TransGLYCIT™

Azide Activation, 100 µg

FOR RESEARCH
USE ONLY

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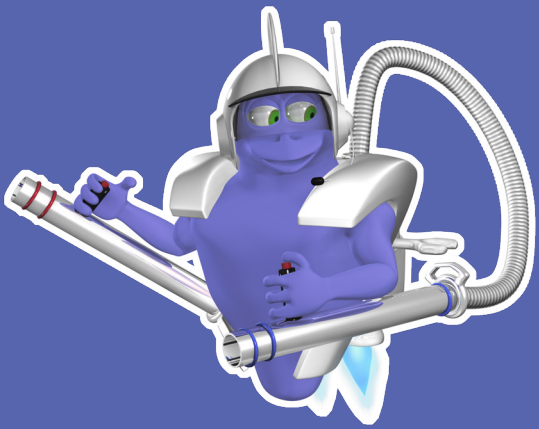
STORE CONTENT AT DIFFERENT TEMPERATURES
(See page 9)



SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCTS

TransGLYCIT™ Azide Activation, 100 µg
Azide activation of 100 µg human IgG1 or IgG4 (T1-AZ1-001)

TransGLYCIT™ Azide Activation hIgG2, 100 µg
Azide activation of 100 µg human IgG2 (T1-AZ2-001)

Last revised April 2022

Antibody Azide Activation using TransGLYCIT

1 Deglycosylation

- **Deglycosylation of IgG Fc** using Immobilized GlycINATOR® to expose the core GlcNAc.

2 Transglycosylation and Azide Activation

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

3 Purification

- **Purification** of the azide-activated antibody and removal of excess reagents.

PRODUCT DESCRIPTION

TransGLYCIT Azide Activation is a transglycosylation kit for site-specific azide activation of human IgG. Upon azide activation using TransGLYCIT, the antibody can be labeled with any click reagent of choice to a degree of labeling (DOL) of four labels per antibody (DOL=4). For a schematic overview of the TransGLYCIT Azide Activation workflow, see Fig. 1.

The IgG N-glycosylation site on the CH2 domain of the heavy chain is first trimmed to the core GlcNAc using GlycINATOR (EndoS2). GlycINATOR is an IgG-specific endoglycosidase hydrolyzing all IgG Fc glycoforms including high-mannose, hybrid-type and bisected N-glycans, and leaving the core GlcNAc attached to the antibody (1). This is followed by transglycosylation using the engineered

glycosynthase TransINATOR (EndoS2mut) which catalyzes the reaction between an azide-containing oxazoline glycoform and the core GlcNAc (2). Finally, the azide-activated antibody is purified and excess reagents are removed using a CaptureSelect™ affinity purification resin*.

With fast and robust enzymatic workflows, TransGLYCIT enables azide activation on the Fc N-glycan sites on human IgG, preparing antibodies for site-specific conjugation that preserves the affinity of the antigen-binding site.

* *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*

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.*

PRODUCT DESCRIPTION

The IgG Fc N-glycan azide activation is performed in the following steps:

1. **Deglycosylation:** Immobilized GlycINATOR hydrolyzes the N-glycans on the Fc-part of the IgG to the inner GlcNAc.

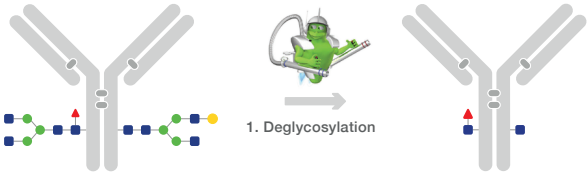
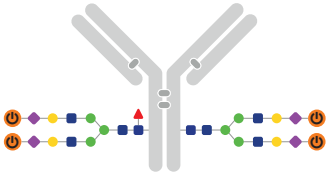


Figure 1. Schematic overview of azide activation of human IgG using the TransGLYCIT Azide Activation product to obtain antibody preparations ready for conjugation using click-chemistry.

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

2. Transglycosylation
followed by
3. Affinity Purification



Content and Storage

TransGLYCIT Azide Activation contains sufficient material to perform Fc azide activation of 100 µg human IgG.

