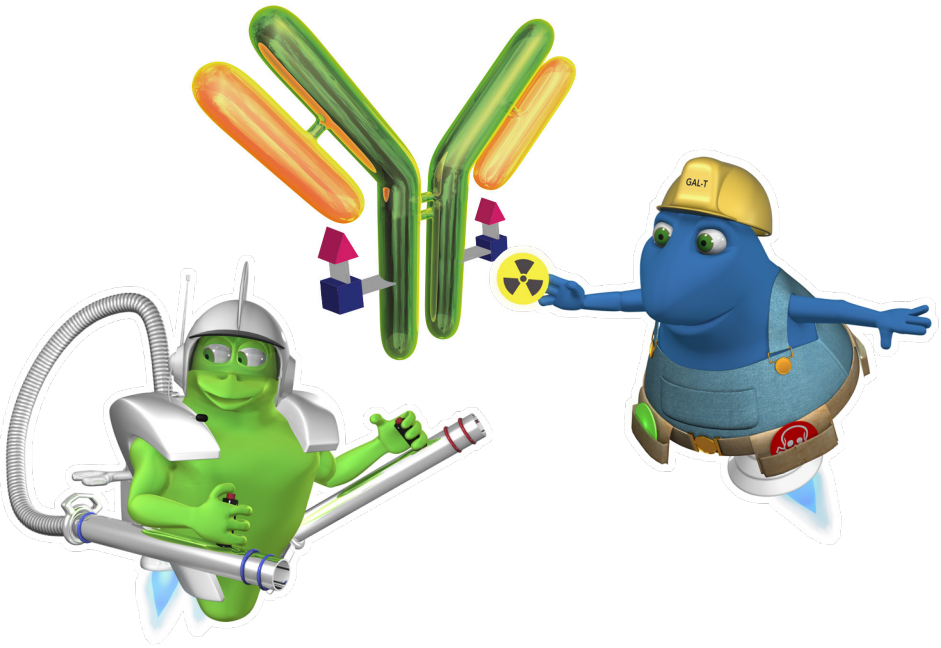
GlyCLICK®

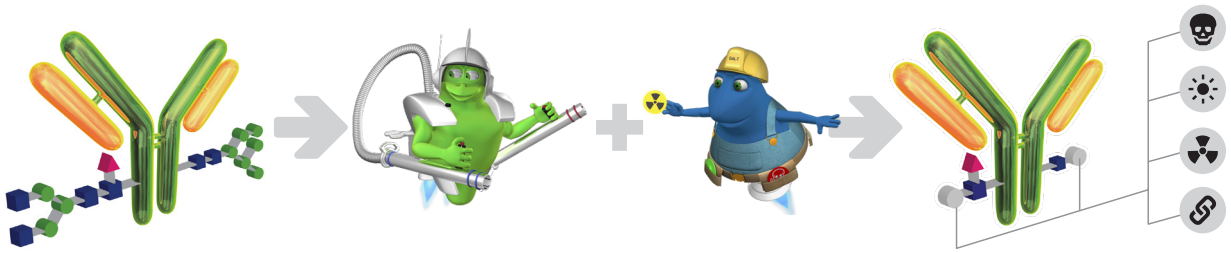


Site-specific Antibody Conjugation Technology

SmartEnzymes™

GENOVIS

GlyCLICK®



GlyCLICK® is a site-specific conjugation technology for IgG.

GlyCLICK enables quantitative conjugation of functional groups site-specifically to the Fc domain of IgG. The GlyCLICK technology utilizes the Fc-specific endoglycosidase activity of GlycINATOR and the GalNAz transfer and click-chemistry of SiteClick®* for robust and precise attachment of labels to IgG. The resulting antibody conjugate has 2.0 labels per antibody and allows sensitive quantitative applications. Conjugation at the Fc domain ensures unaffected antigen binding after IgG labeling. The functional groups available as GlyCLICK kits include AlexaFluor®488, Biotin and DFO. The GlyCLICK Azide Activation kit allows site-specific conjugation of a custom label of choice.
The GlyCLICK technology is applied in both *in vitro* and *in vivo* imaging applications as well as ADC and therapeutic antibody development.

- Human IgG1-4, Fc-fusion proteins, IgG from mouse, rabbit, rat, monkey, sheep, goat, cow and horse
- ~ 3 day protocol
- Available conjugates: Alexa Fluor®488, biotin and DFO. Azide activation kits are available for custom conjugation

GlyCLICK Conjugation Overview

The GlyCLICK technology involves two enzymatic steps and click-chemistry to obtain a site-specific antibody conjugate. The process of GlyCLICK conjugation is schematically illustrated in *Fig. 1*.

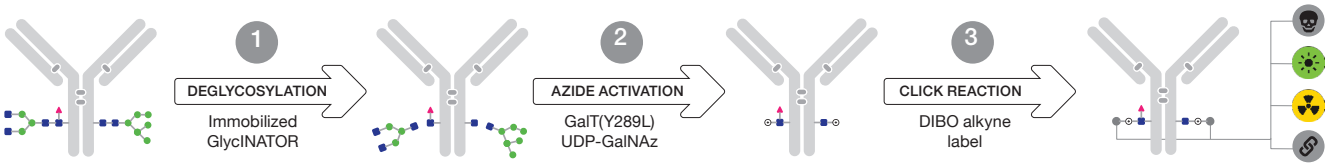


Figure 1. Schematic presentation of the GlyCLICK conjugation process.

