

# **N-Linked Glycan Analysis**

## **USER GUIDE**

for use with:

**GlycanAssure™ APTS Kit**

**GlycanAssure™ Teal™ Kit**

**GlycanAssure™ Turquoise™ Kit**

Catalog Numbers A28676, A28677, A28678

Publication Number 100033998

Revision B

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# About this guide

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**IMPORTANT!** Before using this product, read and understand the information in the “Safety” appendix in this document.

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## Revision history

Revision	Date	Description
A	September 2015	New document for Early Access sites
B	November 2015	General updates for Product Release



# Product information

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## System overview

The GlycanAssure™ System is an integrated glycan analysis platform with three components:

1. **GlycanAssure™ Kits** (described in Pub. no. 100033998) – Provide reagents for sample preparation (to release, purify, and label glycans) and for capillary electrophoresis. Three kits are available; each includes a different dye for glycan labeling:
  - GlycanAssure™ APTS Kit (Cat. no. A28676)
  - GlycanAssure™ Teal™ Kit (Cat. no. A28677)
  - GlycanAssure™ Turquoise™ Kit (Cat. no. A28678)
2. **Applied Biosystems™ 3500/3500xL Genetic Analyzer for Protein Quality Analysis** (POP-7™ polymer, 50-cm capillary array) (described in Pub. no. 100036372) – Runs GlycanAssure™ Data Acquisition Software to collect data for samples prepared with the GlycanAssure™ Kits.
3. **GlycanAssure™ Data Analysis Software** (described in Pub. no. 100036373) – Processes and analyzes glycan data and includes data trending and profile matching features.

## Product description

The GlycanAssure™ Kits rapidly and effectively release and label the oligosaccharides from *N*-linked glycans, then separate the labeled oligosaccharides using capillary electrophoresis to create an oligosaccharide fingerprint or *glycan profile*. You can use the glycan profile to:

- Assess the complexity of the oligosaccharide mixture present on the protein.
- Perform relative quantitation for individual species on a mole percent basis by integrating the absorbance signal from the chromophore label.
- Tentatively identify individual oligosaccharide species.

## How the kits work

The GlycanAssure™ Kits provide reagents for sample preparation and for capillary electrophoresis.

1. The enzyme peptide-*N*-glycosidase F (PNGase F) is used to release glycans from the target glycoprotein.
2. The glycans are purified to prepare them for fluorescent labeling.
3. A fluorophore (APTS, Teal™, or Turquoise™ dye) is used to label the oligosaccharide.
4. For the APTS and Turquoise™ Kits, excess dye is removed.
5. During capillary electrophoresis, samples are loaded into a 3500/3500xL Genetic Analyzer with CE sample loading mixture. The fluorescently labeled glycans are separated with laser-induced fluorescence detection.

## About glycosylation

Glycosylation is one of the most important post-translational modifications (PTM) of eukaryotic cell proteins. Glycan-modified proteins (glycoproteins) are involved in a wide range of biological and physiological processes, such as:

- Cell-to-cell recognition and regulatory functions
- Cellular communication
- Cellular immunity, growth, and development
- Gene expression

Glycoprotein functions are often dependent on the structure of the glycan (carbohydrate) attached to them. Glycans are covalently attached to glycoproteins, primarily through two structural motifs:

- Attached to the amide group of an asparagine, referred to as *N-linked glycans*
- Attached to the hydroxyl group on serine or threonine, referred to as *O-linked glycans*

Both *N-linked* and *O-linked* glycans are investigated as biomarkers, in order to understand changes related to complex organelle development, and as part of therapeutic protein drug development with strong indication that efficacy and safety are affected by changes in glycosylation (Varki, 1993).

## Limitations

Certain carriers used in compound formulation can interfere with the enzymatic deglycosylation or fluorescent labeling steps. These matrix effects can be largely overcome by performing the supplemental wash protocol described in this User Guide. The supplemental wash protocol effectively eliminates interference in many cases.

When matrix effects cannot be overcome with the supplemental wash protocol, we recommend pre-processing samples by spin-column buffer exchange or molecular-sieve washing to remove formulation components.

## Recommended starter kit

If you are using our GlycanAssure™ Kits for the first time, we recommend the GlycanAssure™ APTS Kit as a starter kit. If you find that the glycans cannot be separated using the APTS Kit, try the Teal™ Kit, then the Turquoise™ Kit, to see if those work better.



## Kit contents and storage

### GlycanAssure™ APTS Kit (Cat. no. A28676)



Table 1 GlycanAssure™ Core Reagents

Box Cat. no.	Contents	Cap color	Item Cat. no.	Quantity	Volume	Storage
A28670	CE Loading Buffer	Red	100031742	1	7.2 mL	Room temp. (15°C to 30°C)
	Water (HPLC Grade)	Clear	100031739	1	49 mL	
	Wash Buffer	Clear	100031740	1	10 mL	
	Supplemental Wash Buffer	Blue	100032167	1	3.6 mL	
	Elution Buffer	Green	100031738	1	3.6 mL	

Table 2 GlycanAssure™ Beads

Box Cat. no.	Contents	Cap color	Item Cat. no.	Quantity	Volume	Storage
A28675	GlycanAssure™ Beads	White	100031737	1	4.5 mL	2°C to 8°C

Table 3 GlycanAssure™ APTS Labeling Reagents

Box Cat. no.	Contents <sup>[1]</sup>	Cap color	Item Cat. no.	Quantity	Volume	Storage
A28672	APTS Reagent Mix	Blue	100031736	1	230 µL	-25°C to -15°C
	Reductant	Purple	100031735	1	230 µL	
	PNGase F, Recombinant	White	A28404	1	60 µL	
	PNGase F Buffer	Yellow	100031741	1	500 µL	
	Landmark Red	Red	100031730	1	33 µL	

<sup>[1]</sup> Only the APTS Reagent Mix and Reductant are used for the dye labeling procedure. The other reagents are included in this box because of the storage conditions — all reagents must be stored at -25°C to -15°C.

## GlycanAssure™ Teal™ Kit (Cat. no. A28677)



Table 4 GlycanAssure™ Core Reagents

Box Cat. no.	Contents	Cap color	Item Cat. no.	Quantity	Volume	Storage
A28670	CE Loading Buffer	Red	100031742	1	7.2 mL	Room temp. (15°C to 30°C)
	Water (HPLC Grade)	Clear	100031739	1	49 mL	
	Wash Buffer	Clear	100031740	1	10 mL	
	Supplemental Wash Buffer	Blue	100032167	1	3.6 mL	
	Elution Buffer	Green	100031738	1	3.6 mL	

Table 5 GlycanAssure™ Beads

Box Cat. no.	Contents	Cap color	Item Cat. no.	Quantity	Volume	Storage
A28675	GlycanAssure™ Beads	White	100031737	1	4.5 mL	2°C to 8°C

Table 6 GlycanAssure™ Teal™ Labeling Reagents

Box Cat. no.	Contents <sup>[1]</sup>	Cap color	Item Cat. no.	Quantity	Volume	Storage
A28673	Teal™ dye	Dark green	100031734	1	60 µL	-25°C to -15°C
	Teal™ Labeling Buffer	Light green	100031733	1	800 µL	
	PNGase F, Recombinant	White	A28404	1	60 µL	
	PNGase F Buffer	Yellow	100031741	1	500 µL	
	Landmark Red	Red	100031730	1	33 µL	

<sup>[1]</sup> Only the Teal™ dye and Teal™ Labeling Buffer are used for the dye labeling procedure. The other reagents are included in this box because of the storage conditions — all reagents must be stored at -25°C to -15°C.

**GlycanAssure™  
Turquoise™ Kit  
(Cat. no. A28678)**



**Table 7** GlycanAssure™ Core Reagents

Box Cat. no.	Contents	Cap color	Item Cat. no.	Quantity	Volume	Storage
A28670	CE Loading Buffer	Red	100031742	1	7.2 mL	Room temp. (15°C to 30°C)
	Water (HPLC Grade)	Clear	100031739	1	49 mL	
	Wash Buffer	Clear	100031740	1	10 mL	
	Supplemental Wash Buffer	Blue	100032167	1	3.6 mL	
	Elution Buffer	Green	100031738	1	3.6 mL	

**Table 8** GlycanAssure™ Beads

Box Cat. no.	Contents	Cap color	Item Cat. no.	Quantity	Volume	Storage
A28675	GlycanAssure™ Beads	White	100031737	1	4.5 mL	2°C to 8°C

**Table 9** GlycanAssure™ Turquoise™ Labeling Reagents

Box Cat. no.	Contents <sup>[1]</sup>	Cap color	Item Cat. no.	Quantity	Volume	Storage
A28674	Turquoise™ dye	Light blue	100031732	1	60 µL	-25°C to -15°C
	Turquoise™ Labeling Buffer	Dark green	100031731	1	800 µL	
	PNGase F, Recombinant	White	A28404	1	60 µL	
	PNGase F Buffer	Yellow	100031741	1	500 µL	
	Landmark Red	Red	100031730	1	33 µL	

<sup>[1]</sup> Only the Turquoise™ dye and Turquoise™ Labeling Buffer are used for the dye labeling procedure. The other reagents are included in this box because of the storage conditions — all reagents must be stored at -25°C to -15°C.

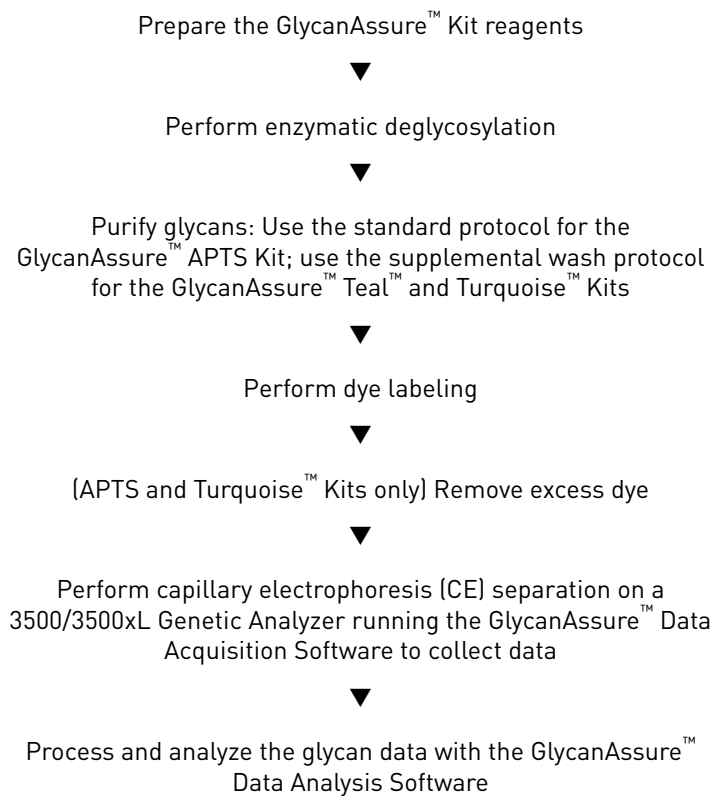
## Materials and equipment required but not included

Unless otherwise indicated, all materials are available through **thermofisher.com**, Fisher Scientific (**www.fisherscientific.com**), or other major laboratory supplier (MLS).

