TransGLYCIT™

Afucosylated 1 mg

FOR RESEARCH
USE ONLY

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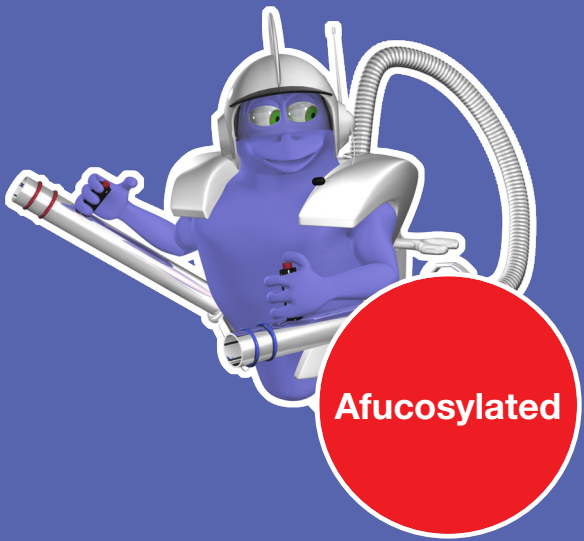
STORE CONTENT AT DIFFERENT TEMPERATURES
(See page 9)



SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

TransGLYCIT™ G0 Afucosylated 1 mg (T1-G0A-010)
N-glycan remodeling of up to 1 mg IgG with
G0 glycoform and core afucosylation

TransGLYCIT™ G1 Afucosylated 1 mg (T1-G1A-010)
N-glycan remodeling of up to 1 mg IgG with
G1 glycoform and core afucosylation

TransGLYCIT™ G2 Afucosylated 1 mg (T1-G2A-010)
N-glycan remodeling of up to 1 mg IgG with
G2 glycoform and core afucosylation

TransGLYCIT™ G2S2 Afucosylated 1 mg (T1-S2A-010)
N-glycan remodeling of up to 1 mg IgG with
G2S2 glycoform and core afucosylation

Last revised August 2021

N-glycan Remodeling Using TransGLYCIT Afucosylated

1 Deglycosylation

- **Deglycosylation and defucosylation of IgG Fc** using Immobilized GlycINATOR® and FucosEXO™ 16 to expose the core GlcNAc and remove the α1-6 linked core fucose.

2 Transglycosylation

- **Transglycosylation** using the glycosynthase TransINATOR™ to transfer the oxazoline reactive glycofrom to the core GlcNAc.

3 Purification

- **Purification** of the antibody with a defined glycoform and removal of excess reagents.

PRODUCT DESCRIPTION

TransGLYCIT Afucosylated is a transglycosylation kit for N-glycan remodeling of human IgG Fc to obtain antibody preparations with a homogenous glycoform without the α1-6 linked core fucose.

The IgG N-glycosylation site on the CH2 domain of the heavy chain is first trimmed to the core GlcNAc using GlycINATOR (EndoS2) and the α1-6 linked core fucose is removed by FucosEXO 16. Subsequent transglycosylation with a defined glycoform is performed using the engineered glycosynthase TransINATOR (EndoS2mut).

GlycINATOR is an IgG specific endoglycosidase, hydrolyzing all IgG Fc glycoforms including high-mannose, hybrid-type and bisected N-glycans, leaving only the core GlcNAc attached to the antibody. The FucosEXO 16 is a fucosidase acting on the α1-6 linked core fucose and requires hydrolysis of the glycan using GlycINATOR to access its substrate (1). The TransINATOR enzyme is an

engineered glycosynthase catalyzing the transglycosylation reaction between oxazoline reactive glycoforms and the core GlcNAc (2).

TransGLYCIT is available with either G0, G1, G2 or G2S2 oxazoline glycoform for transglycosylation of human IgG. Finally, the antibody with a single homogenous glycoform is purified and excess reagents are removed using a CaptureSelect™ affinity purification resin*.

TransGLYCIT enables specific remodeling of the Fc N-glycan on human IgG for preparation of antibodies with defined and homogenous glycoforms using fast and robust enzymatic workflows. Using TransGLYCIT Afucosylated, afucosylated antibodies can be obtained for direct comparison of antibodies with or without the core fucose.

* *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*

PRODUCT DESCRIPTION

The IgG Fc N-glycan remodeling is performed in three steps:

1. **Deglycosylation and defucosylation:** Immobilized GlycINATOR hydrolyzes the N-glycans on the Fc-part of the IgG to the inner GlcNAc. Immobilized FucosEXO 16 removes the α1-6 linked core fucose.

