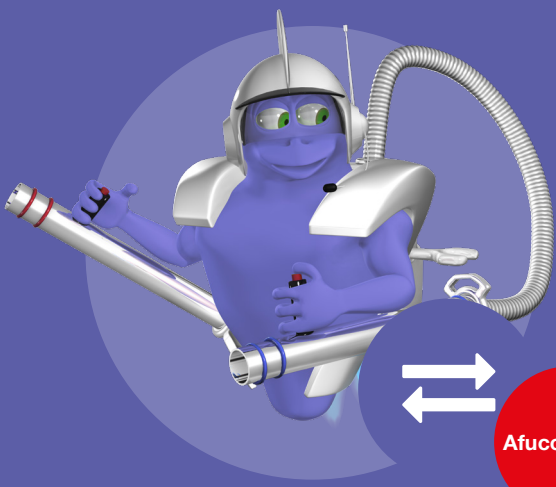
SmartEnzymes™

GENOVIS



TransGLYCIT®

Remodeling Afucosylated 1 mg

STORE AT
DIFFERENT
TEMPERATURES

FOR RESEARCH USE ONLY

Instructions for Use

TransGLYCIT® Remodeling Afucosylated G0 1 mg
(T1-G0A-010)
Process 1 mg human IgG

TransGLYCIT® Remodeling Afucosylated G1 1 mg
(T1-G1A-010)
Process 1 mg human IgG

TransGLYCIT® Remodeling Afucosylated G2 1 mg
(T1-G2A-010)
Process 1 mg human IgG

TransGLYCIT® Remodeling Afucosylated G2S2 1 mg
(T1-S2A-010)
Process 1 mg human IgG

TransGLYCIT® Remodeling Afucosylated Man5 1 mg
(T1-M5A-010)
Process 1 mg human IgG

DOWNLOAD INSTRUCTIONS FOR USE
www.genovis.com/ifu-T1-G0A-010

Glycan Remodeling of Human IgG with the G0, G1, G2, G2S2 or Man5 Glycoform, with Core Afucosylation

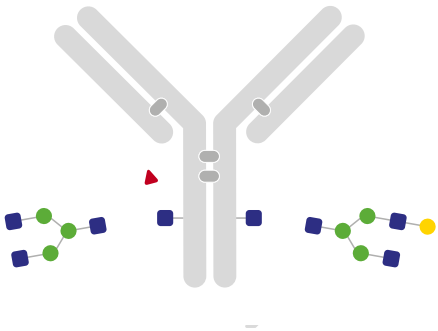
TransGLYCIT Remodeling Afucosylated is a platform technology for efficient transglycosylation of native human IgG in a few hours. The robust workflow transglycosylates the antibody Fc N-glycans using enzymatic remodeling, resulting in a homogenous pool of antibodies carrying a defined glycoform. The technology is available in kits to generate antibodies carrying the G0, G1, G2, G2S2 or Man5 glycan profiles, with core afucosylation.

TransGLYCIT Remodeling Afucosylated contains sufficient material to transglycosylate 1 mg human IgG.

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

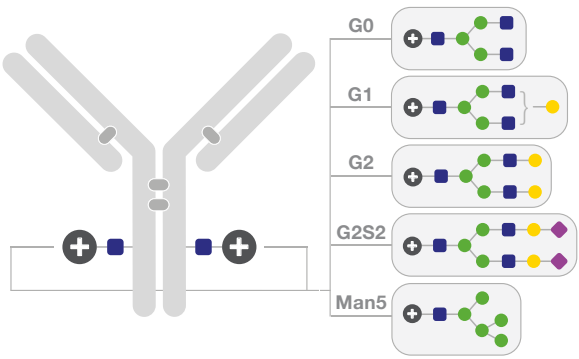
1. **Deglycosylation:** GlycINATOR/FucosEXO 16 Immobilized hydrolyzes the N-glycans on the Fc part of the IgG to the inner GlcNAc, and removes any core fucose present.
2. **Transglycosylation:** The engineered glycosynthase TransINATOR™ catalyzes the transglycosylation reaction between the oxazoline-reactive glycoform and the core GlcNAc.
3. **Purification:** The N-glycan-remodeled antibody is purified, and excess reagents are removed using affinity chromatography.

* 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.



1. Deglycosylation

GlycINATOR™ / FucosEXO® 16



2. Transglycosylation

TransINATOR™ + Oxazoline Glycoform

3. Purification

Figure 1. Schematic overview of the TransGLYCIT Remodeling Afucosylated technology.

Preparations

Important Information
Before you begin, briefly centrifuge the tubes.

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap and loosen the lid (do *not* remove the lid).
- Let the GlycINATOR/FucosEXO 16 Immobilized column equilibrate to room temperature before use.

