GlycanAssure™ HyPerformance APTS Kit

USER GUIDE

N-glycan purification and APTS labeling of glycoproteins

Catalog Numbers A33953, A33952, A38927, and A38928

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0016959

Revision	Date	Description
D.0	12 December 2018	Updated references to Cat. No. A38263. It is not an orderable part, but is included in Cat. Nos. A38927 and A38928.
C.0	2 May 2018	Added new kits, Cat. Nos. A38927 and A38928.
B.0	21 March 2018	Revised product description. Clarified the recommended input amounts. Aligned text and formatting with related documentation.
A.0	24 January 2018	New document.

Contents

1 Product information

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

Product description

The GlycanAssure™ HyPerformance APTS Kit is an *N*-glycan rapid-release, APTS labeling, and cleanup kit for glycoprotein sample preparation. The kit includes reagents for a simple three-step workflow for rapid *N*-glycan release, APTS glycan labeling with reductive amination, and excess dye cleanup with Magnetic Beads. The kit is designed for glycoprotein input amounts of 20–100 µg with sample concentrations ≥1 mg/mL.

The resulting labeled *N*-glycans can be analyzed with:

- Liquid chromatography (LC) on the Thermo Scientific™ Vanquish™ UHPLC System with Chromeleon™ Chromatography Data System (CDS) Software
- Capillary electrophoresis (CE) on the Thermo Fisher Scientific 3500/3500xL Genetic Analyzer with GlycanAssure™ Data Acquisition Software and GlycanAssure™ Data Analysis Software

Benefits of the kit include:

- The entire workflow can be performed in <1.5 hours, with a hands-on time of <1 hour.
- The kit can be used with 1.5-mL microcentrifuge tubes or 96-well reaction plates.
- Rapid deglycosylation of complex glycoproteins occurs in 10 minutes.
- Released glycans are directly labeled without the need for glycan purification.
- The Magnetic Bead-based cleanup preserves sialylated glycans while effectively removing free dye from the labeling step.

Contents and storage

Table 1 GlycanAssure™ HyPerformance APTS Kit

The kit includes reagents for rapid deglycosylation, labeling, and cleanup. The kit is provided in three boxes.

Contents	Amount		Storage
	Cat. No. A33953 (24 samples)	Cat. No. A33952 (96 samples)	
GlycanAssure™ HP Buffer Kit			
Denaturation Buffer	1 × 70 µL	4 × 70 µL	Room temp. (15°C to 30°C)
Elution Buffer	3 × 2 mL	12 × 2 mL	
Wash Buffer	1 × 5 mL	4 × 5 mL	
GlycanAssure™ HP Beads Kit			
Water (HPLC grade)	1 × 10 mL	4 × 10 mL	2°C to 8°C
Magnetic Beads	1 × 5 mL	4 × 5 mL	
GlycanAssure™ HP Core Kit			
Denaturant	1 × 200 µL	4 × 200 µL	–25°C to –15°C
PNGase F, Recombinant (peptide-N-glycosidase)	1 × 60 µL	4 × 60 µL	
Digestion Buffer	1 × 100 µL	4 × 100 µL	
APTS Reagent Mix	1 × 330 µL	4 × 330 µL	
Reductant	1 × 60 µL	4 × 60 µL	

Table 2 GlycanAssure™ HyPerformance APTS Kit with the GlycanAssure™ CE Module

The kit includes reagents for rapid deglycosylation, labeling, cleanup, and capillary electrophoresis. The kit is provided in four boxes.

