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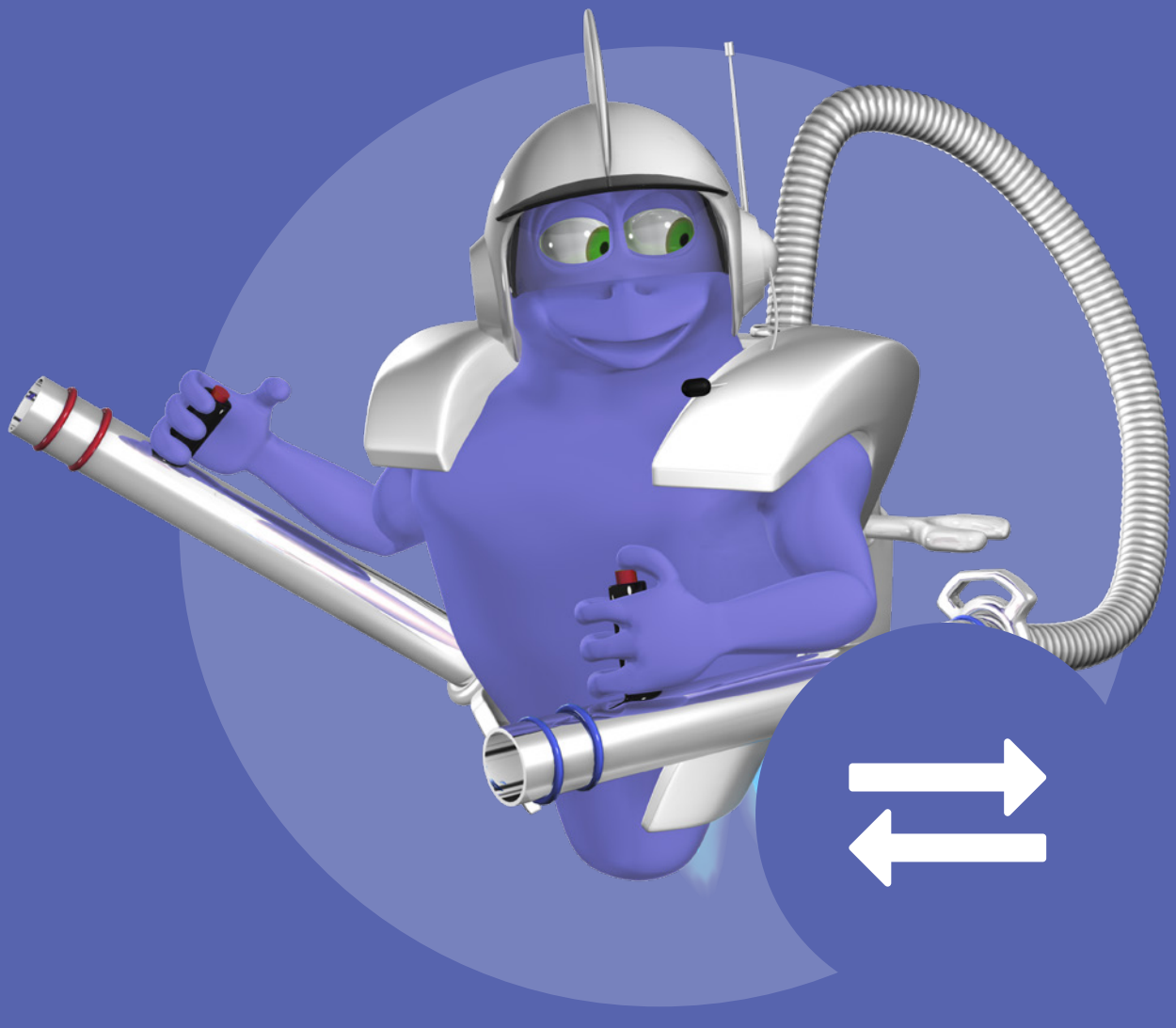

TransGLYCIT™

Transglycosylation of IgG

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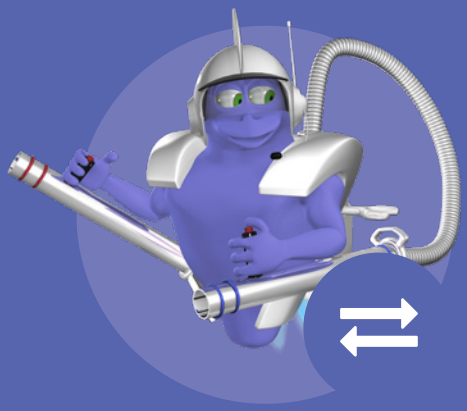
TransGLYCIT™