Item	Recommended cat. no.
<b>Enzymatic deglycosylation</b>	
One of the following thermal cyclers: <ul style="list-style-type: none"> <li>GeneAmp™ PCR System 9600</li> <li>GeneAmp™ PCR System 9700</li> <li>Veriti™ Thermal Cycler</li> </ul>	Contact Technical Support
Fisher Scientific™ Vortex Mixer, Digital	S96518
Eppendorf™ 5804R Series Centrifuge with Rotor Packages, or equivalent <b>Note:</b> Do not used a fixed-angle rotor.	Various cat. nos. at <b>www.fisherscientific.com</b>
Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL	022364111
Nunc™ 1.3-mL DeepWell™ Plates with Shared-Wall Technology	260252
Human IgG Isotype Control (10 mg; the concentration is 5 mg/mL)	02-7102
<b>Glycan purification</b>	
Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL	022364111
16-position Magnetic Stand	4457858
Magnetic Stand-96	AM10027
Nunc™ 1.3-mL DeepWell™ Plates with Shared-Wall Technology	260252
100% Acetonitrile (HPLC-grade)	A998-4
<b>Dye labeling</b>	
One of the following thermal cyclers: <ul style="list-style-type: none"> <li>GeneAmp™ PCR System 9600</li> <li>GeneAmp™ PCR System 9700</li> <li>Veriti™ Thermal Cycler</li> </ul>	Contact Technical Support
Fisher Scientific™ Vortex Mixer, Digital	S96518
Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL	022364111

Item	Recommended cat. no.
Aluminum block, or equivalent <b>Note:</b> We recommend the cooling chambers from Diversified Biotech.	Diversified Biotech, CHAM-1000 or CHAM-8000
<b>Excess dye removal</b>	
16-position Magnetic Stand	4457858
Magnetic Stand-96	AM10027
100% Acetonitrile (HPLC-grade)	A998-4
<b>Capillary electrophoresis</b>	
3500/3500xL Genetic Analyzer	Contact Technical Support
Septa for 3500/3500xL Genetic Analyzers, 96-well	4412614
Retainer and Base Set (Standard) for 3500/3500xL Genetic Analyzers, 96-well	4410228
3500 Genetic Analyzer 8-Capillary Array, 50-cm	4404685
3500xL Genetic Analyzer 24-Capillary Array, 50-cm	4404689
MicroAmp™ Optical 96-Well Reaction Plates	4306737
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Clear Adhesive Film	4306311
GeneScan™ 600 LIZ™ Dye Size Standard v2.0	4408399
<b>Other materials</b>	
Timer	MLS
Pipettes: <ul style="list-style-type: none"> <li>For tube methods – Pipetman™ 20-μL, 200-μL, and 1-mL pipettes, or equivalent</li> <li>For plate methods – 20-μL, 200-μL, and 1-mL multichannel pipettes, electronic multistep pipettes, single-channel multistep pipettes</li> </ul>	MLS
Aerosol-resistant pipette tips	MLS
For plate methods: reagent trough and funnel	MLS
Powder-free disposable gloves	MLS

## Workflow overview for all kits



# 2

## Prepare reagents

- Reagents required for each kit ..... 15
- Prepare the deglycosylation reagents ..... 15
- Prepare the GlycanAssure™ Beads ..... 16
- Prepare the purification and dye removal reagents ..... 17
- Prepare the dye labeling reagents ..... 18
- Determine the optimum protein sample input ..... 18
- Determine the number of reactions ..... 19

### Reagents required for each kit

Prepare the required reagents for your GlycanAssure™ Kit:

Reagents	Required?		
	GlycanAssure™ APTS Kit	GlycanAssure™ Teal™ Kit	GlycanAssure™ Turquoise™ Kit
Deglycosylation (glycan release) reagents	Yes	Yes	Yes
GlycanAssure™ Beads	Yes	Yes	Yes
Purification reagents	Yes	Yes	Yes
Dye labeling reagents	Yes	Yes	Yes
Dye removal reagents	Yes	No	Yes

### Prepare the deglycosylation reagents

#### PNGase F, Recombinant

1. Briefly spin to bring droplets on the cap and sides of the tube down into the tube.
2. Pipet up and down 10 times to thoroughly mix.

Proceed immediately to the enzymatic deglycosylation procedure for your kit.

**PNGase F Buffer**

1. Thaw.

**Note:** Thawed PNGase F Buffer can be stored for up to 1 month at 2°C to 8°C until use.

2. Briefly vortex, then spin-down.

**Prepare the GlycanAssure™ Beads**

1. Thoroughly resuspend the GlycanAssure™ Beads by vortexing or shaking the bottle. Be sure that the bead suspension is homogenous.

**Note:** If the beads sit undisturbed for >5 minutes, mix again as described above. Alternatively, swirl the bottle every 3 minutes to keep the beads in suspension.

2. Prepare a 2X and/or 1X bead suspension, as needed:

Bead suspension	Required for...	Kit
2X (page 16)	Glycan purification	All three kits
	APTS dye removal	GlycanAssure™ APTS Kit
1X (page 17)	Turquoise™ dye removal	GlycanAssure™ Turquoise™ Kit

**2X bead suspension**

The 2X bead suspension is used for:

- Glycan purification for all three kits (standard or supplemental wash protocols)
- Dye removal for the APTS Kit

This procedure makes 0.5 mL of 2X bead suspension, which is enough for ~50 reactions.

**Note:** For the Teal™ Kit or Turquoise™ Kit, this procedure makes enough 2X bead suspension for 50 glycan purification reactions. For the APTS Kit, this procedure makes enough 2X bead suspension for 25 purification reactions and 25 dye removal reactions.

1. Transfer 1.0 mL of the GlycanAssure™ Beads into a 1.5-mL tube, then briefly spin the tube to bring the contents to the bottom.
2. Place the tube on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Use a pipette to carefully remove the liquid, avoiding bead carryover, then remove the tubes from the magnetic stand.
3. Add 1 mL of HPLC-grade water. Wash by vortexing for ~10 seconds until thoroughly mixed, then briefly spin the tube to bring the contents to the bottom.
4. Place the tube back on the magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Use a pipette to carefully remove the liquid, avoiding bead carryover, then remove the tubes from the magnetic stand.



5. Repeat step 3 and step 4 one more time.
6. Add 450  $\mu\text{L}$  of HPLC-grade water to create a 2X bead suspension.

---

**STOPPING POINT** You can store the 2X bead suspension at 2°C to 8°C for up to 2 weeks before initial use.

---

### 1X bead suspension

The 1X bead suspension is used for dye removal for the Turquoise™ Kit. This procedure makes enough 1X bead suspension for ~50 reactions.

1. Transfer 1.0 mL of the GlycanAssure™ Beads into a 1.5-mL tube, then briefly spin the tube to bring the contents to the bottom.
2. Place the tube on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Use a pipette to carefully remove the liquid, avoiding bead carryover, then remove the tubes from the magnetic stand.
3. Add 1 mL of HPLC-grade water. Wash by quickly vortexing, then briefly spin the tube to bring the contents to the bottom.
4. Place the tube back on the magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Use a pipette to carefully remove the liquid, avoiding bead carryover, then remove the tubes from the magnetic stand.
5. Repeat step 3 and step 4 one more time.
6. Add 950  $\mu\text{L}$  of HPLC-grade water to create a 1X bead suspension.

---

**STOPPING POINT** You can store the 1X bead suspension at 2°C to 8°C for up to 2 weeks before initial use.

---

## Prepare the purification and dye removal reagents

### Wash Buffer

1. Add 90 mL of 100% acetonitrile (HPLC-grade) to the Wash Buffer bottle.
2. On the bottle, write the date and indicate that the acetonitrile has been added.

### Supplemental Wash Buffer

1. Add 2.4 mL of 100% acetonitrile (HPLC-grade) to the Supplemental Wash Buffer bottle.
2. On the bottle, write the date and indicate that the acetonitrile has been added.

### Elution Buffer

1. Add 2.4 mL of 100% acetonitrile (HPLC-grade) to the Elution Buffer bottle.
2. On the bottle, write the date and indicate that the acetonitrile has been added.

## Prepare the dye labeling reagents

### GlycanAssure™ APTS Kit

1. Thaw the APTS Reagent Mix and Reductant.
2. Thoroughly mix each reagent, then briefly spin to bring the contents to the bottom.
3. Keep the reagents on ice until use.

---

**IMPORTANT!** Do not keep the reagents on ice for >2 hours. Open and close the caps quickly, then place in the freezer immediately after use.

---

### GlycanAssure™ Teal™ Kit

1. Thaw the Teal™ dye.
2. Remove the Teal™ Labeling Buffer from the freezer just before use; the buffer does not require thawing.
3. Thoroughly mix each reagent, then briefly spin to bring the contents to the bottom.
4. Keep the reagents on ice until use.

---

**IMPORTANT!** Do not keep the reagents on ice for >2 hours. Open and close the caps quickly, then place in the freezer immediately after use.

---

### GlycanAssure™ Turquoise™ Kit

1. Thaw the Turquoise™ dye.
2. Remove the Turquoise™ Labeling Buffer from the freezer just before use; the buffer does not require thawing.
3. Thoroughly mix each reagent, then briefly spin to bring the contents to the bottom.
4. Keep the reagents on ice until use.

---

**IMPORTANT!** Do not keep the reagents on ice for >2 hours. Open and close the caps quickly, then place in the freezer immediately after use.

---

## Determine the optimum protein sample input

The optimum protein sample input is based on the glycan profile. You will need to experiment for the glycan that you are using. We recommend the following guidelines:

- Prepare the protein sample with a method that allows processing of 2 to 50 µg of input. We recommend 25 µg as an ideal input quantity.
- Use one or both of the following controls, processed with the same method that is used for the protein samples:
  - Negative control (no-protein control), using the sample carrier buffer
  - "Blank" control, using water

## Determine the number of reactions

Determine the number of reactions for your experiment. An example sample set is provided below:

- **Blank (water)** – To test for contamination of the target from the sample preparation reagents or from human error. We recommend HPLC-grade water or deionized water.
- **Negative control (formulation buffer)** – To test for contamination of the target. You can use the formulation buffer, without the protein sample.
- **(Optional) Positive control** – IgG sample with a known glycan profile. We recommend Human IgG Isotype Control (Cat. no. 02-7102).
- **Reference sample** – For comparison purposes; for example, an internal reference standard.
- **Unknown samples** – The target protein of interest.