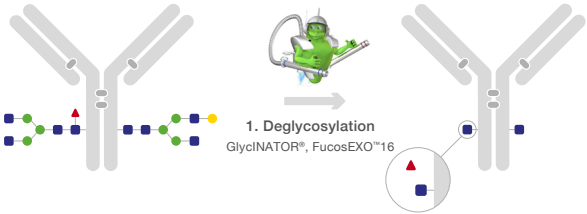
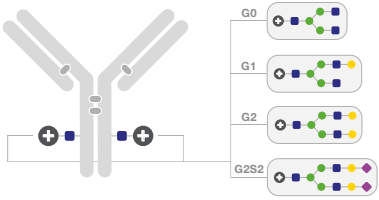


Figure 1. Schematic overview of N-glycan remodeling of human IgG using the TransGLYCIT Afucosylated products to obtain antibody preparations with a defined and homogenous glycoform and core afucosylation.

2. **Transglycosylation:** TransINATOR catalyzes the attachment of the selected oxazoline reactive glycoform to the core GlcNAc.
3. **Purification:** The N-glycan remodeled antibody is purified, and excess reagents are removed using affinity chromatography.

2. Transglycosylation
TransINATOR™, Glycoform



Content and Storage

TransGLYCIT Afucosylated contains enzymes and reagents to perform Fc N-glycan remodeling of up to 1 mg human IgG.

TransGLYCIT Afucosylated is shipped cold, and components should be stored at storage temperatures according to Table 1 upon arrival.

Before you begin, briefly centrifuge the tubes.

Do not freeze Immobilized GlycINATOR & FucosEXO 16 column or CaptureSelect column!

TransGLYCIT Afucosylated is for R&D use only.

Table 1. Content and storage temperatures of TransGLYCIT Afucosylation components

Name	Amount	Store at
Immobilized GlycINATOR & FucosEXO 16	1 piece	4 °C to 8 °C
TransINATOR	1 vial solid	-25°C to -5°C
CaptureSelect Fc(ms) Microspin	1 piece	4 °C to 8 °C
Oxazoline glycoform G0 or Oxazoline glycoform G1 or Oxazoline glycoform G2 or Oxazoline glycoform G2S2	1 vial solid	-25°C to -5°C

Quality Control

TransGLYCIT Afucosylated is tested to meet the specification and for lot-to-lot consistency.

Equipment Required

- Centrifuge for microcentrifuge tubes
- End-over-end mixer or similar

Additional Materials Required

- Antibody in 1 × PBS, pH 7.4, free of carrier proteins. 1 mg human IgG in a maximum volume of 100 µl
- Centrifuge tubes: 1.5-2 ml
- Phosphate Buffer Saline (PBS): 10 mM Sodium Phosphate, 150 mM Sodium Chloride, pH 7.4
- ddH_2O
- Elution buffer: 0.1 M Glycine, pH 2.5
- Neutralization buffer: 1 M Tris, pH 8.0

Protocol for N-glycan Remodeling of up to 1 mg Human IgG

1 Deglycosylation and Defucosylation: Hydrolysis of the N-glycan on the Antibody Fc Domain and Removal of α1-6 Linked Core Fucose.

The antibody solution should be in PBS buffer pH 7.4, maximum 1 mg in 100 µl.

Time required: 15 min hands-on, 60 min hands-off.

Materials from kit:

- Immobilized GlycINATOR & FucosEXO 16 Microspin column.
- Let the Immobilized GlycINATOR & FucosEXO 16 column equilibrate to room temperature before use.
- The lid and the cap of the spin column are used during the incubation.
- Before the centrifugations, remove the bottom cap and slightly open the lid.

1.1 Break off the bottom plastic cap of the GlycINATOR & FucosEXO 16 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 PBS buffer on top of the resin. Centrifuge the column at 200 × g for 1 min and discard the flow-through.

1.6 Perform step 1.5 **three times.**

1.7 Re-insert the bottom cap at the bottom of the spin column.

1.8 Adjust the antibody sample volume (containing 1 mg of antibody) to 100 µl using PBS and immediately add the antibody solution to the column.