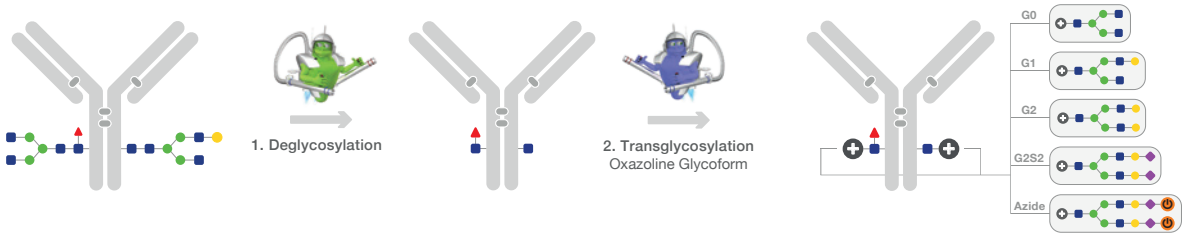


Transglycosylation of IgG

TransGLYCIT is a platform that enables efficient and site-specific human IgG glycan remodeling and conjugation. With TransGLYCIT, antibodies with defined and homogenous glycoforms or site-specific azide activation are generated using fast and robust enzymatic workflows. With the optional FucosEXO™ 16 enzyme in the glycan remodeling workflow, afucosylated antibodies carrying the Fc glycoform of choice (G0, G1, G2 or G2S2) can be obtained for comparison of antibodies with or without core fucose. With the azide activation workflow, antibodies are prepared for conjugation with any label of choice using click-chemistry.

- Human IgG1, IgG2 and IgG4
- Transglycosylation occurs at the Fc N-glycan sites
- 3-6 hour workflow
- G0, G1, G2, G2S2 or azide-activated glycoform is included

The Transglycosylation Workflow



Deglycosylation: The Fc N-glycans are trimmed to the core GlcNAc using the IgG-specific Immobilized GlycINATOR® (EndoS2) enzyme that hydrolyses all Fc glycoforms, including high-mannose, hybrid, complex and bisecting glycans. The degree of fucosylation is the same as on the original IgG.

Note that for the glycan remodeling products, it is possible to obtain afucosylated G0, G1, G2 or G2S2 glycoforms using TransGLYCIT Afucosylated, where the Immobilized FucosEXO™ 16 enzyme hydrolyzes the α1,6-linked core fucose prior to the transglycosylation step.

Transglycosylation: The engineered glycosynthase TransINATOR™ catalyzes the transglycosylation reaction between the oxazoline reactive G0, G1, G2, G2S2 or azide-activated glycoform and the core GlcNAc.

Product Formats

TransGLYCIT™
Glycan remodeling of human IgG with the G0, G1, G2 or G2S2 glycoform