# Methods for the GlycanAssure™ APTS Kit

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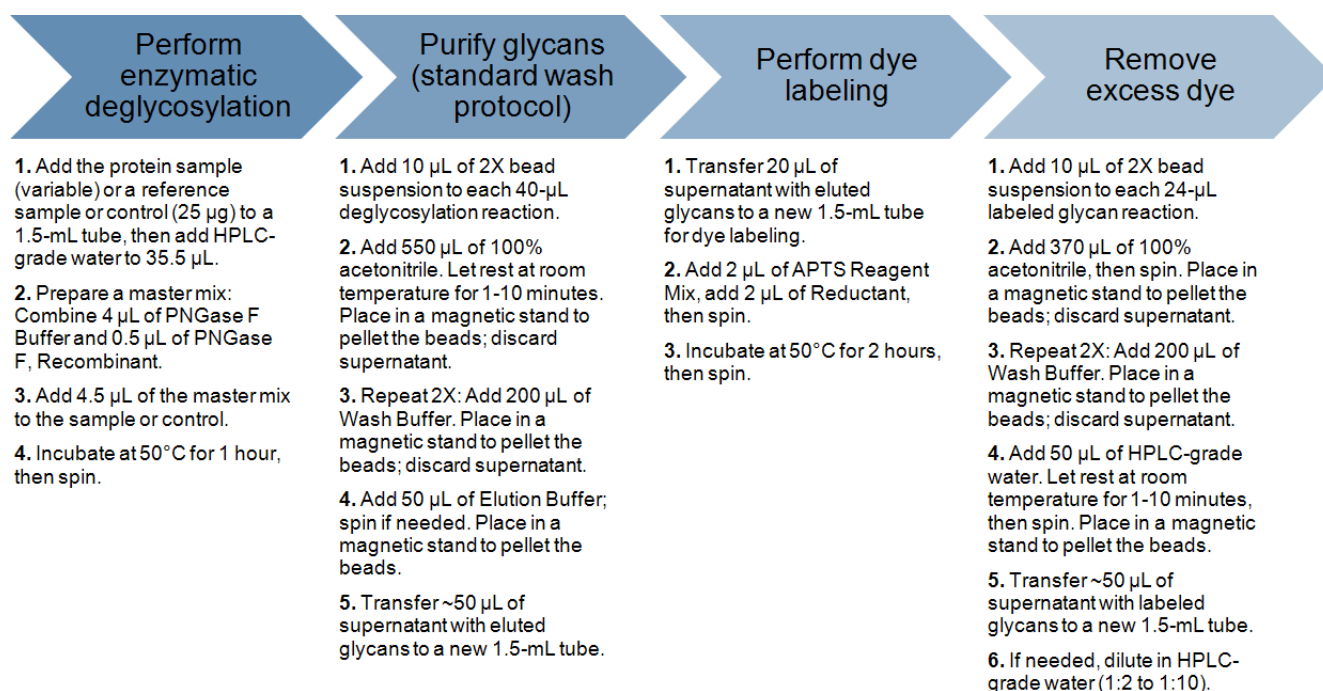
## Section 3.1 Methods for 1.5-mL microcentrifuge tubes

This section provides procedures for performing the kit methods in 1.5-mL microcentrifuge tubes:



If you are using plates, see page 26.

### GlycanAssure™ APTS Kit – workflow for 1.5-mL tubes



## About volumes

The volumes provided in each method are for one reaction. When calculating for your total number of reactions, be sure to include ~10% overage to account for the loss that occurs during reagent transfer.

## Perform enzymatic deglycosylation (1.5-mL tubes)

1. Determine your total number of reactions.
2. Prepare individual reactions in 1.5-mL tubes:

**Note:** We recommend Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL.

- a. Add the appropriate amount of sample or control to each 1.5-mL tube:

Component	Amount for 1 reaction
Protein sample	Variable <sup>[1]</sup>
OR	
Reference sample or control <sup>[2]</sup>	25 µg

<sup>[1]</sup> The optimal input amount depends on the glycoprotein of interest. The optimal input amount must be determined empirically by your laboratory. See “Determine the optimum protein sample input” on page 18.

<sup>[2]</sup> The control can be blank (water), negative (no-protein), or positive. For the positive control, we recommend Human IgG Isotype Control (Cat. no. 02-7102).

- b. Add sufficient HPLC-grade water to bring the total volume per reaction to 35.5 µL. (The final volume will be 40 µL after the master mix is prepared and added; see step 3 and step 4 below.)
3. Prepare a master mix:
  - a. Combine the following in a separate (fresh) 1.5-mL tube:

Component	Volume for 1 reaction <sup>[1]</sup>	Example: Volume for 96 reactions, including 10% overage
PNGase F Buffer	4.0 µL	422.4 µL
PNGase F, Recombinant	0.5 µL	52.8 µL

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

- b. Pipet up and down 6 times to mix.

---

**IMPORTANT!** Proceed immediately to step 4 and step 5. Alternatively, you can hold the master mix on ice, but do not hold for > 30 minutes.

---

4. Add 4.5 µL of the master mix to each 1.5-mL tube, pipet up and down to mix, then close the tube caps.

5. Place the tubes in a heater block or oven and incubate at 50°C for 1 hour.
6. Remove from incubation, then spin to bring the condensate to the bottom.

---

**STOPPING POINT** You can freeze the deglycosylation reactions at –25°C to –15°C overnight.

---

When ready, proceed to the purification method below.

## Purify glycans – standard wash protocol (1.5-mL tubes)

**Note:** As an alternative to the standard wash protocol for the GlycanAssure™ APTS Kit, you can purify glycans by following the supplemental wash protocol designed for the GlycanAssure™ Teal™ and Turquoise™ Kits. See “Purify glycans – supplemental wash protocol (1.5-mL tubes)” on page 35.

1. Add the 2X bead suspension (prepared per the procedure on page 16) to the PNGase F deglycosylation reactions, as follows:
  - a. Vortex the 2X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Add 10 µL of the resuspended 2X beads to each 40-µL deglycosylation reaction.
  - c. Pipet up and down to mix, then tap the tube to thoroughly suspend the beads.
2. Using a 1-mL pipette, add 550 µL of 100% acetonitrile to each tube, then immediately pipet up and down at least 6 times. After adding the acetonitrile to the last tube, let the tubes rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
3. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
4. Add 200 µL of Wash Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet.
5. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
6. Repeat step 4 and step 5 one more time.
7. Add 50 µL of Elution Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet. If needed, spin the tubes to bring the contents to the bottom.

- Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu$ L pipette, carefully transfer the supernatant containing eluted glycans to new 1.5-mL tubes (~50  $\mu$ L per tube), avoiding bead carryover.

**Note:** We recommend Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL.

---

**STOPPING POINT** The supernatant containing eluted glycans can be stored at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for up to 1 week. Alternatively, the eluted glycans can be dried and stored in the dark at room temperature for up to 1 month.

---

When ready, proceed to the dye labeling method below.

## Perform dye labeling (1.5-mL tubes)

- For each labeling reaction: Transfer 20  $\mu$ L of the supernatant containing eluted glycans to a new 1.5-mL microcentrifuge tube.

**Note:** We recommend Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL.

- Add the following to each reaction:

Component	Volume for 1 reaction
APTS Reagent Mix	2.0 $\mu$ L
Reductant	2.0 $\mu$ L

- Pipet up and down to mix, close the tube caps, then briefly spin to bring the solution to the bottom.
- Place all tubes in a heater block or oven and incubate at  $50^{\circ}\text{C}$  for 2 hours.

**IMPORTANT!** Secure the tube caps or apply weight to prevent the tubes from opening. We recommend placing a pre-heated ( $50^{\circ}\text{C}$ ) aluminum block (or equivalent) on the tubes. The heated block helps prevent condensation.

---

- Remove from incubation, then briefly spin to bring the condensate to the bottom.

---

**STOPPING POINT** The labeled glycans can be stored in the dark at room temperature overnight.

---

When ready, proceed to the excess dye removal method below.



## Remove excess dye (1.5-mL tubes)

1. Add the 2X bead suspension (prepared per the procedure on page 16) to the labeled glycans, as follows:
  - a. Vortex the 2X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Add 10  $\mu\text{L}$  of the resuspended 2X beads to each 24- $\mu\text{L}$  labeled glycan reaction.
  - c. Gently tap the tube to mix.
2. Using a 1-mL pipette, add 370  $\mu\text{L}$  of 100% acetonitrile to each tube, pipet up and down at least 4 times, then briefly spin.
3. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
4. Add 200  $\mu\text{L}$  of Wash Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet.
5. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
6. Repeat step 4 and step 5 one more time.
7. Add 50  $\mu\text{L}$  of HPLC-grade water to each tube to resuspend the pellet, then vortex or vigorously tap the tube to mix. After adding the water to the last tube, let the tubes rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
8. Briefly spin the tubes to bring the solution to the bottom.
9. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu\text{L}$  pipette, carefully transfer the supernatant containing labeled glycans to new tubes (~50  $\mu\text{L}$  per tube), avoiding bead carryover.
10. If off-scale peaks are seen in initial runs: Dilute as needed (1:2 to 1:10) in HPLC-grade water.

---

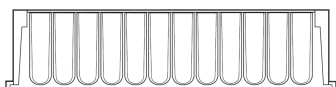
**STOPPING POINT** The labeled glycans can be stored in the dark at 2°C to 8°C for up to 1 day or at -25°C to -15°C for longer periods. However, we recommend proceeding immediately to “Perform CE separation” on page 56.