Contents	Amount		Storage
	Cat. No. A38927 (24 samples)	Cat. No. A38928 (96 samples)	
GlycanAssure™ HP Buffer Kit			
Denaturation Buffer	1 × 70 µL	4 × 70 µL	Room temp. (15°C to 30°C)
Elution Buffer	3 × 2 mL	12 × 2 mL	
Wash Buffer	1 × 5 mL	4 × 5 mL	
GlycanAssure™ HP Beads Kit			
Water (HPLC grade)	1 × 10 mL	4 × 10 mL	2°C to 8°C
Magnetic Beads	1 × 5 mL	4 × 5 mL	

Contents	Amount		Storage
	Cat. No. A38927 (24 samples)	**Cat. No. A38928 (96 samples)**	
GlycanAssure™ HP Core Kit			
Denaturant	1 × 200 µL	4 × 200 µL	–25°C to –15°C
PNGase F, Recombinant (peptide-N-glycosidase)	1 × 60 µL	4 × 60 µL	
Digestion Buffer	1 × 100 µL	4 × 100 µL	
APTS Reagent Mix	1 × 330 µL	4 × 330 µL	
Reductant	1 × 60 µL	4 × 60 µL	
GlycanAssure™ CE Module (Cat. No. A38263)			
GlycanAssure™ Landmark Red	1 × 33 µL	1 × 33 µL	–25°C to –15°C
GlycanAssure™ Loading Buffer	1 × 7.2 mL	1 × 7.2 mL	Room temp. (15°C to 30°C)
GlycanAssure™ LIZ™ Dye Size Standard	1 × 220 µL	1 × 220 µL	2°C to 8°C

Required materials not supplied

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

Item	Source
Fisherbrand™ Standard Mini Centrifuge	Fisher Scientific™ 12-006-901
Fisher Scientific™ Vortex Mixer	02-215-365
Robbins Scientific™ 400 Incubator (convection oven)	1040-60-1
Eppendorf™ Thermomixer™ Temperature Control Device (heat block)	05-412-500
Eppendorf™ Snap-Cap Microcentrifuge Flex-Tube™ Tubes, 1.5-mL (for use with Cat. No. 12321D)	022364111
DynaMag™-2 Magnet (16-position magnetic stand; for use with Cat. No. 022364111)	12321D
Thermo Scientific™ 0.8-mL Plate (for low-volume recovery with elution volumes of 20–600 µL; for use with Cat. No. AM10050)	AB-0765 or AB-0859
Ambion™ 96-well Magnetic-Ring Stand (for use with Cat. Nos. AB-0765 and AB-0859)	AM10050
Nunc™ 1.3-mL DeepWell™ Plates with Shared-Wall Technology (for >600-µL elution volumes; for use with Cat. No. AM10027)	260252
Magnetic Stand-96 (for use with Cat. No. 260252)	AM10027
Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with Nylon Membrane	164-0020
Pipettes: • For tube methods—2–20-µL, 20–200-µL, and 100–1,000-µL pipettes • For plate methods—2–20-µL, 20–200-µL, and 100–1,000-µL multichannel pipettes, electronic multistep pipettes, single-channel multistep pipettes	MLS
Waste bottle (to collect dye removal waste)	MLS
100% acetonitrile, HPLC grade	ACROS Organics™ AC610010040
Formic acid, 99.5+%, Optima™ LC/MS grade	Fisher Chemical A117-50
Water, HPLC grade	MLS

2 Prepare materials

Before first use of the kit

Prepare the Wash Buffer

You only need to prepare the Wash Buffer once, before first use of the kit.

1. Using a serological pipette, add 45 mL of 100% acetonitrile (HPLC-grade) to the Wash Buffer bottle, then cap the bottle.
2. Swirl to mix.
3. **IMPORTANT!** On the bottle label, write the date and check the box to indicate that the 100% acetonitrile has been added.

STOPPING POINT You can store the prepared Wash Buffer at room temperature for up to 6 months.

Note: To prevent the acetonitrile from evaporating, do not leave the bottle open for extended periods of time.

Prepare the Magnetic Beads

You only need to prepare the Magnetic Beads one time, before first use of the kit.

Note: If you will be using the kit over several months, prepare only the volume of beads that are required for your current application.

IMPORTANT! Ensure that the Magnetic Beads are prepared and ready for dye removal as soon as labeling is complete.

IMPORTANT! If you ordered multiples kits, do not combine Magnetic Beads from different lots. Check the lot number that is provided on each GlycanAssure™ HP Beads Kit box.

1. Thoroughly resuspend the Magnetic Beads by vortexing for 1 minute, or until the bead suspension is a homogeneous brown color and no beads are sticking to the bottle.