Additional Materials Required

- Phosphate-buffered saline (PBS)[1]: 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4
- Antibody in 1 × PBS, pH 7.4, free of carrier proteins. 1 mg human IgG in a maximum volume of 100 µl
- Microcentrifuge tubes: 1.5-2 ml
- ddH_2O
- Elution buffer: 0.1 M glycine, pH 2.5
- Neutralization buffer: 1 M Tris, pH 8.0

1. PBS including 2.7 mM KCl can also be used.

Glycan Remodeling of Human IgG

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

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

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

Materials from kit:

- GlycINATOR/FucosEXO 16 Immobilized Microspin column

1.1 Let the GlycINATOR/FucosEXO 16 Immobilized column equilibrate to room temperature before use. Break off the bottom plastic cap of the GlycINATOR/FucosEXO 16 Immobilized column (save the cap) and slightly open the lid. Place the column in a microcentrifuge tube.

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

1.3 Discard the flow-through.

1.4 Place the column in the microcentrifuge tube.

1.5 Add 300 µl PBS on top of the resin. Centrifuge 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 1 mg of antibody) to 100 µl using PBS and add the antibody solution to the column.

1.9 Seal the column with the lid.

1.10 Fully suspend 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 60 min.

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

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

1.14 Seal the column with the bottom cap. Add 100 µl 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 new microcentrifuge tube. Loosen the lid.

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

1.18 Seal the column with 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 new 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: Oxazoline Glycoform Attachment

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

Materials from kit:

- TransINATOR
- 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 by end-over-end mixing at room temperature (22-24°C) for 60 min[2]. When 15 minutes of the incubation time remains, start the equilibration of the CaptureSelect Fc(ms) column as described in steps 3.1 to 3.11.

3. Removal of Excess Reagents

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

Materials from kit:

- CaptureSelect™ Fc(ms) Microspin column

Additional materials:

- PBS[1]: 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4
- Elution buffer: 0.1 M glycine, pH 2.5
- Neutralization buffer: 1 M Tris, pH 8.0

Equilibration

3.1 Break off the bottom plastic cap of the CaptureSelect Fc(ms) column (save the cap) and place the column in a microcentrifuge tube. Loosen the lid.

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

3.3 Discard the flow-through.

3.4 Re-insert the bottom cap at the bottom of the spin column. Place the column in the microcentrifuge tube.

3.5 Add 300 µl PBS on top of the resin.

3.6 Seal the column with the lid.

3.7 Fully suspend the resin, mix it by inversion.

3.8 Remove the bottom cap and loosen the lid.

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

3.10 Repeat steps 3.4-3.9 two times.

3.11 Seal the spin column with the bottom cap.

2. Most antibodies are fully transglycosylated after 60 min of incubation, however, optimization may be required for some antibodies. A longer incubation time is required for human IgG2.

Binding of the N-glycan-remodeled Antibody

3.12 Apply the sample (from step 2.4) on top of the resin and seal the column with the lid.

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

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

Wash

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

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

3.17 Seal the column with the bottom cap.

3.18 Add 300 µl PBS on top of the resin.

3.19 Seal the column with the lid.

3.20 Fully suspend the resin, mix it by inversion.

3.21 Remove the bottom cap, place the column in a microcentrifuge tube and loosen the lid.

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

3.23 Repeat steps 3.17-3.22 three times.

Elution of Purified N-glycan-remodeled Antibody

3.24 Prepare **four** microcentrifuge tubes each with 20 µl 1 M Tris pH 8.0.

3.25 Seal the spin column with the bottom cap.

3.26 Add 100 µl of 0.1 M glycine, pH 2.5, on top of the resin, and seal the column with the lid.

3.27 Fully suspend the resin, mix it by inversion.

3.28 Remove the bottom cap and place the column in a microcentrifuge tube containing Tris. Loosen the lid.

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

3.30 Repeat steps 3.25-3.29 three times.

3.31 Pool the eluted fractions and make sure the pH is neutralized.

3.32 The N-glycan-remodeled antibody can now be stored at +4–8°C for one month. For storage times longer than one month, storage at -20°C is recommended.

CONTENT AND STORAGE

TransGLYCIT Remodeling Afucosylated contains several components.

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

Do not freeze:

- GlycINATOR/FucosEXO 16 Immobilized Microspin column
- CaptureSelect™ Fc(ms) Microspin column

TransGLYCIT Remodeling Afucosylated is for R&D use only.

Table 1. Content and Storage Temperatures of TransGLYCIT Remodeling Afucosylated Components

Name	Amount	Store at
GlycINATOR/FucosEXO 16 Immobilized Microspin	1 piece	4 - 8 °C
TransINATOR	1 vial solid	(-25)-(-5)°C
CaptureSelect Fc(ms) Microspin	1 piece	4 - 8 °C
Oxazoline glycoform (G0, G1, G2, G2S2 or Man5)	1 vial solid	(-25)-(-5)°C

QUALITY CONTROL

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

YOU MIGHT ALSO BE INTERESTED IN

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

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

USA & Canada
Genovis Inc.
10919 Technology Place Suite C, San Diego, CA 92127, USA
Phone: 1-855-782-0084 (toll free)
Fax: 1-858-524-3006

EMEA & Asia
Genovis AB
Box 4, SE-244 21 Kävlinge, Sweden
Phone: +46 46 10 12 30
Fax: +46 46 12 80 20

support@genovis.com
www.genovis.com

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