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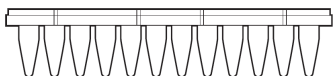
## Section 3.2 Methods for plates

This section provides procedures for performing the kit methods in:

- Deep-well plates

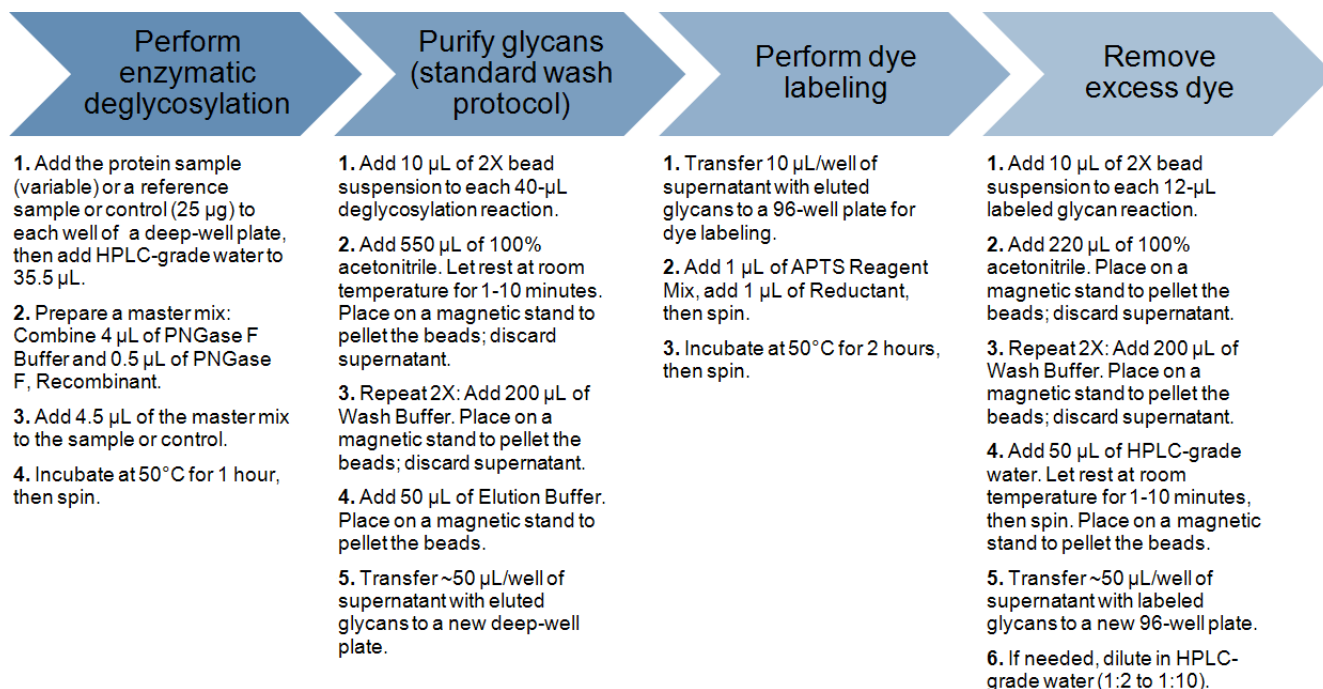


- 96-well plates



If you are using 1.5-mL microcentrifuge tubes, see page 21.

## GlycanAssure™ APTS Kit – workflow for deep-well and 96-well plates



## About volumes

The volumes provided in each method are for one reaction. When calculating for your total number of reactions, be sure to include ~10% overage to account for the loss that occurs during reagent transfer.

## Perform enzymatic deglycosylation (deep-well plates)

1. Determine your total number of reactions.
2. Prepare individual reactions in a deep-well plate:

**Note:** We recommend Nunc™ 1.3-mL DeepWell™ Plates with Shared-Wall Technology.

- a. Add the appropriate amount of sample or control to each well:

Component	Amount for 1 reaction
Protein sample	Variable <sup>[1]</sup>
OR	
Reference sample or control <sup>[2]</sup>	25 µg

<sup>[1]</sup> The optimal input amount depends on the glycoprotein of interest. The optimal input amount must be determined empirically by your laboratory. See "Determine the optimum protein sample input" on page 18

<sup>[2]</sup> The control can be blank (water), negative (no-protein), or positive. For the positive control, we recommend Human IgG Isotype Control (Cat. no. 02-7102).

- b. Add sufficient HPLC-grade water to bring the total volume per reaction to 35.5 µL. (The final volume will be 40 µL after the master mix is prepared and added; see step 3 and step 4 below.)

3. Prepare a master mix:

- a. Combine the following in a 1.5-mL tube:

Component	Volume for 1 reaction <sup>[1]</sup>	Example: Volume for 96 reactions, including 10% overage
PNGase F Buffer	4.0 µL	422.4 µL
PNGase F, Recombinant	0.5 µL	52.8 µL

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

- b. Pipet up and down 6 times to mix.

---

**IMPORTANT!** Proceed immediately to step 4 and step 5. Alternatively, you can hold the master mix on ice, but do not hold for > 30 minutes.

---

4. Add 4.5 µL of the master mix to each well, pulse-vortex on medium speed (300 to 500 rpm) to mix, then seal the plate.

5. Place the plate in a heater block, oven, or thermal cycler and incubate at 50°C for 1 hour.
6. Remove from incubation, then spin at 1000 rpm for 1 minute to bring the condensate to the bottom.

---

**STOPPING POINT** You can freeze the deglycosylation reactions at –25°C to –15°C overnight.

---

When ready, proceed to the purification method below.

## Purify glycans – standard wash protocol (deep-well plates)

**Note:** As an alternative to the standard wash protocol for the GlycanAssure™ APTS Kit, you can purify glycans by following the supplemental wash protocol designed for the GlycanAssure™ Teal™ and Turquoise™ Kits. See “Purify glycans – supplemental wash protocol (deep-well plates)” on page 40.

---

**IMPORTANT!** To avoid cross-contamination or sample loss, be careful when performing the vortex steps in this procedure. Vortexing at high speeds can cause the reagents to splash into neighboring wells.

---

**Note:** This procedure requires a multichannel pipette to discard supernatant to a solvent waste bottle. We recommend using a reagent trough and attaching a funnel to the waste bottle.

1. Add the 2X bead suspension (prepared per the procedure on page 16) to the PNGase F deglycosylation reactions, as follows:
  - a. Vortex the 2X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Using an electronic multistep pipette, add 10 µL of the resuspended 2X beads to each 40-µL deglycosylation reaction.
  - c. Vortex the plate on medium speed (300 to 500 rpm).
2. Using a 1-mL multichannel pipette, add 550 µL of 100% acetonitrile to each well, then immediately pipet up and down at least 6 times. After adding the acetonitrile to the last well, let the plate rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
3. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
4. Using a 200-µL multichannel pipette, add 200 µL of Wash Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.

5. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
6. Repeat step 4 and step 5 one more time.
7. Using a 200- $\mu$ L multichannel pipette, add 50  $\mu$ L of Elution Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.
8. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu$ L multichannel pipette, carefully transfer the supernatant containing eluted glycans to a new deep-well plate (~50  $\mu$ L per well), avoiding bead carryover.

**Note:** We recommend Nunc™ 1.3-mL DeepWell™ Plates with Shared-Wall Technology.

---

**STOPPING POINT** The supernatant containing eluted glycans can be stored at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for up to 1 week. Alternatively, the eluted glycans can be dried and stored in the dark at room temperature for up to 1 month.

---

When ready, proceed to the dye labeling method below.

## Perform dye labeling (96-well plates)

1. For each labeling reaction: Using a 20- $\mu$ L multichannel pipette, transfer 10  $\mu$ L of the supernatant containing eluted glycans to a new 96-well plate (10  $\mu$ L per well).

**Note:** We recommend MicroAmp™ Optical 96-Well Reaction Plates.

2. Using an electronic multistep pipette, add the following to each reaction:

Component	Volume for 1 reaction
APTS Reagent Mix	1.0 $\mu$ L
Reductant	1.0 $\mu$ L

3. Seal the plate:

If you are using...	Then seal with...
A thermal cycler in step 5	Clear adhesive film
An oven in step 5	Optical adhesive film

4. Vortex to mix well, then briefly spin to bring the solution to the bottom.

5. Place the plate in a thermal cycler or oven and incubate at 50°C for 2 hours.

---

**IMPORTANT!** If you are using an oven, apply weight to prevent the plate from opening. We recommend placing a pre-heated (50°C) aluminum block (or equivalent) on the plate to weigh-down the adhesive film. The heated block helps prevent condensation.

---

6. Remove from incubation, then spin at 1000 rpm for 1 minute to bring the condensate to the bottom.

---

**STOPPING POINT** The labeled glycans can be stored in the dark at room temperature overnight.

---

When ready, proceed to the excess dye removal method below.

## Remove excess dye (96-well plates)

**Note:** This procedure requires a multichannel pipette to discard supernatant to a solvent waste bottle. We recommend using a reagent trough and attaching a funnel to the waste bottle.

1. Add the 2X bead suspension (prepared per the procedure on page 16) to the labeled glycans, as follows:
  - a. Vortex the 2X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Using an electronic multistep pipette, add 10  $\mu\text{L}$  of the resuspended 2X beads to each 12- $\mu\text{L}$  labeled glycan reaction.
  - c. Using a 20- $\mu\text{L}$  multichannel pipette set at 10  $\mu\text{L}$ , pipet up and down at least 4 times to mix.
2. Using a 1-mL multichannel pipette, add 220  $\mu\text{L}$  of 100% acetonitrile to each well, then pipet up and down at least 4 times to mix.
3. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
4. Using a 200- $\mu\text{L}$  multichannel pipette, add 200  $\mu\text{L}$  of Wash Buffer (prepared per the procedure on page 17) to each well, then pipet up and down at least 4 times to resuspend the pellet.
5. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu\text{L}$  multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
6. Repeat step 4 and step 5 one more time.

7. Using a 200- $\mu$ L multichannel pipette, add 50  $\mu$ L of HPLC-grade water to each well to resuspend the pellet, then pipet up and down to mix. After adding the water to the last well, let the plate rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
8. Briefly spin the plate to bring the solution to the bottom.
9. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu$ L multichannel pipette, carefully transfer the supernatant containing labeled glycans to a new 96-well plate (~50  $\mu$ L per well), avoiding bead carryover.  
**Note:** We recommend MicroAmp™ Optical 96-Well Reaction Plates.
10. If off-scale peaks are seen in initial runs: Dilute as needed (1:2 to 1:10) in HPLC-grade water.

---

**STOPPING POINT** The labeled glycans can be stored in the dark at 2°C to 8°C for up to 1 day or at -25°C to -15°C for longer periods. However, we recommend proceeding immediately to “Perform CE separation” on page 56.