TransGLYCIT Azide Activation is shipped cold. Upon arrival, the content should be stored according to storage temperatures listed in Table 1.

Before you begin, briefly centrifuge the tubes.

Do not freeze the Immobilized GlycINATOR or CaptureSelect columns!

TransGLYCIT Azide Activation is for R&D use only.

Table 1. Content and storage temperatures of TransGLYCIT Azide Activation components.

Name	Amount	Store at
Immobilized GlycINATOR, Microspin	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 Azide	T1-AZ1-001: 1 vial solid T1-AZ2-001: 2 vials solid	-25°C to -5°C

Quality Control

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

Equipment Required

- Centrifuge for microcentrifuge tubes
- Rotation mixer or similar

Additional Materials Required

- Antibody in 1× PBS, pH 7.4, free of carrier proteins. 100 µg 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 Azide Activation of 100 µg Human IgG

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

The antibody solution should be in PBS buffer pH 7.4, 100 µg in 100 µl.

Time required: 15 min hands-on, 30 min hands-off.

Materials from kit:

- Immobilized GlycINATOR Microspin column
- Let the Immobilized GlycINATOR 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 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 with the last centrifugation for 1½ min.

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

1.8 Adjust the antibody sample volume (containing 100 µg 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 by lightly tapping on the column.

1.11 Incubate the column with rotational mixing at room temperature for 30 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 antibody sample.

1.14 Attach the bottom cap. Add 20 µl of PBS and seal the column with the lid.

1.15 Lightly tap the column to mix the content.

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 antibody sample.

1.18 Perform step 1.14-1.17 two times

1.19 Pool the collected deglycosylated antibody material.

2 Transglycosylation: Attachment of Oxazoline glycoform Azide

Time required: 15 min hands-on, 75-210 min hands-off.

Materials from kit:

- TransINATOR
- Oxazoline glycoform Azide

2.1 Reconstitute TransINATOR in 20 µl ddH_2O.

2.2 Reconstitute the oxazoline glycoform azide (Ox-Az) with the pooled deglycosylated sample from step 1.19 according to Table 2 below.

Table 2. Protocol parameters for different IgG subclasses.

Product No	IgG Subclass	Reconstitution of Ox-Az
T1-AZ1-001	hIgG1	Reconstitute by adding all of the pooled sample into the Ox-Az vial
T1-AZ2-001	hIgG2	Reconstitute by adding all of the pooled sample into one Ox-Az vial. Transfer the content to the second vial of Ox-Az to reconstitute second vial of oxazoline glycoform azide.
T1-AZ1-001	hIgG4	Reconstitute by adding all of the pooled sample into the Ox-Az vial

2.3 Add TransINATOR to the sample from step 2.2 according to Table 2 below.

2.4 Close the vial with the lid. Mix by gentle inversion of the vial several times. Spin down the sample to the bottom of the vial.

2.5 Incubate protected from light at 22-24°C (room temperature) according to Table 2 below. During the last 15 min of incubation, start equilibration of the CaptureSelect Fc(ms) column as described in steps 3.1 to 3.7 on page 16.

TransINATOR (µl)	Incubation time (min)
2	75
4	210
2	90

3 Removal of Excess Reagents

Time required: 30 min hands-on, 25 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** with the last centrifugation for 1½ min.

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

Binding of the Azide-activated Antibody

3.8 Add the sample from step 2.5 and seal the column with the lid.

3.9 Fully suspend the resin by lightly tapping the column.

3.10 Incubate the column protected from light with rotational mixing at room temperature for 25 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 Azide-activated Antibody

3.16 Prepare **five** collection tubes with 4 µl of 1 M Tris pH 8.0.

3.17 Seal the column with the bottom cap.

3.18 Add 20 µl of 0.1 M Glycine pH 2.5 directly onto the resin and seal the column with the lid.

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

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

3.21 Perform step 3.17-3.20 **five times.**

3.22 Pool the collected eluates.

3.23 The azide-activated antibody can now be stored protected from light at +4–8°C for at least one month.

Related Products

TransGLYCIT™
Generation of 1 mg human IgG with the G0, G1, G2 or G2S2 glycoform.

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