2. Immediately transfer the appropriate volume of bead suspension (Table 3) to a 1.5-mL microcentrifuge tube, then briefly centrifuge to bring the contents to the bottom.

   Table 3 Bead suspension

Number of samples	Volume of bead suspension
8	1.0 mL
24	3.0 mL
48	6.0 mL
96	12.0 mL

3. Place the tube on the DynaMag™-2 Magnet for at least 30 seconds to capture the beads.
   Ensure that the solution is clear (not light brown/brown with beads) before continuing.

4. Using a pipette, carefully remove the liquid, then discard the liquid to a waste bottle. Avoid bead carryover.

5. Remove the tube from the DynaMag™-2 Magnet.

6. Add the appropriate volume of water (Table 4), vortex for ~20 seconds to mix, then briefly centrifuge to bring the contents to the bottom.

   Table 4 Water (HPLC grade)

Number of samples	Volume of Water (HPLC grade)
8	1.0 mL
24	3.0 mL
48	6.0 mL
96	12.0 mL

7. Place the tube on the DynaMag™-2 Magnet to capture the beads for at least 30 seconds.
8. Using a pipette, carefully remove the liquid, then discard the liquid to a waste bottle.
9. Repeat step 5 to step 8 one more time.

   IMPORTANT! Ensure that you wash the beads twice before continuing.

10. Remove the tube from the DynaMag™-2 Magnet.
11. Add the appropriate volume of water (Table 5), then vortex to mix.

Table 5 Water (HPLC grade)

Number of samples	Volume of Water (HPLC grade)
8	450 µL
24	1.35 mL
48	2.70 mL
96	5.40 mL

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

Before each use of the kit

Prepare heat sources

- For 1.5-mL microcentrifuge tubes:
  - If you use a heat block for denaturation, digestion, and labeling, preheat two heat blocks: One to 80°C and the other to 55°C.
  - If you use a heat block for denaturation, and a convection oven for digestion and labeling: Preheat the heat block to 80°C and the convection oven to 50°C.
- For 96-well reaction plates: Preheat a convection oven to 80°C for denaturation. After denaturation is performed, adjust the oven to 50°C for digestion and labeling.
- *(CE only)* Preheat a heat block or convection oven to 60°C for preparing the CE sample loading mixture.

Prepare glycoprotein: Recommended input amounts

For routine analysis, we recommend a glycoprotein input amount of 20–100 µg.

Prepare the Digestion Buffer

1. Thaw at room temperature.
2. Briefly vortex, then centrifuge to bring the contents to the bottom.

Prepare the Denaturant

1. Thaw at room temperature for a minimum of 0.5–1 hour.

   IMPORTANT! Do not thaw for <0.5 hour.