---



# Methods for GlycanAssure™ Teal™ Kit

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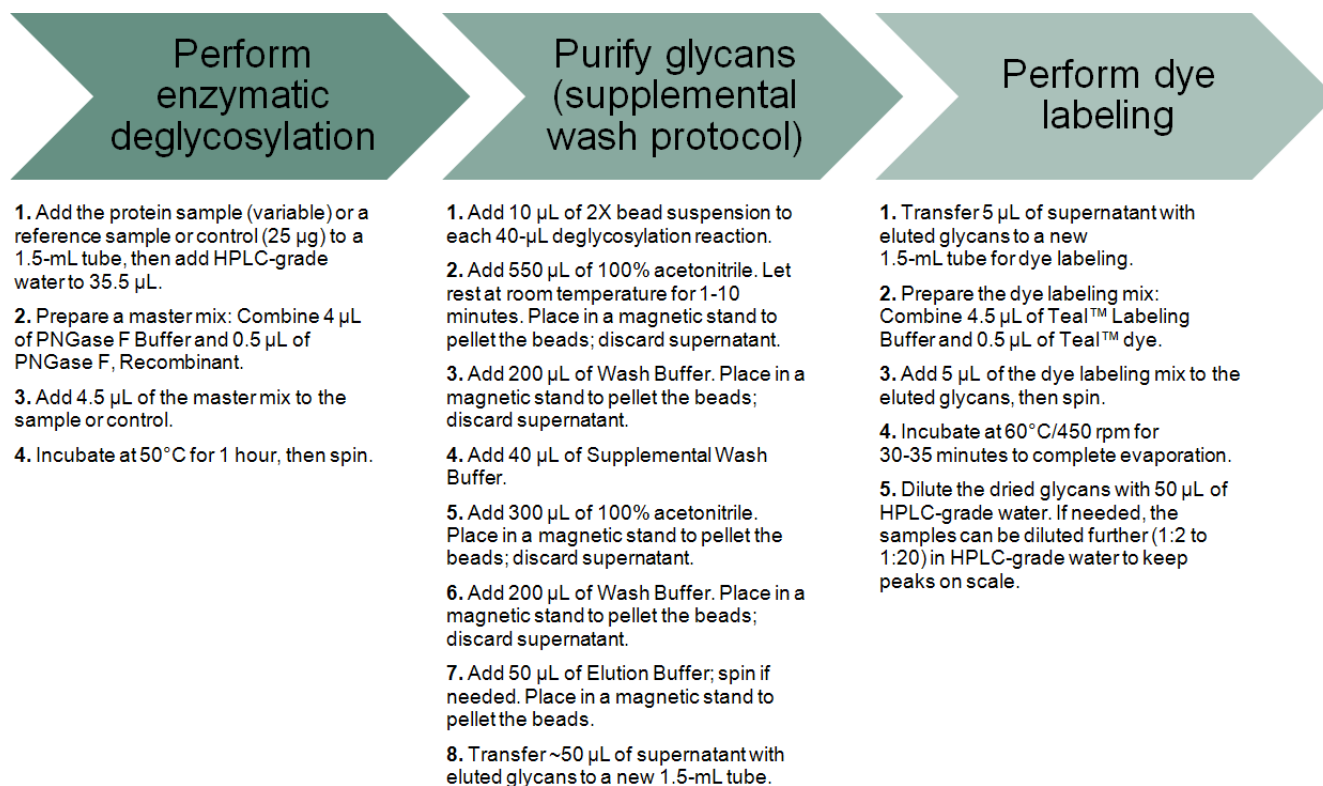
## Section 4.1 Methods for 1.5-mL microcentrifuge tubes

This section provides procedures for performing the kit methods in 1.5-mL microcentrifuge tubes:



If you are using plates, see page 38.

### GlycanAssure™ Teal™ Kit – workflow for 1.5-mL tubes



## About volumes

The volumes provided in each method are for one reaction. When calculating for your total number of reactions, be sure to include ~10% overage to account for the loss that occurs during reagent transfer.

## Perform enzymatic deglycosylation (1.5-mL tubes)

1. Determine your total number of reactions.

2. Prepare individual reactions in 1.5-mL tubes:

**Note:** We recommend Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL.

a. Add the appropriate amount of sample or control to each 1.5-mL tube:

Component	Amount for 1 reaction
Protein sample	Variable <sup>[1]</sup>
OR	
Reference sample or control <sup>[2]</sup>	25 µg

<sup>[1]</sup> The optimal input amount depends on the glycoprotein of interest. The optimal input amount must be determined empirically by your laboratory. See “Determine the optimum protein sample input” on page 18.

<sup>[2]</sup> The control can be blank (water), negative (no-protein), or positive. For the positive control, we recommend Human IgG Isotype Control (Cat. no. 02-7102).

b. Add sufficient HPLC-grade water to bring the total volume per reaction to 35.5 µL. (The final volume will be 40 µL after the master mix is prepared and added; see step 3 and step 4 below.)

3. Prepare a master mix:

a. Combine the following in a separate (fresh) 1.5-mL tube:

Component	Volume for 1 reaction <sup>[1]</sup>	Example: Volume for 96 reactions, including 10% overage
PNGase F Buffer	4.0 µL	422.4 µL
PNGase F, Recombinant	0.5 µL	52.8 µL

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

b. Pipet up and down 6 times to mix.

---

**IMPORTANT!** Proceed immediately to step 4 and step 5. Alternatively, you can hold the master mix on ice, but do not hold for > 30 minutes.

---

4. Add 4.5 µL of the master mix to each 1.5-mL tube, pipet up and down to mix, then close the tube caps.

5. Place the tubes in a heater block or oven and incubate at 50°C for 1 hour.
6. Remove from incubation, then spin to bring the condensate to the bottom.

---

**STOPPING POINT** You can freeze the deglycosylation reactions at –25°C to –15°C overnight.

---

When ready, proceed to the purification method below.

## Purify glycans – supplemental wash protocol (1.5-mL tubes)

1. Add the 2X bead suspension (prepared per the procedure on page 16) to the PNGase F deglycosylation reactions, as follows:
  - a. Vortex the 2X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Add 10 µL of the resuspended 2X beads to each 40-µL deglycosylation reaction.
  - c. Pipet up and down to mix, then tap the tube to thoroughly suspend the beads.
2. Using a 1-mL pipette, add 550 µL of 100% acetonitrile to each tube, then immediately pipet up and down at least 6 times. After adding the acetonitrile to the last tube, let the tubes rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
3. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
4. Add 200 µL of Wash Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet.
5. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
6. Add 40 µL of Supplemental Wash Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet.
7. Using a 1-mL pipette, add 300 µL of 100% acetonitrile to each tube, then immediately pipet up and down at least 6 times.

8. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
9. Add 200  $\mu\text{L}$  of Wash Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet.
10. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
11. Add 50  $\mu\text{L}$  of Elution Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet. If needed, spin the tubes to bring the solution to the bottom.
12. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu\text{L}$  pipette, carefully transfer the supernatant containing eluted glycans to new 1.5-mL tubes (~50  $\mu\text{L}$  per tube), avoiding bead carryover.

**Note:** We recommend Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL.

---

**STOPPING POINT** The supernatant containing eluted glycans can be stored at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for up to 1 week. Alternatively, the eluted glycans can be dried and stored in the dark at room temperature for up to 1 month.

---

When ready, proceed to the dye labeling method below.

## Perform dye labeling (1.5-mL tubes)

1. For each labeling reaction: Transfer 5  $\mu\text{L}$  of the supernatant containing eluted glycans to a new 1.5-mL microcentrifuge tube.  
**Note:** We recommend Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL.
2. Freshly prepare the dye labeling mix: Combine the components listed in the table below, then mix well.

Component	Volume for 1 reaction <sup>[1]</sup>
Teal™ Labeling Buffer	4.5 $\mu\text{L}$
Teal™ dye	0.5 $\mu\text{L}$

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

3. Add 5  $\mu$ L of the dye labeling mix to each tube of eluted glycans, then pipet up and down at least 6 times to mix.
4. Close the tube caps, then briefly spin to bring the solution to the bottom.
5. Working under a fume hood or on a well-ventilated workbench, place all tubes in a shaking heater block set to 60°C, **carefully open all of the tube caps**, then incubate as follows:
  - Incubate at 450 rpm for 30 minutes.
  - After 30 minutes, check the reactions. If needed, incubate for an additional 5 minutes to complete evaporation/dryness.

---

**STOPPING POINT** You can proceed immediately to the dilution step below, or store the dried samples. The dried samples are extremely stable and can be stored in the dark at room temperature for up to 30 days or at –25°C to –15°C for longer periods.

---

6. Dilute each tube of dried glycans with 50  $\mu$ L of HPLC-grade water to prepare for capillary electrophoresis.

**Note:** If needed, the samples can be diluted further (1:2 to 1:20) in HPLC-grade water to keep peaks on scale.

---

**STOPPING POINT** You can store the diluted glycans at –25°C to –15°C for up to 30 days.

---

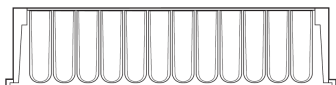
When ready, proceed to “Perform CE separation” on page 56.

**Note:** The Teal™ Kit does not require excess dye removal.

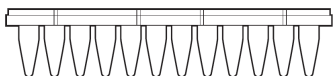
## Section 4.2 Methods for plates

This section provides procedures for performing the kit methods in:

- Deep-well plates

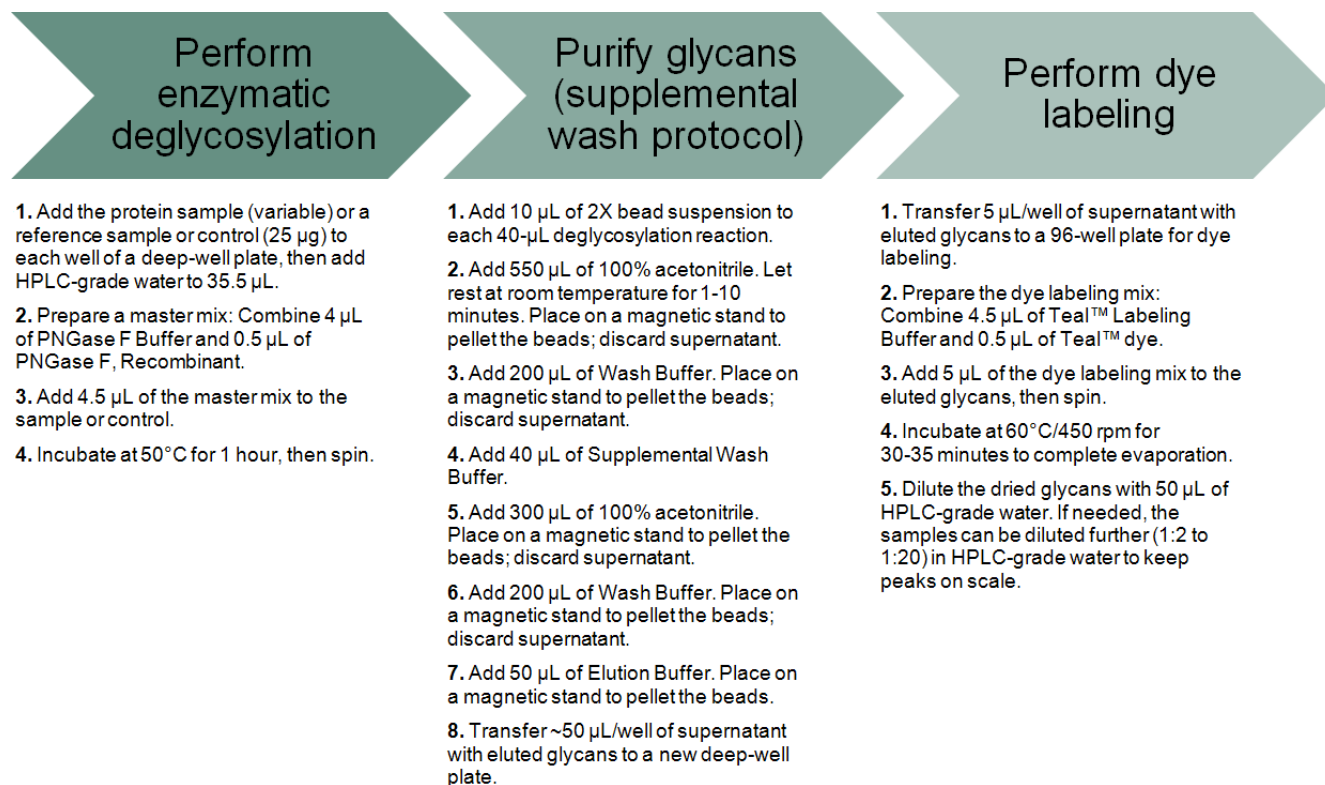


- 96-well plates



If you are using 1.5-mL microcentrifuge tubes, see page 33.

