin, briefly centrifuge tubes. Always wear suitable laboratory protective clothing and gloves when handling the reagents. **Keep in mind:** The DBCO-toxin is toxic. Sodium azide must be avoided throughout the protocol.

- Let the GlycINATOR Immobilized and Desalting Spin columns 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. 2 mg of IgG in a maximum volume of 250 μ 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.
- 1×TBS: 10 ml 1×TBS is prepared by adding 0.5 ml of 20×TBS to 9.5 ml of ddH₂O. Vortex briefly to mix.
- Centrifuge tubes: 1.5-2 ml and 15 ml.
- Dimethyl sulfoxide (DMSO) for reconstitution of DBCO-modified toxin.
- ddH₂O.
- Elution buffer: 0.1 M glycine, pH 2.5.
- Neutralization buffer: 1 M Tris, pH 8.0.

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 μ l.

If the sample volume is 250 μ l but needs a buffer exchange (if it contains phosphate or azide), concentrate the sample to < 150 μ l and then follow the steps in section “B. Buffer Exchange”.

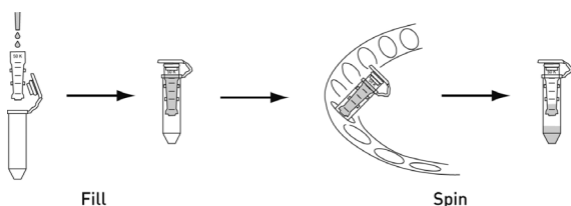


Figure 2. Antibody concentration step.

- A.1 Add 500 μ l of ddH₂O to the Antibody concentrator (0.5 ml, 50K) and cap the device as shown in Figure 2.
- 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 150 μ 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.*

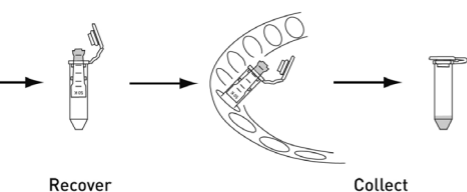
- A.6 Invert the Antibody concentrator into a new collection tube as shown in Figure 2.
- A.7 Centrifuge at 1000 \times g for 3 min to collect the concentrated IgG. After collection, the volume of concentrated IgG should be approximately 100-150 μ l in the collection tube.

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 Let the Desalting Spin column (0.5 ml, 40K) equilibrate to room temperature before use. Break off the bottom closure of the Desalting Spin column. 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 300 μ l of 1 \times TBS on top of the resin. Centrifuge the column 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 (100-150 μ l) on top of the resin.
- B.8 Centrifuge at 700 \times g for 2 min and collect the flow-through containing the antibody in 1 \times TBS.



Site-specific Conjugation of IgG with MMAE or PNU

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

Make sure that the antibody solution is 2 mg IgG in 250 μ l of 1 \times TBS (pH 7.4, without azide).

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

Materials from kit:

- 1 \times TBS (prepared from 20 \times 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 \times 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 μ l 1 \times TBS on top of the resin. Centrifuge the column at 200 \times 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 (250 μ l) to the column.
 - 1.8 Seal the column with the lid.
 - 1.9 Fully resuspend 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 2 h.
 - 1.11 Remove the bottom cap and place the column in a new microcentrifuge tube. Loosen the lid.
 - 1.12 Centrifuge at 1000 \times g for 1 min to collect the processed material.
 - 1.13 Insert the bottom cap. Add 100 μ l 1 \times 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 \times 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 and adjust the sample volume to 550 μ l with 1 \times TBS.

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 7 µl Buffer additive to the pooled fractions from step 1.18.
- 2.2 Transfer the 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 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)
 - Desalting Spin column (2 ml, 40K)
- 3.1 Let the Desalting Spin column (2 ml, 40K) equilibrate to room temperature before use. Twist off the bottom cap of the Desalting Spin column and place the column in a 15 ml centrifuge tube. Loosen the lid.
 - 3.2 Centrifuge at 700 × g for 2 min to remove the storage solution. Discard the flow-through.
 - 3.3 Place the column in the centrifuge tube.
 - 3.4 Add 1 ml 1× TBS on top of the resin. Centrifuge at 700 × g for 2 min and discard the flow-through.
 - 3.5 Perform step 3.4 two additional times. The last time, centrifuge for 3 min.
 - 3.6 Place the column in a new 15 ml centrifuge tube.
 - 3.7 Apply the azide-activated antibody sample from step 2.5 on top of the resin.
 - 3.8 Centrifuge at 700 × 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 +4-8°C protected from light for conjugation of a label at a later time.