2. Just before use, gently vortex.

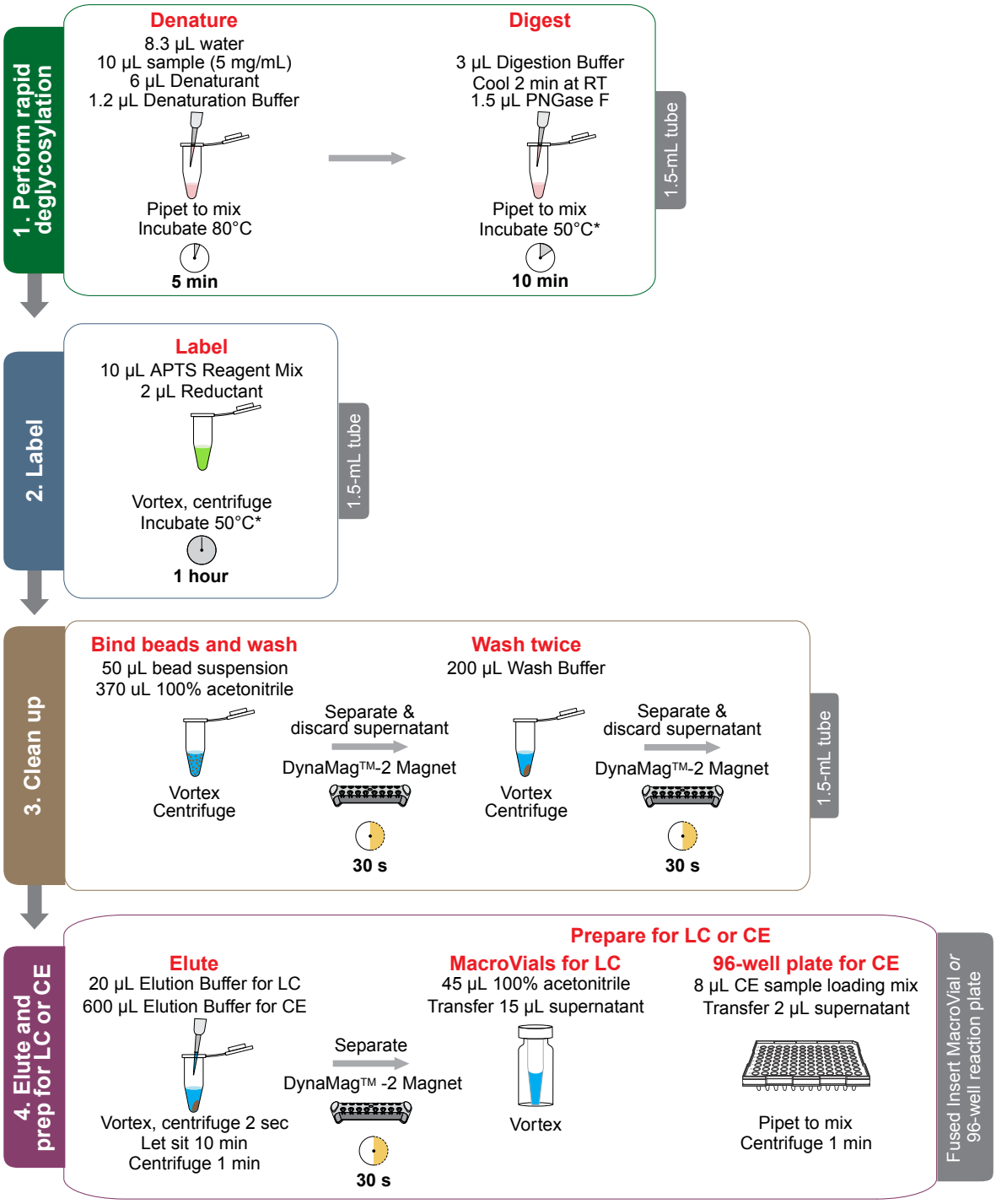
Prepare the Reductant

1. Thaw at room temperature.
2. Vortex, then centrifuge to bring the contents to the bottom.

3 Prepare *N*-glycan samples using tubes

Workflow for 1.5-mL microcentrifuge tubes

The volumes provided in the figure below are for one reaction at a glycoprotein input amount of 50 µg and a concentration of 5 mg/mL. For other input amounts and concentrations, see Table 6.



* If you are using a convection oven, set the temperature to 50°C. If you are using a heat block, set the temperature to 55°C.

Perform rapid deglycosylation

Denature

1. In a 1.5-mL microcentrifuge tube, combine the denaturation components (Table 6):
   a. Add the water (if needed) and glycoprotein sample to each tube.
   b. Gently vortex the tube of thawed Denaturant (equilibrated to room temperature), then add the Denaturant to the microcentrifuge tube.
   c. Vortex the Denaturation Buffer tube (do not centrifuge), add the buffer to the microcentrifuge tube, then thoroughly pipet to mix.

Note: Alternatively, you can combine the Denaturant, Denaturation Buffer, and water to prepare a denaturation mixture for multiple samples. Use Table 6 to calculate the total volume that is required for all samples, then include 20% overage to account for the loss that occurs during pipetting.