### GlycanAssure™ Teal™ Kit – workflow for deep-well and 96-well plates



## About volumes

The volumes provided in each method are for one reaction. When calculating for your total number of reactions, be sure to include ~10% overage to account for the loss that occurs during reagent transfer.

## Perform enzymatic deglycosylation (deep-well plates)

1. Determine your total number of reactions.
2. Prepare individual reactions in a deep-well plate:

**Note:** We recommend Nunc™ 1.3-mL DeepWell™ Plates with Shared-Wall Technology.

- a. Add the appropriate amount of sample or control to each well:

Component	Amount for 1 reaction
Protein sample	Variable <sup>[1]</sup>
OR	
Reference sample or control <sup>[2]</sup>	25 µg

<sup>[1]</sup> The optimal input amount depends on the glycoprotein of interest. The optimal input amount must be determined empirically by your laboratory. See "Determine the optimum protein sample input" on page 18

<sup>[2]</sup> The control can be blank (water), negative (no-protein), or positive. For the positive control, we recommend Human IgG Isotype Control (Cat. no. 02-7102).

- b. Add sufficient HPLC-grade water to bring the total volume per reaction to 35.5 µL. (The final volume will be 40 µL after the master mix is prepared and added; see step 3 and step 4 below.)
3. Prepare a master mix:
    - a. Combine the following in a 1.5-mL tube:

Component	Volume for 1 reaction <sup>[1]</sup>	Example: Volume for 96 reactions, including 10% overage
PNGase F Buffer	4.0 µL	422.4 µL
PNGase F, Recombinant	0.5 µL	52.8 µL

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

- b. Pipet up and down 6 times to mix.

---

**IMPORTANT!** Proceed immediately to step 4 and step 5. Alternatively, you can hold the master mix on ice, but do not hold for > 30 minutes.

---

4. Add 4.5 µL of the master mix to each well, pulse-vortex on medium speed (300 to 500 rpm) to mix, then seal the plate.

5. Place the plate in a heater block, oven, or thermal cycler and incubate at 50°C for 1 hour.
6. Remove from incubation, then spin at 1000 rpm for 1 minute to bring the condensate to the bottom.

---

**STOPPING POINT** You can freeze the deglycosylation reactions at –25°C to –15°C overnight.

---

When ready, proceed to the purification method below.

## Purify glycans – supplemental wash protocol (deep-well plates)

---

**IMPORTANT!** To avoid cross-contamination or sample loss, be careful when performing the vortex steps in this procedure. Vortexing at high speeds can cause the reagents to splash into neighboring wells.

---

**Note:** This procedure requires a multichannel pipette to discard supernatant to a solvent waste bottle. We recommend using a reagent trough and attaching a funnel to the waste bottle.

1. Add the 2X bead suspension (prepared per the procedure on page 16) to the PNGase F deglycosylation reactions, as follows:
  - a. Vortex the 2X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Using an electronic multistep pipette, add 10 µL of the resuspended 2X beads to each 40-µL deglycosylation reaction.
  - c. Vortex the plate on medium speed (300 to 500 rpm).
2. Using a 1-mL multichannel pipette, add 550 µL of 100% acetonitrile to each well, then immediately pipet up and down at least 6 times. After adding the acetonitrile to the last well, let the plate rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
3. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
4. Using a 200-µL multichannel pipette, add 200 µL of Wash Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.
5. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.



6. Using a 200- $\mu$ L multichannel pipette, add 40  $\mu$ L of Supplemental Wash Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.
7. Using a 1-mL multichannel pipette, add 300  $\mu$ L of 100% acetonitrile to each well, then immediately pipet up and down at least 6 times.
8. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
9. Using a 200- $\mu$ L multichannel pipette, add 200  $\mu$ L of Wash Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.
10. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
11. Using a 200- $\mu$ L multichannel pipette, add 50  $\mu$ L of Elution Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.
12. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu$ L multichannel pipette, carefully transfer the supernatant containing eluted glycans to a new deep-well plate (~50  $\mu$ L per well), avoiding bead carryover.

**Note:** We recommend Nunc™ 1.3-mL DeepWell™ Plates with Shared-Wall Technology.

---

**STOPPING POINT** The supernatant containing eluted glycans can be stored at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for up to 1 week. Alternatively, the eluted glycans can be dried and stored in the dark at room temperature for up to 1 month.

---

When ready, proceed to the dye labeling method below.

## Perform dye labeling (96-well plates)

1. For each labeling reaction: Using a 20- $\mu$ L multichannel pipette, transfer 5  $\mu$ L of the supernatant containing eluted glycans to a new 96-well plate (5  $\mu$ L per well).

**Note:** We recommend MicroAmp™ Optical 96-Well Reaction Plates.

2. Freshly prepare the dye labeling mix: Combine the components listed in the table below, then mix well.

Component	Volume for 1 reaction <sup>[1]</sup>
Teal™ Labeling Buffer	4.5 $\mu$ L
Teal™ dye	0.5 $\mu$ L

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

3. Using a single-channel multistep pipette, add 5  $\mu$ L of the dye labeling mix to each well of eluted glycans, then use a 20- $\mu$ L multichannel pipette to pipet up and down at least 6 times to mix.
4. Seal the plate, then briefly spin to bring the solution to the bottom.
5. Working under a fume hood or on a well-ventilated workbench, place the plate in a shaking heater block or thermal cycler set to 60°C, **carefully open the plate**, then incubate as follows:
  - Incubate at 450 rpm for 30 minutes.
  - After 30 minutes, check the reactions. If needed, incubate for an additional 5 minutes to complete evaporation/dryness.

---

**STOPPING POINT** You can proceed immediately to the dilution step below, or store the dried samples. The dried samples are extremely stable and can be stored in the dark at room temperature for up to 30 days or at -25°C to -15°C for longer periods.

---

6. Dilute each well of dried glycans with 50  $\mu$ L of HPLC-grade water to prepare for capillary electrophoresis.

**Note:** If needed, the samples can be diluted further (1:2 to 1:20) in HPLC-grade water to keep peaks on scale.

---

**STOPPING POINT** You can store the diluted glycans at -25°C to -15°C for up to 30 days.

---

When ready, proceed to “Perform CE separation” on page 56.

**Note:** The Teal™ Kit does not require excess dye removal.



# Methods for the GlycanAssure™ Turquoise™ Kit

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## Section 5.1 Methods for 1.5-mL microcentrifuge tubes

This section provides procedures for performing the kit methods in 1.5-mL microcentrifuge tubes:



If you are using plates, see page 50.

### GlycanAssure™ Turquoise™ Kit – workflow for 1.5-mL tubes

Perform enzymatic deglycosylation	Purify glycans (supplemental wash protocol)	Perform dye labeling	Remove excess dye
<ol style="list-style-type: none"> <li>1. Add the protein sample (variable) or a reference sample or control (25 µg) to a 1.5-mL tube, then add HPLC-grade water to 35.5 µL.</li> <li>2. Prepare a master mix: Combine 4 µL of PNGase F Buffer and 0.5 µL of PNGase F, Recombinant.</li> <li>3. Add 4.5 µL of the master mix to the sample or control.</li> <li>4. Incubate at 50°C for 1 hour, then spin.</li> </ol>	<ol style="list-style-type: none"> <li>1. Add 10 µL of 2X bead suspension to each 40-µL deglycosylation reaction.</li> <li>2. Add 550 µL of 100% acetonitrile. Let rest at room temp. for 1-10 minutes. Place in a magnetic stand to pellet the beads; discard supernatant.</li> <li>3. Add 200 µL of Wash Buffer. Place in a magnetic stand to pellet the beads; discard supernatant.</li> <li>4. Add 40 µL of Supplemental Wash Buffer.</li> <li>5. Add 300 µL of 100% acetonitrile. Place in a magnetic stand to pellet the beads; discard supernatant.</li> <li>6. Add 200 µL of Wash Buffer. Place in a magnetic stand to pellet the beads; discard supernatant.</li> <li>7. Add 50 µL of Elution Buffer; spin if needed. Place in a magnetic stand to pellet the beads.</li> <li>8. Transfer ~50 µL of supernatant with eluted glycans to a new 1.5-mL tube.</li> </ol>	<ol style="list-style-type: none"> <li>1. Transfer 5 µL of supernatant with eluted glycans to a new 1.5-mL tube for dye labeling.</li> <li>2. Prepare the dye labeling mix: Combine 4.5 µL of Turquoise™ Labeling Buffer and 0.5 µL of Turquoise™ dye.</li> <li>3. Add 5 µL of the dye labeling mix to the eluted glycans, then spin.</li> <li>4. Incubate at 60°C/450 rpm for 30-35 minutes to complete evaporation.</li> </ol>	<ol style="list-style-type: none"> <li>1. Add 20 µL of 1X bead suspension to each labeled and dried glycan reaction.</li> <li>2. Add 220 µL of 100% acetonitrile, then spin. Place in a magnetic stand to pellet the beads; discard supernatant.</li> <li>3. Repeat 2X: Add 200 µL of Wash Buffer. Place in a magnetic stand to pellet the beads; discard supernatant.</li> <li>4. Add 50 µL of HPLC-grade water. Let rest at room temperature for 1-10 minutes, then spin. Place in a magnetic stand to pellet the beads.</li> <li>5. Transfer ~50 µL of supernatant with labeled glycans to a new 1.5-mL tube.</li> <li>6. If needed, dilute in HPLC-grade water (1:2 to 1:20).</li> </ol>

## About volumes

The volumes provided in each method are for one reaction. When calculating for your total number of reactions, be sure to include ~10% overage to account for the loss that occurs during reagent transfer.

### Perform enzymatic deglycosylation (1.5-mL tubes)

1. Determine your total number of reactions.
2. Prepare individual reactions in 1.5-mL tubes:

**Note:** We recommend Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL.

- a. Add the appropriate amount of sample or control to each 1.5-mL tube:

Component	Amount for 1 reaction
Protein sample	Variable <sup>[1]</sup>
OR	
Reference sample or control <sup>[2]</sup>	25 µg

<sup>[1]</sup> The optimal input amount depends on the glycoprotein of interest. The optimal input amount must be determined empirically by your laboratory. See “Determine the optimum protein sample input” on page 18.