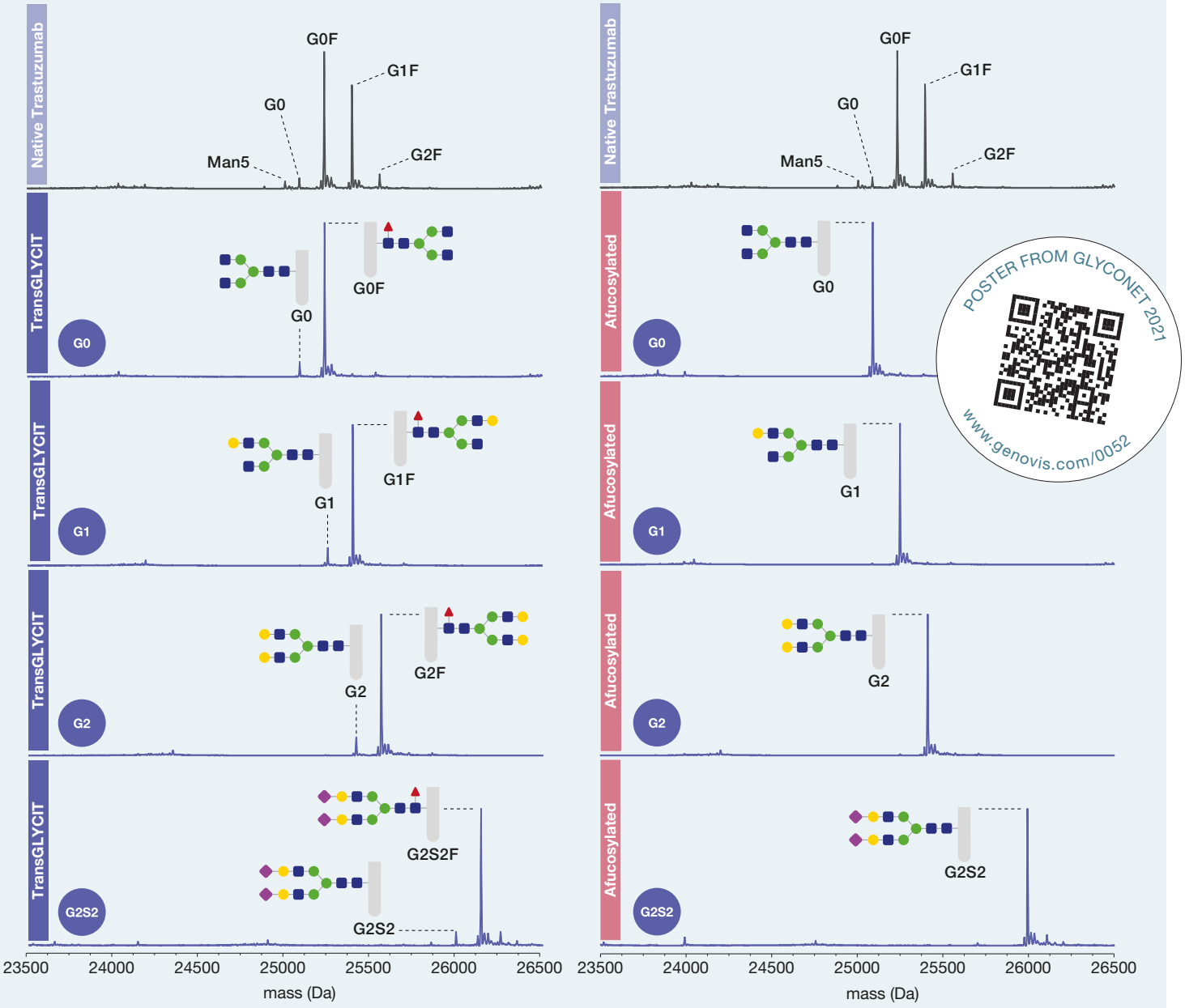
TransGLYCIT™ Azide Activation
Azide activation of human IgG1/IgG4 or IgG2

TransGLYCIT™ Afucosylated
Glycan remodeling of human IgG with the G0, G1, G2 or G2S2 glycoform and core afucosylation

Generating Agalactosylated, Galactosylated, or Sialylated Fc Glycoforms using TransGLYCIT™

Glycoengineering of the IgG Fc N-glycan profile is important for the development of next-generation therapeutic antibodies with enhanced or silenced effector functions. TransGLYCIT is used to generate antibodies carrying agalactosylated (G0), galactosylated (G1, G2) or sialylated (G2S2) glycan structures. With the option to generate glycan structures lacking the core fucose using the TransGLYCIT Afucosylated kit, antibodies that show increased binding to activating FcγIIIa receptors and thus an elevated ADCC response can be obtained for direct comparison between fucosylated and afucosylated antibodies.