4. Conjugation with DBCO-modified Toxin

Time required: 10 min hands-on, followed by overnight incubation.

Materials from kit:

- DBCO-modified toxin

- 4.1 Reconstitute the DBCO-modified toxin in 26 μ l of DMSO.
- 4.2 Transfer the azide-activated antibody from step 3.9 to a 1.5ml microcentrifuge tube and add all of the DBCO-modified toxin from step 4.1. Mix 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 purification.

5. Removal of Excess Toxin Reagent

Time required: 1 h.

Materials from kit:

- Two CaptureSelect[™] Fc** Microspin columns, 0.5 ml
- 1× TBS or PBS may be used for the purification of the conjugated antibody. 20× TBS is provided in the kit for convenience.

Additional materials:

- Elution buffer: 0.1 M glycine, pH 2.5
- Neutralization buffer: 1 M Tris, pH 8.0

Equilibration

- 5.1 Break off the bottom plastic caps of the CaptureSelect[™] Fc columns (save the caps) and place the columns in microcentrifuge tubes. Loosen the lids.
- 5.2 Centrifuge at 200× g for 1 min to remove the storage solution.
- 5.3 Insert the bottom caps.
- 5.4 Add 400 μ l 1× TBS to each column.
- 5.5 Seal the columns with the lids.
- 5.6 Fully suspend the resin, mix by inversion.
- 5.7 Remove the bottom caps and loosen the lids.
- 5.8 Centrifuge at 200× g for 1 min.
- 5.9 Perform steps 5.3-5.8 two additional times.
- 5.10 Insert the bottom caps.

** Made with Thermo Scientific[™] CaptureSelect[™] resin from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

Binding of the IgG Conjugate

- 5.11 Equally divide the antibody conjugate from step 4.5 and add to the CaptureSelect™ Fc columns. Seal the columns with the lids.
- 5.12 Fully suspend the resin, mix by inversion and make sure there is a flow in the columns.
- 5.13 Incubate the columns with end-over-end mixing at room temperature for 30 min.

Wash

- 5.14 Remove the bottom caps and place the columns in microcentrifuge tubes. Loosen the lids.
- 5.15 Centrifuge at 200×g for 1 min.
- 5.16 Insert the bottom caps.
- 5.17 Add 400µl 1×TBS to each column.
- 5.18 Seal the columns with the lids.
- 5.19 Fully suspend the resin, mix by inversion.
- 5.20 Remove the bottom caps, place the columns in microcentrifuge tubes and loosen the lids.
- 5.21 Centrifuge at 200×g for 1 min.
- 5.22 Perform steps 5.16-5.21 three additional times.

Elution of the Purified Conjugated IgG

- 5.23 Prepare two microcentrifuge tubes with 10µl of 1 M Tris, pH 8.0.
- 5.24 Insert the bottom caps.
- 5.25 Add 50µl of 0.1 M glycine, pH 2.5 to each of the CaptureSelect™ Fc columns and seal the columns with the lids.
- 5.26 Fully suspend the resin by inverting the columns a couple of times.
- 5.27 Remove the bottom caps and place the columns in the microcentrifuge tubes containing Tris. Loosen the lids.
- 5.28 Centrifuge at 200×g for 1 min to elute the conjugated antibody.
- 5.29 Perform steps 5.23-5.28 four additional times with centrifugation at 1000×g for 1 min.
- 5.30 Pool the eluted fractions and make sure the pH is neutralized.
- 5.31 The antibody conjugate can now be stored protected from light at +4-8 °C.

CONTENT AND STORAGE

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

GlyCLICK ADC 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, 40K	1 piece	+4-8°C
Antibody concentrator, 0.5 ml, 50K	1 piece	+4-25°C
Collection tube for antibody concentrator	2 pieces	+4-25°C
Desalting Spin column, 2 ml, 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)	3 × 2 ml	+4-8°C
Buffer additive	1 × 50 µl	+4-8°C Protect from light
β-1,4-galactosyltransferase GalT (Y289L)	1 × 40 µl	+4-8°C Protect from light
DBCO-modified toxin:		
DBCO-Val-Ser(GlcA)-EDA-PNU (L1-T01-200) or DBCO-Val-Ser(GlcA)-PAB-MMAE (L1-T02-200)	1 vial solid	(-25)-(-5)°C Protect from light
CaptureSelect™ Fc, Microspin	2 pieces	+4-8°C

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