<sup>[2]</sup> The control can be blank (water), negative (no-protein), or positive. For the positive control, we recommend Human IgG Isotype Control (Cat. no. 02-7102).

- b. Add sufficient HPLC-grade water to bring the total volume per reaction to 35.5 µL. (The final volume will be 40 µL after the master mix is prepared and added; see step 3 and step 4 below.)
3. Prepare a master mix:
    - a. Combine the following in a separate (fresh) 1.5-mL tube:

Component	Volume for 1 reaction <sup>[1]</sup>	Example: Volume for 96 reactions, including 10% overage
PNGase F Buffer	4.0 µL	422.4 µL
PNGase F, Recombinant	0.5 µL	52.8 µL

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

- b. Pipet up and down 6 times to mix.

---

**IMPORTANT!** Proceed immediately to step 4 and step 5. Alternatively, you can hold the master mix on ice, but do not hold for > 30 minutes.

---

4. Add 4.5 µL of the master mix to each 1.5-mL tube, pipet up and down to mix, then close the tube caps.

5. Place the tubes in a heater block or oven and incubate at 50°C for 1 hour.
6. Remove from incubation, then spin to bring the condensate to the bottom.

---

**STOPPING POINT** You can freeze the deglycosylation reactions at –25°C to –15°C overnight.

---

When ready, proceed to the purification method below.

## Purify glycans – supplemental wash protocol (1.5-mL tubes)

1. Add the 2X bead suspension (prepared per the procedure on page 16) to the PNGase F deglycosylation reactions, as follows:
  - a. Vortex the 2X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Add 10 µL of the resuspended 2X beads to each 40-µL deglycosylation reaction.
  - c. Pipet up and down to mix, then tap the tube to thoroughly suspend the beads.
2. Using a 1-mL pipette, add 550 µL of 100% acetonitrile to each tube, then immediately pipet up and down at least 6 times. After adding the acetonitrile to the last tube, let the tubes rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
3. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
4. Add 200 µL of Wash Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet.
5. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
6. Add 40 µL of Supplemental Wash Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet.
7. Using a 1-mL pipette, add 300 µL of 100% acetonitrile to each tube, then immediately pipet up and down at least 6 times.

8. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
9. Add 200  $\mu$ L of Wash Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet.
10. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
11. Add 50  $\mu$ L of Elution Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet. If needed, spin the tubes to bring the solution to the bottom.
12. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu$ L pipette, carefully transfer the supernatant containing eluted glycans to new 1.5-mL tubes (~50  $\mu$ L per tube), avoiding bead carryover.

**Note:** We recommend Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL.

---

**STOPPING POINT** The supernatant containing eluted glycans can be stored at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for up to 1 week. Alternatively, the eluted glycans can be dried and stored in the dark at room temperature for up to 1 month.

---

When ready, proceed to the dye labeling method below.

## Perform dye labeling (1.5-mL tubes)

1. For each labeling reaction: Transfer 5  $\mu\text{L}$  of the supernatant containing eluted glycans to a new 1.5-mL microcentrifuge tube.

**Note:** We recommend Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL.

2. Freshly prepare the dye labeling mix: Combine the components listed in the table below, then mix well.

Component	Volume for 1 reaction <sup>[1]</sup>
Turquoise™ Labeling Buffer	4.5 $\mu\text{L}$
Turquoise™ dye	0.5 $\mu\text{L}$

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

3. Add 5  $\mu\text{L}$  of the dye labeling mix to each tube of eluted glycans, then pipet up and down at least 6 times to mix.
4. Close the tube caps, then briefly spin to bring the solution to the bottom.
5. Working under a fume hood or on a well-ventilated workbench, place all tubes in a shaking heater block set to 60°C, **carefully open all of the tube caps**, then incubate as follows:
  - Incubate at 450 rpm for 30 minutes.
  - After 30 minutes, check the reactions. If needed, incubate for an additional 5 minutes to complete evaporation/dryness.

---

**STOPPING POINT** The dried samples are extremely stable and can be stored in the dark at room temperature for up to 30 days or at -25°C to -15°C for longer periods.

---

When ready, proceed to the excess dye removal method below.

## Remove excess dye (1.5-mL tubes)

1. Add the 1X bead suspension (prepared per the procedure on page 17) to the labeled glycans, as follows:
  - a. Vortex the 1X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Add 20  $\mu\text{L}$  of the resuspended 1X beads to each labeled and dried glycan reaction.
  - c. Gently tap the tube to mix.
2. Using a 1-mL pipette, add 220  $\mu\text{L}$  of 100% acetonitrile to each tube, pipet up and down at least 4 times, then briefly spin.



3. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
4. Add 200  $\mu\text{L}$  of Wash Buffer (prepared per the procedure on page 17) to each tube, then pipet up and down to resuspend the pellet.
5. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the tubes from the magnetic stand.
6. Repeat step 4 and step 5 one more time.
7. Add 50  $\mu\text{L}$  of HPLC-grade water to each tube to resuspend the pellet, then vortex or vigorously tap the tube to mix. After adding the water to the last tube, let the tubes rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
8. Briefly spin the tubes to bring the solution to the bottom.
9. Place the tubes in a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu\text{L}$  pipette, carefully transfer the supernatant containing labeled glycans to new tubes (~50  $\mu\text{L}$  per tube), avoiding bead carryover.
10. If off-scale peaks are seen in initial runs: Dilute as needed (1:2 to 1:20) in HPLC-grade water.

---

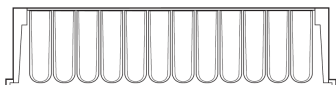
**STOPPING POINT** The labeled glycans can be stored in the dark at 2°C to 8°C for up to 1 day or at -25°C to -15°C for longer periods. However, we recommend proceeding immediately to “Perform CE separation” on page 56.

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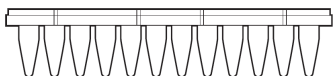
## Section 5.2 Methods for plates

This section provides procedures for performing the kit methods in:

- Deep-well plates

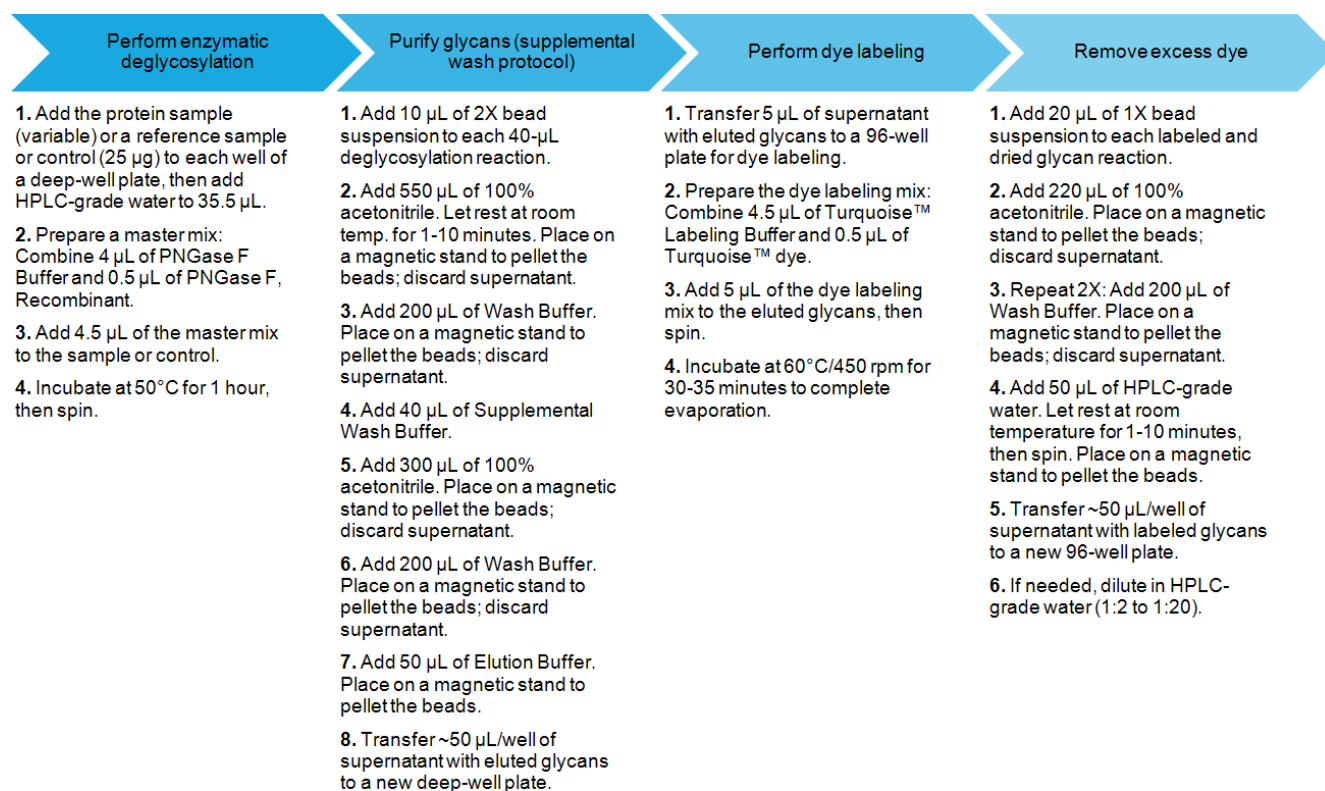


- 96-well plates



If you are using 1.5-mL microcentrifuge tubes, see page 44.

## GlycanAssure™ Turquoise™ Kit – workflow for deep-well and 96-well plates



## About volumes

The volumes provided in each method are for one reaction. When calculating for your total number of reactions, be sure to include ~10% overage to account for the loss that occurs during reagent transfer.

## Perform enzymatic deglycosylation (deep-well plates)

1. Determine your total number of reactions.
2. Prepare individual reactions in a deep-well plate:

**Note:** We recommend Nunc™ 1.3-mL DeepWell™ Plates with Shared-Wall Technology.

- a. Add the appropriate amount of sample or control to each well:

Component	Amount for 1 reaction
Protein sample	Variable <sup>[1]</sup>
OR	
Reference sample or control <sup>[2]</sup>	25 µg

<sup>[1]</sup> The optimal input amount depends on the glycoprotein of interest. The optimal input amount must be determined empirically by your laboratory. See "Determine the optimum protein sample input" on page 18

<sup>[2]</sup> The control can be blank (water), negative (no-protein), or positive. For the positive control, we recommend Human IgG Isotype Control (Cat. no. 02-7102).