1.9 Seal the column with the lid.

1.10 Fully suspend the resin, mix it by inversion and make sure there is a flow in the column.

1.11 Incubate the column with end-over-end mixing at room temperature for 60 min.

1.12 Remove the bottom cap and place the column in a clean microcentrifuge tube. Loosen the top lid.

1.13 Centrifuge the column at 1000 × g for 1 min to collect the deglycosylated and afucosylated antibody sample.

1.14 Attach the bottom cap. Add 100 µl of PBS 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 clean microcentrifuge tube. Loosen the lid.

1.17 Centrifuge at 1000 × g for 1 min to collect the deglycosylated and afucosylated antibody sample.

1.18 Attach the bottom cap. Add 50 µl of PBS and seal the column with the lid.

1.19 Invert the column a couple of times.

1.20 Remove the bottom cap and place the column in a clean microcentrifuge tube. Loosen the lid.

1.21 Centrifuge at 1000 × g for 1 min to collect the deglycosylated and afucosylated antibody sample.

1.22 Pool the collected deglycosylated and afucosylated antibody material.

2 Transglycosylation: Attachment of Selected Oxazoline Glycoform

Time required: 15 min hands-on, 60 min hands-off.

Materials from kit:

- TransINATOR
- Selected oxazoline glycoform

2.1 Reconstitute TransINATOR in 20 µl ddH_2O.

2.2 Reconstitute the oxazoline glycoform in 10 µl ddH_2O.

2.3 Add TransINATOR and the oxazoline glycoform to the deglycosylated and afucosylated antibody sample from step 1.22.

2.4 Incubate with end-over-end mixing at room temperature for 60 min[1].

3 Removal of Excess Reagents

Time required: 30 min hands-on, 20 min hands-off.

Materials from kit:

- CaptureSelect Fc(ms) microspin column

Equilibration

3.1 Break off the bottom plastic cap of the CaptureSelect column (save the cap) and slightly open the lid. Place the column in a microcentrifuge collection tube.

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

3.3 Discard the flow-through.

3.4 Place the column in the collection tube.

3.5 Add 300 µl of PBS buffer on top of the resin. Centrifuge the column at 200 × g for 1 min and discard the flow-through.

3.6 Perform step 3.5 **three times.**

3.7 Re-insert the bottom cap at the bottom of the spin column.

Binding of the N-glycan Remodeled Antibody

3.8 Add the sample from step 2.4 and seal the column with the lid.

3.9 Fully suspend the resin, mix it by inversion and make sure there is a flow in the column.

3.10 Incubate the column with end-over-end mixing at room temperature for 20 min.

Wash

3.11 Remove the bottom cap and place the column in a microcentrifuge tube. Slightly open the lid.

3.12 Centrifuge the column at 200 × g for 1 min and discard the flow-through.

3.13 Add 300 µl of PBS buffer on top of the resin. Fully suspend the resin, mix it by inversion.

3.14 Centrifuge the column at 200 × g for 1 min and discard the flow-through.

3.15 Perform steps 3.13-3.14 **four times.**

Elution of Purified, N-glycan Remodeled Antibody

3.16 Prepare **four** collection tubes with 10 µl of 1 M Tris pH 8.0.

3.17 Seal the column with the bottom cap.

3.18 Add 100 µl of 0.1 M Glycine pH 2.5 and seal the column with the lid.

3.19 Fully suspend the resin by inverting the columns a couple of times.

3.20 Remove the bottom cap and place the column in a collection tube containing Tris. Slightly open the top lid.

3.21 Centrifuge the column at 1000 × g for 1 min to elute the antibody.

3.22 Perform step 3.17-3.21 **four times.**

3.23 Pool the collected eluates.

3.24 The N-glycan remodeled antibody can now be stored at +4–8°C.

Notes

1. Longer incubation time may be required for human IgG2.

Related Products

TransGLYCIT™
Transglycosylation of up to 1 mg of human IgG with a selected glycoform (either G0, G1, G2 or G2S2).

FabRICATOR®
Digestion of IgG below the hinge.

GlyCLICK® Azide Activation kit
Azide activation of native IgG for site-specific conjugation.

References

1. *Sjögren, J. et al., 2015. EndoS and EndoS2 hydrolyze Fc-glycans on therapeutic antibodies with different glycoform selectivity and can be used for rapid quantification of high-mannose glycans. Glycobiology, 25(10), pp.1053–1063.*

2. *Li, T. et al., 2016. Glycosynthase Mutants of Endoglycosidase S2 Show Potent Transglycosylation Activity and Remarkably Relaxed Substrate Specificity for Antibody Glycosylation Remodeling. J Biol Chem, 291(32), pp.16508–16518.*

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