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GlyCLICK®

ADC

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Instructions for Use

GlyCLICK® ADC MMAE 2 mg (L1-T02-200)
Process 2 mg of IgG

GlyCLICK® ADC PNU 2 mg (L1-T01-200)
Process 2 mg of IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-L1-T0

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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 for IgG from several species and subclasses. Fc N-glycan remodeling by complete deglycosylation of the antibody allows for site-specific conjugation using robust click-chemistry, resulting in a degree of label (DOL) or antibody-drug ratio (DAR) of 2.0.

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 procedure 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 is a reliable tool for conjugation of toxins to generate antibody-drug conjugates from any 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 to form a stable and homogenous antibody drug conjugate.
4. **Purification:** Excess toxin reagent is removed from the antibody-drug conjugate by affinity chromatography.

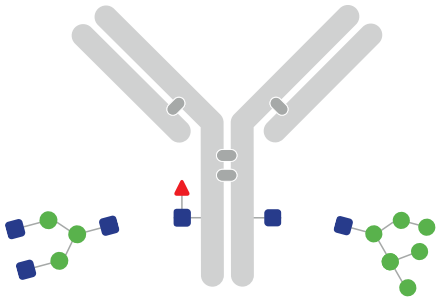
YOU MIGHT ALSO BE INTERESTED IN

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

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

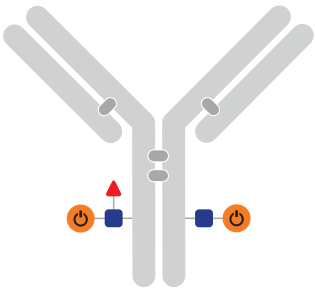
GlyCLICK® Fluorophore
Site-specific conjugation of IgG with Alexa Fluor® 488, 555 or 647

* 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.



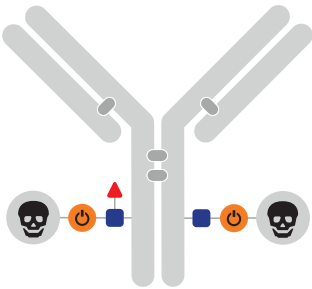
1. Deglycosylation

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 these reagents. **Keep in mind:** The DBCO-toxin is toxic. 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).

Equipment Required

- Centrifuge with swinging bucket rotor for 15 ml tubes accomodating desalting columns.
- Centrifuge for 1.5-2 ml microcentrifuge tubes.
- Incubator or water bath for 25°C and 30°C.
- End-over-end mixer.

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 (40K) for buffer exchange and a small concentrator (50K) are provided for convenience.
- Centrifuge tubes: 1.5-2 ml and 15 ml.
- Dimethyl sulfoxide (DMSO) for reconstitution of DBCO-modified toxin.
- ddH_2O.
- 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 (Fig. 2)

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 "Buffer exchange with Desalting Spin Column, 0.5 ml".

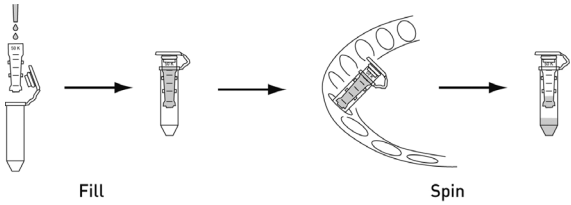


Figure 2. Antibody concentration step.

A.1 Add 500 µl of ddH_2O to the small antibody concentrator and cap the device as shown in Figure 2.

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 small 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 small 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 with Desalting Spin Column, 0.5 ml

This step **is required if:**

- The IgG is in a phosphate-containing buffer (e.g. PBS),

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. 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.

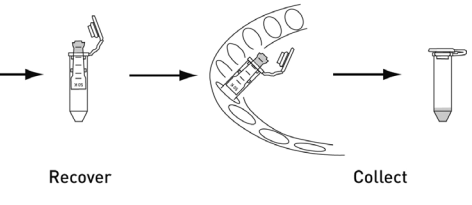
B.5 Add 300 µl of 1× TBS buffer on top of the resin. Centrifuge the column at 1500 × g for 1 min and discard the flow-through.

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 with MMAE or PNU

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

The IgG solution should be in 1× TBS buffer pH 7.4, with no azide. Max 2 mg 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
- Let the GlycINATOR Immobilized column equilibrate to room temperature before use
- The lid and the cap of the spin column are used during the incubation

1.1 Break off the bottom plastic cap of the GlycINATOR 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 of 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 at the bottom of the spin column.

1.8 Adjust the antibody sample volume (containing 2 mg of 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 of 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 1.14-1.17 one more 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 of 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

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 min to remove the storage solution. Discard the flow-through.

3.3 Place the column in the collection tube.

3.4 Add 1 ml of 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 the column 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 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 µl of DMSO.

4.2 Transfer the azide-activated antibody from step 3.9 to a 1.5 ml centrifuge tube and add all of the DBCO-modified toxin 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 purification.

5. Removal of Excess Toxin Reagent

Time required: 1 h.

Materials from kit:

- Two CapureSelect™ 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 collection tubes. Loosen the lids.

5.2 Centrifuge at 200 × g for 1 min to remove the storage solution.

5.3 Re-insert the bottom caps at the bottom of the spin columns.

5.4 Add 400 µl 1× TBS to each column.

5.5 Seal the columns with the lids.

5.6 Fully suspend the resin, mix it by inversion.

5.7 Remove the bottom caps and loosen the lids.

5.8 Centrifuge at 200 × g for 1 min.

5.9 Repeat steps 5.3-5.8 two times.

5.10 Seal the spin columns with the bottom caps.

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 it 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 collection tubes. Loosen the lids.

5.15 Centrifuge the columns at 200 × g for 1 min.

5.16 Seal the columns with the bottom caps.

5.17 Add 400 µl 1× TBS to each column.

5.18 Seal the columns with the lids.

5.19 Fully suspend the resin, mix it by inversion.

5.20 Remove the bottom caps, place the columns in collection tubes and loosen the lids.

5.21 Centrifuge at 200 × g for 1 min.

5.22 Repeat steps 5.16-5.21 three times.

Elution of the Purified Conjugated IgG

5.23 Prepare two collection tubes with 10 µl of 1 M Tris, pH 8.0.

5.24 Seal the columns with the bottom caps.

5.25 Add 50 µl 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 collection tubes containing Tris. Loosen the lids.

5.28 Centrifuge the columns at 200 × g for 1 min to elute the conjugated antibody.

5.29 Repeat steps 5.23-5.28 four 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 box is shipped cold, and the components should be stored at different temperatures upon arrival (see Table 1).

Do not freeze:

- Desalting Spin columns
- GlycINATOR Immobilized column
- CaptureSelect™ columns**
- GalT enzyme

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, 40 K	1 piece	4 °C to 8 °C
Antibody concentrator (incl 2 collection tubes), 0.5 ml, 50 K	1 piece	4 °C to 25 °C
Desalting Spin column, 2 ml, 40 K	1 piece	4 °C to 8 °C
GlycINATOR Immobilized Microspin column	1 piece	4 °C to 8 °C
UDP-GalNAz	1 vial solid	4 °C to 8 °C Protect from light
20× TBS pH 7.4 (0.5 M)	3 × 2 ml	4 °C to 8 °C
Buffer additive	1 × 50 µl	4 °C to 8 °C Protect from light
β-1,4-galactosyltransferase (Y289L) (GalT)	1 × 40 µl	4 °C to 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 °C to -5°C Protect from light
CaptureSelect Fc, Microspin	2 pieces	4 °C to 8 °C

** 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.

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