- b. Add sufficient HPLC-grade water to bring the total volume per reaction to 35.5 µL. (The final volume will be 40 µL after the master mix is prepared and added; see step 3 and step 4 below.)
3. Prepare a master mix:
    - a. Combine the following in a 1.5-mL tube:

Component	Volume for 1 reaction <sup>[1]</sup>	Example: Volume for 96 reactions, including 10% overage
PNGase F Buffer	4.0 µL	422.4 µL
PNGase F, Recombinant	0.5 µL	52.8 µL

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

- b. Pipet up and down 6 times to mix.

---

**IMPORTANT!** Proceed immediately to step 4 and step 5. Alternatively, you can hold the master mix on ice, but do not hold for > 30 minutes.

---

4. Add 4.5 µL of the master mix to each well, pulse-vortex on medium speed (300 to 500 rpm) to mix, then seal the plate.

5. Place the plate in a heater block, oven, or thermal cycler and incubate at 50°C for 1 hour.
6. Remove from incubation, then spin at 1000 rpm for 1 minute to bring the condensate to the bottom.

---

**STOPPING POINT** You can freeze the deglycosylation reactions at –25°C to –15°C overnight.

---

When ready, proceed to the purification method below.

## Purify glycans – supplemental wash protocol (deep-well plates)

---

**IMPORTANT!** To avoid cross-contamination or sample loss, be careful when performing the vortex steps in this procedure. Vortexing at high speeds can cause the reagents to splash into neighboring wells.

---

**Note:** This procedure requires a multichannel pipette to discard supernatant to a solvent waste bottle. We recommend using a reagent trough and attaching a funnel to the waste bottle.

1. Add the 2X bead suspension (prepared per the procedure on page 16) to the PNGase F deglycosylation reactions, as follows:
  - a. Vortex the 2X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Using an electronic multistep pipette, add 10 µL of the resuspended 2X beads to each 40-µL deglycosylation reaction.
  - c. Vortex the plate on medium speed (300 to 500 rpm).
2. Using a 1-mL multichannel pipette, add 550 µL of 100% acetonitrile to each well, then immediately pipet up and down at least 6 times. After adding the acetonitrile to the last well, let the plate rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
3. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
4. Using a 200-µL multichannel pipette, add 200 µL of Wash Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.
5. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.

6. Using a 200- $\mu$ L multichannel pipette, add 40  $\mu$ L of Supplemental Wash Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.
7. Using a 1-mL multichannel pipette, add 300  $\mu$ L of 100% acetonitrile to each well, then immediately pipet up and down at least 6 times.
8. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
9. Using a 200- $\mu$ L multichannel pipette, add 200  $\mu$ L of Wash Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.
10. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
11. Using a 200- $\mu$ L multichannel pipette, add 50  $\mu$ L of Elution Buffer (prepared per the procedure on page 17) to each well, then vortex the plate on medium speed (300 to 500 rpm) to resuspend the pellet.
12. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu$ L multichannel pipette, carefully transfer the supernatant containing eluted glycans to a new deep-well plate (~50  $\mu$ L per well), avoiding bead carryover.

**Note:** We recommend Nunc™ 1.3-mL DeepWell™ Plates with Shared-Wall Technology.

---

**STOPPING POINT** The supernatant containing eluted glycans can be stored at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  for up to 1 week. Alternatively, the eluted glycans can be dried and stored in the dark at room temperature for up to 1 month.

---

When ready, proceed to the dye labeling method below.

## Perform dye labeling (96-well plates)

1. For each labeling reaction: Using a 20- $\mu$ L multichannel pipette, transfer 5  $\mu$ L of the supernatant containing eluted glycans to a new 96-well plate (5  $\mu$ L per well).

**Note:** We recommend MicroAmp™ Optical 96-Well Reaction Plates.

2. Freshly prepare the dye labeling mix: Combine the components listed in the table below, then mix well.

Component	Volume for 1 reaction <sup>[1]</sup>
Turquoise™ Labeling Buffer	4.5 $\mu$ L
Turquoise™ dye	0.5 $\mu$ L

<sup>[1]</sup> Multiply by your total number of reactions, then include ~10% overage to account for the loss that occurs during reagent transfer.

3. Using a single-channel multistep pipette, add 5  $\mu$ L of the dye labeling mix to each well of eluted glycans, then use a 20- $\mu$ L multichannel pipette to pipet up and down at least 6 times to mix.
4. Seal the plate, then briefly spin to bring the solution to the bottom.
5. Working under a fume hood or on a well-ventilated workbench, place the plate in a shaking heater block or thermal cycler set to 60°C, **carefully open the plate**, then incubate as follows:
  - Incubate at 450 rpm for 30 minutes.
  - After 30 minutes, check the reactions. If needed, incubate for an additional 5 minutes to complete evaporation/dryness.

---

**STOPPING POINT** The dried samples are extremely stable and can be stored in the dark at room temperature for up to 30 days or at -25°C to -15°C for longer periods.

---

When ready, proceed to the excess dye removal method below.

## Remove excess dye (96-well plates)

**Note:** This procedure requires a multichannel pipette to discard supernatant to a solvent waste bottle. We recommend using a reagent trough and attaching a funnel to the waste bottle.

1. Add the 1X bead suspension (prepared per the procedure on page 17) to the labeled glycans, as follows:
  - a. Vortex the 1X bead suspension on medium speed (300 to 500 rpm) until completely resuspended (10 to 20 seconds).
  - b. Using an electronic multistep pipette, add 20  $\mu$ L of the resuspended 1X beads to each labeled and dried glycan reaction.
  - c. Using a 20- $\mu$ L multichannel pipette set at 10  $\mu$ L, pipet up and down at least 4 times to mix.

2. Using a 1-mL multichannel pipette, add 220  $\mu\text{L}$  of 100% acetonitrile to each well, then pipet up and down at least 4 times to mix.
3. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 1-mL multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
4. Using a 200- $\mu\text{L}$  multichannel pipette, add 200  $\mu\text{L}$  of Wash Buffer (prepared per the procedure on page 17) to each well, then pipet up and down at least 4 times to resuspend the pellet.
5. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu\text{L}$  multichannel pipette, carefully transfer the supernatant to a solvent waste bottle to discard, then remove the plate from the magnetic stand.
6. Repeat step 4 and step 5 one more time.
7. Using a 200- $\mu\text{L}$  multichannel pipette, add 50  $\mu\text{L}$  of HPLC-grade water to each well to resuspend the pellet, then pipet up and down to mix. After adding the water to the last well, let the plate rest at room temperature for 1 minute (minimum) to 10 minutes (maximum).
8. Briefly spin the plate to bring the solution to the bottom.
9. Place the plate on a magnetic stand to pellet the beads. The beads should adhere within 30 seconds; be sure that the solution is clear (not light brown/brown with beads) before continuing. Using a 200- $\mu\text{L}$  multichannel pipette, carefully transfer the supernatant containing labeled glycans to a new 96-well plate (~50  $\mu\text{L}$  per well), avoiding bead carryover.  
**Note:** We recommend MicroAmp™ Optical 96-Well Reaction Plates.
10. If off-scale peaks are seen in initial runs: Dilute as needed (1:2 to 1:20) in HPLC-grade water.

---

**STOPPING POINT** The labeled glycans can be stored in the dark at 2°C to 8°C for up to 1 day or at -25°C to -15°C for longer periods. However, we recommend proceeding immediately to “Perform CE separation” on page 56.

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# 6

## Perform capillary electrophoresis (CE) separation

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### About the size standard and Landmark Red

The CE sample loading mixture includes the following:

- GeneScan™ 600 LIZ™ Dye Size Standard v2.0 – Provides an internal standard that is used for peak alignment
- Landmark Red – Provides two identifier peaks that are required for processing by the GlycanAssure™ Data Analysis Software

### Perform CE separation

1. Prepare the CE sample loading mixture:
  - a. Combine the following in a microcentrifuge tube:

Component	Volume for 24 wells
GeneScan™ 600 LIZ™ Dye Size Standard v2.0	2.0 µL
CE Loading Buffer	200.0 µL
Landmark Red	2.0 µL <sup>[1]</sup>

<sup>[1]</sup> Use 2.0 µL as a starting point. The Landmark Red volume can be decreased or increased as needed.

- b. Close the tube cap, mix well, then spin to bring the solution to the bottom.
  - c. Place the tube in a heater block or oven and incubate at 60°C for 5 minutes, then cool to room temperature.
2. Load the MicroAmp™ Optical 96-Well Reaction Plate:
  - a. Add 8 µL per well of the CE sample loading mixture to the first three columns of the plate (24 wells).
  - b. Add 2 µL per well of the labeled glycans (APTS, Teal™, or Turquoise™ dyes).
3. Pipet up and down at least 6 times to mix.



4. Seal the reaction plate with the Septa for 3500/3500xL Genetic Analyzers.
5. Briefly spin the plate at 1000 rpm for 1 minute to bring the solution to the bottom.
6. Load the plate into the Retainer and Base Set (Standard) for 3500/3500xL Genetic Analyzers.
7. Load the plate/retainer onto the appropriate position (Plate A or Plate B) of a 3500/3500xL Genetic Analyzer.
8. In the GlycanAssure™ Data Acquisition Software, click **Run Setup** to set up the run.
9. Start the capillary electrophoresis run.

For detailed information on performing the run, refer to the *3500/3500xL Genetic Analyzer with GlycanAssure™ Data Acquisition Software User Guide*.

## Perform data analysis

For information on performing data analysis, refer to the *GlycanAssure™ Data Analysis Software v1.0 User Guide*.



# Safety



**WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
  - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
-

## Chemical safety



**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
  - Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
  - Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
  - Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
  - Handle chemical wastes in a fume hood.
  - Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
  - After emptying a waste container, seal it with the cap provided.
  - Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
  - Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
  - **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
-

## Biological hazard safety



**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:  
[www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf](http://www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf)
  - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:  
[www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf](http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf)
-

# Documentation and support

## Related documentation

Document	Publication number
<i>3500/3500xL Genetic Analyzer with GlycanAssure™ Data Acquisition Software User Guide</i>	100036372
<i>GlycanAssure™ Data Analysis Software v1.0 User Guide</i>	100036373
<i>GlycanAssure™ Data Acquisition Software Help</i>	MAN0014023
<i>GlycanAssure™ Data Analysis Software Help</i>	MAN0014024
<i>GlycanAssure™ System Quick Reference</i>	100038224

Portable document format (PDF) versions of this guide and the documents listed above are available at **thermofisher.com**.

**Note:** To open the user documentation available from our web site, use the Adobe™ Reader™ software available from **www.adobe.com**.

## Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at [www.thermofisher.com/us/en/home/global/terms-and-conditions.html](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have any questions, please contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).

# References

Varki, A. 1993. Biological Roles of Oligosaccharides: All the Theories are Correct. *Glycobiology* 3(2):97–130.

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