1. Immobilized GlycINATOR hydrolyzes the Fc-glycans of IgG and exposes the core GlcNAc.
2. The enzyme β-1,4-galactosyltransferase GalT (Y289L) transfers GalNAz to the exposed GlcNAc, rendering the antibody azide-activated.
3. Through a strain-promoted copper-free click reaction using a DIBO-alkyne label (cyclooctyne), the functional group is conjugated to the two azides of GalNAz at the Fc domains of the antibody.

Available DIBO-Functionalized Labels

Label	Name	Examples of Applications
Fluorophore	AlexaFluor® 488	FACS, IHC, *in vitro* cell imaging
Affinity	Biotin	Immuno assays, ELISA, western blot
Chelator	Deferoxamine (DFO)	Immuno imaging, *in vivo*

* SiteClick® is provided under an intellectual property license from Life Technologies Corporation. The trademark SiteClick® is the property of Life Technologies Corporation.
See Legal and Disclaimers, p. 39.

Fc-specific and Complete GlyCLICK Conjugation

The GlyCLICK conjugation of the desired label occurs only at the azide-activated sites on the Fc region, ensuring site-specific conjugation. Trastuzumab was conjugated with AlexaFluor®488 using GlyCLICK and digested using FabRICATOR, resulting in F(ab')2 and Fc/2 fragments. The fluorescence signal was detected only from the Fc domain, indicating site-specific conjugation (*Fig. 2*).

The efficacy of the GlyCLICK process was studied by conjugating monomethyl auristatin E (MMAE) to trastuzumab using GlyCLICK and analyze the Fc/2 fragment using LC-MS. The native (*Fig. 3a*) and deglycosylated (*Fig. 3b*) Fc/2 show the hydrolysis of trastuzumab. After conjugation of MMAE, the Fc/2 displays a mass shift of one toxin and linker indicating a complete conjugation to the Fc/2 and a drug to antibody ratio of 2.0 (*Fig. 3c*).

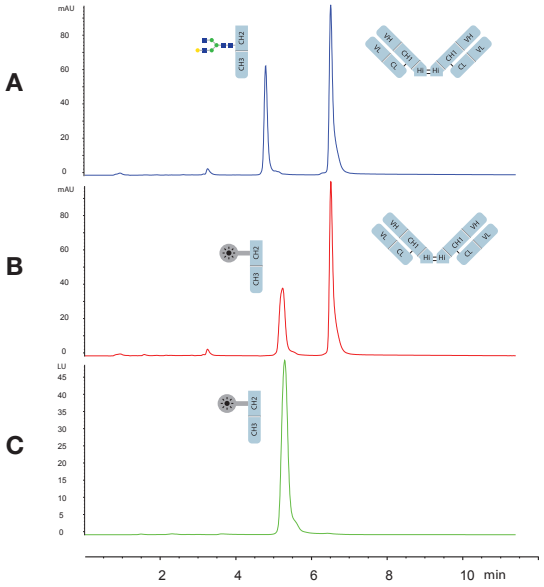


Figure 2. RP-HPLC analysis of trastuzumab. **a)** Trastuzumab digested with FabRICATOR into F(ab')2 and Fc/2 fragments. **b)** Trastuzumab conjugated with Alexa Fluor®488 prior to FabRICATOR digestion into F(ab')2 and Fc/2 fragments. **c)** Fluorescence signal of b).

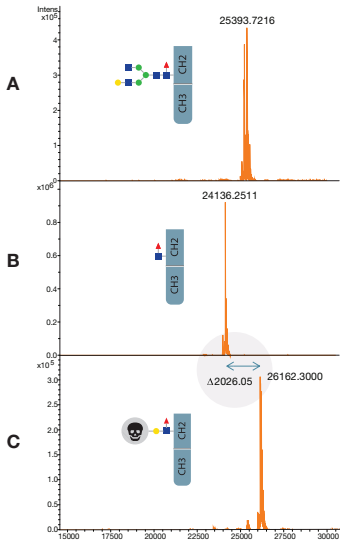


Figure 3. LC/MS analysis of trastuzumab conjugated with MMAE (monomethyl auristatin E) using GlyCLICK, showing the Fc-specific attachment of one toxin per Fc/2 fragment. **a)** Native, **b)** deglycosylated, **c)** conjugated trastuzumab.

GlyCLICK®

GlyCLICK contains all reagents and materials needed to azide activate or label the IgG.

	Product ID	Description	Size	EUR	USD
	L1-F01-025	GlyCLICK Alexa Fluor® 488	Conjugates 250 µg IgG	885	940
	L1-C01-025	GlyCLICK DFO	Conjugates 250 µg IgG	885	940
	L1-A01-025	GlyCLICK Biotin	Conjugates 250 µg IgG	885	940
	L1-AZ1-025	GlyCLICK Azide Activation	Activates 250 µg IgG	790	835
	L1-AZ1-100	GlyCLICK Azide Activation	Activates 1 x 10 mg IgG	4,900	5,900

How to Order

Genovis accepts orders from all over the world. Orders from US and Canada are handled by Genovis Inc, and orders from EMEA and Asia are handled by Genovis AB.
You can place your order via email, fax or telephone, or you can order directly online using a credit card.

US and Canada

Send your purchase order to:
orders.us@genovis.com

Order phone (toll free):
1-855-782-0084
Order fax:
1-858-524-3006

Place your order online:
www.genovis.com/store

EMEA and Asia

Send your purchase order to:
order@genovis.com

Order phone:
0046 (0)46 10 12 30
Order fax:
0046 (0)46 12 80 20

Place your order online:
www.genovis.com/store