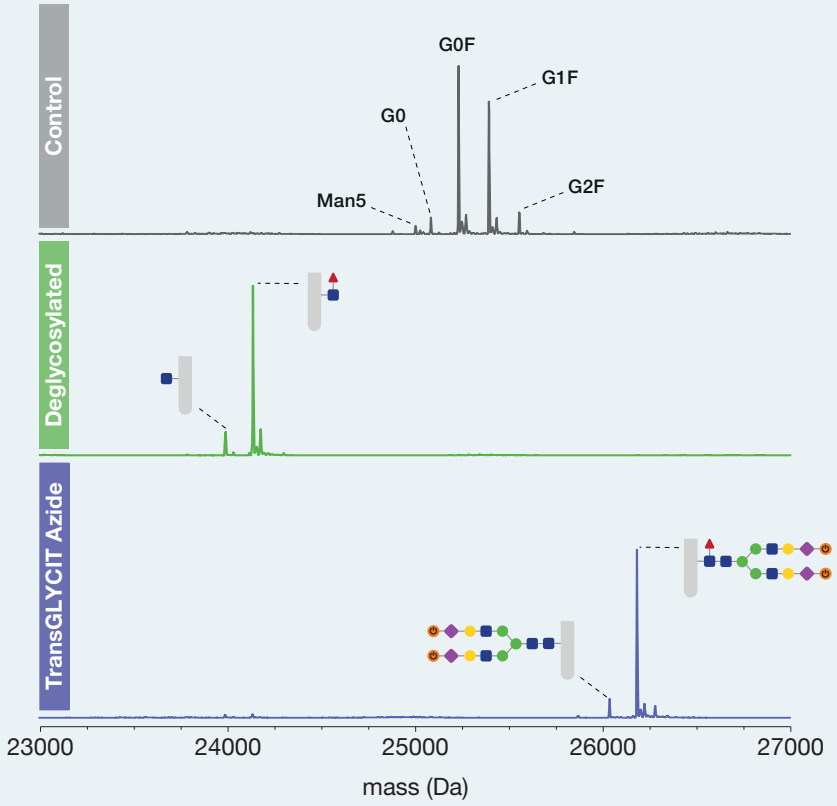
Here, the glycan profile of the therapeutic antibody trastuzumab was remodeled to carry either fucosylated or afucosylated G0, G1, G2 or G2S2 glycoforms. The resulting mass spectra show the heterogenous glycan profile of native trastuzumab and the mass shifts after complete transglycosylation of the antibody to generate G0, G1, G2 or G2S2 using TransGLYCIT. Homogenous afucosylated glycan profiles were generated using the TransGLYCIT Afucosylated kits.



Transglycosylation using TransGLYCIT. Deconvoluted mass spectra of the Fc/2 fragment of native trastuzumab (top) and after transglycosylation with TransGLYCIT (left panel) or TransGLYCIT Afucosylated (right panel). The mAb was digested with FabRICATOR and the subunits were analyzed by reversed-phase LC-MS on a Waters™ BioAccord™ system equipped with a Waters™ BioResolve™ RP mAb column (2.1 × 50 mm).

Site-specific Azide Activation of Human IgG using TransGLYCIT™ Azide Activation

Site-specific Fc N-glycan conjugation is a powerful tool with applications ranging from biomedical research to the development of diagnostic methods and antibody-drug conjugates (ADCs). A reliable conjugation technology is crucial to obtain a homogenous and reproducible conjugate while preserving the stability and immunoreactivity of the final product. Using the TransGLYCIT Azide Activation technology, human IgG is azide-activated by transglycosylation within a few hours. Here, TransGLYCIT Azide Activation was used for site-specific azide activation of trastuzumab using the modified N-glycan oxazoline carrying two azide functionalities. The reaction generated an IgG with four azide-activated sites for subsequent conjugation, two sites on each Fc/2.



Azide activation of trastuzumab using the TransGLYCIT Azide Activation workflow. Deconvoluted mass spectra of the Fc/2 fragment of native trastuzumab (top), after deglycosylation by GlycINATOR (middle) and after transglycosylation by TransINATOR with the oxazoline glycoform azide as the substrate (bottom). The mAb was digested with FabRICATOR and the subunits were analyzed by reversed-phase LC-MS on a Waters™ BioAccord™ system equipped with a Waters™ BioResolve™ RP mAb column (2.1×50mm).

TransGLYCIT™

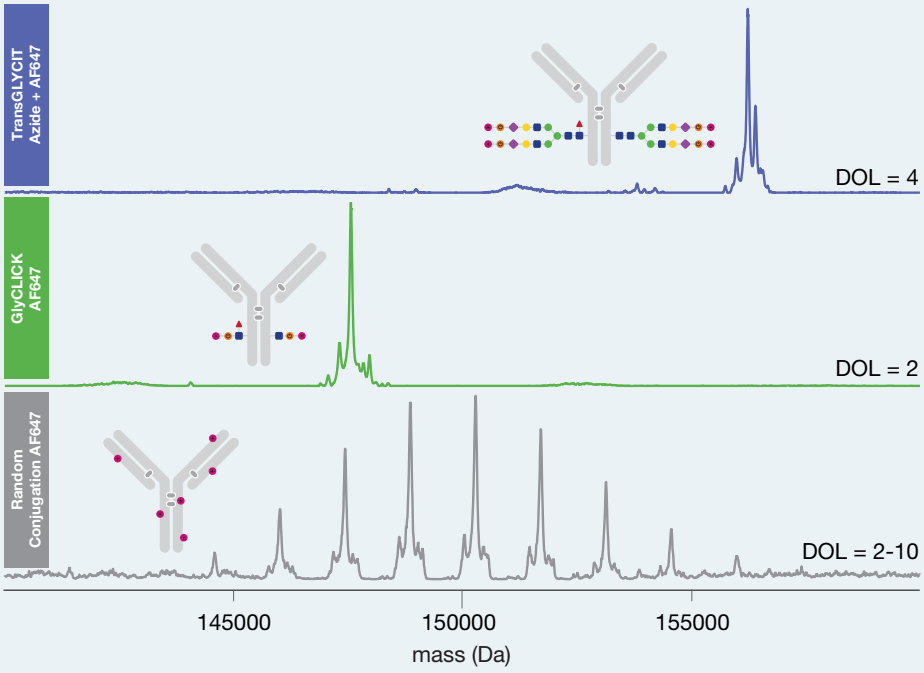
PRODUCT	DESCRIPTION	ID
TransGLYCIT G0, 1 mg	Generates 1 mg human IgG with the G0 glycoform	T1-G0F-010
TransGLYCIT G1, 1 mg	Generates 1 mg human IgG with the G1 glycoform	T1-G1F-010
TransGLYCIT G2, 1 mg	Generates 1 mg human IgG with the G2 glycoform	T1-G2F-010
TransGLYCIT G2S2, 1 mg	Generates 1 mg human IgG with the G2S2 glycoform	T1-S2F-010

TransGLYCIT™ Azide Activation

PRODUCT	DESCRIPTION	ID
TransGLYCIT Azide Activation, 100 µg	Activates 100 µg human IgG1 or IgG4	T1-AZ1-001
TransGLYCIT Azide Activation hIgG2, 100 µg	Activates 100 µg human IgG2	T1-AZ2-001

Comparison of Site-specific and Random Conjugation Technologies

The azide-activated Fc N-glycans on antibodies modified using the TransGLYCIT Azide Activation workflow can be used for site-specific conjugation with a label of choice via click chemistry, resulting in an antibody homogeneously labeled with four labels per molecule. Using site-specific conjugation instead of random conjugation technologies is favorable for preserving affinity and generating conjugates where quantitative properties are desired. To illustrate the impact of different labeling methods on the degree of labeling (DOL), we here analyzed trastuzumab conjugated with a fluorophore using either TransGLYCIT, GlyCLICK or random labeling by NHS-chemistry. The TransGLYCIT and GlyCLICK workflows resulted in homogeneously labeled samples with defined DOL 4 or DOL 2, respectively, whereas the random technique resulted in a heterogeneously labeled sample with a DOL varying from 2 to 10.



Conjugation of trastuzumab using site-specific and random technologies. Deconvoluted mass spectra of intact trastuzumab modified using TransGLYCIT Azide Activation followed by site-specific conjugation by strain-promoted azide alkyne click chemistry using DBCO-AF647 (DOL=4; top), conjugated using GlyCLICK AlexaFluor®647 (DOL=2; middle), and randomly labeled using NHS-activated AlexaFluor 647 resulting in heterogenous labeling (DOL=2-10; bottom). The randomly labeled sample was analyzed after deglycosylation by GlycINATOR. The intact masses of the trastuzumab conjugates were analyzed using a Bruker Impact II ESI-QTOF MS.

TransGLYCIT™ Afucosylated

PRODUCT	DESCRIPTION	ID
TransGLYCIT G0 Afucosylated, 1 mg	Generates 1 mg afucosylated human IgG with the G0 glycoform	T1-G0A-010
TransGLYCIT G1 Afucosylated, 1 mg	Generates 1 mg afucosylated human IgG with the G1 glycoform	T1-G1A-010
TransGLYCIT G2 Afucosylated, 1 mg	Generates 1 mg afucosylated human IgG with the G2 glycoform	T1-G2A-010
TransGLYCIT G2S2 Afucosylated, 1 mg	Generates 1 mg afucosylated human IgG with the G2S2 glycoform	T1-S2A-010

SmartEnzymes™

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