Table 6 Denaturation components

Component	Volume for 1 reaction					
	20-µg input			50-µg input		
	1 mg/mL	2 mg/mL	5 mg/mL	2 mg/mL	3 mg/mL	5 mg/mL
Water (HPLC grade)	0.00 µL	8.30 µL	14.30 µL	0.00 µL	1.63 µL	8.30 µL
Glycoprotein sample	20.00 µL	10.00 µL	4.00 µL	25.00 µL	16.67 µL	10.00 µL
Denaturant	6.50 µL	6.00 µL	6.00 µL	7.80 µL	6.00 µL	6.00 µL
Denaturation Buffer	1.30 µL	1.20 µL	1.20 µL	1.60 µL	1.20 µL	1.20 µL
Total volume	**27.80 µL**	**25.50 µL**	**25.50 µL**	**34.40 µL**	**25.50 µL**	**25.50 µL**

Note: For 80-µg and 100-µg input amounts, see Appendix B, "Additional glycoprotein input amounts".

2. Leave the microcentrifuge tube caps open, place the tubes in a heat block, then incubate at 80°C for 5 minutes.

3. *If precipitation is observed:*
   a. Discard the reactions.
   b. Prepare new reactions with the following changes:
      - Double the volume of Denaturation Buffer.
      - Decrease the volume of water to obtain the total volume that is provided in Table 6.
   c. Repeat step 2.

Digest

1. Remove the 1.5-mL microcentrifuge tubes from incubation, add 3 µL of Digestion Buffer to each tube, then let cool for 2 minutes.
2. Gently vortex the PNGase F tube, then briefly centrifuge to bring the contents to the bottom.
3. Add 1.5 µL of PNGase F to each microcentrifuge tube, then thoroughly pipet to mix.

   Note: Alternatively, you can combine the Digestion Buffer and PNGase F to prepare a digestion mixture for multiple samples. Calculate the total volume required for all samples, then include 20% overage to account for the loss that occurs during pipetting. After removing the microcentrifuge tubes from incubation, let the tubes cool for 2 minutes before adding the digestion mixture.
4. Leave the microcentrifuge tube caps open, place the tubes in a heat block at 55°C or a convection oven at 50°C, then incubate for 10 minutes.

STOPPING POINT We recommend that you immediately proceed to labeling. However, if needed, you can immediately freeze the deglycosylation reactions, then store them at –25°C to –15°C for several hours.

Label

IMPORTANT! Ensure that the Magnetic Beads are prepared and ready for use as soon as labeling is complete. See "Prepare the Magnetic Beads" on page 10.

1. Remove the 1.5-mL microcentrifuge tubes from incubation.
2. Vortex the APTS Reagent Mix and Reductant tubes, then centrifuge each tube to bring the contents to the bottom.
3. Add 10.0 µL of APTS Reagent Mix to each microcentrifuge tube, then thoroughly pipet to mix.
4. Add 2.0 µL of Reductant to each microcentrifuge tube, then thoroughly pipet to mix.

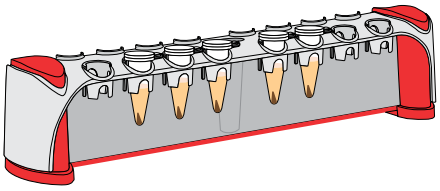
   Alternatively, you can vortex to mix, then briefly centrifuge.
5. Tightly cap the APTS Reagent Mix and Reductant tubes, then return these tubes to storage at –25°C to –15°C.
6. Leave the microcentrifuge tube caps open, place the tubes in a heat block at 55°C or a convection oven at 50°C, then incubate for 1 hour.

   IMPORTANT! Keep the tubes uncapped.

Clean up

Bind beads and wash

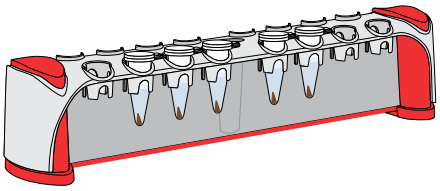
1. Remove the tube rack from the top of the DynaMag™-2 Magnet, then place it on a lab bench.
2. Remove the 1.5-mL microcentrifuge tubes from incubation, then position the tubes in the tube rack.



3. Add 50 µL of the bead suspension (prepared as described in "Prepare the Magnetic Beads" on page 10) to each tube, then cap the tubes.
4. Center the bottom of the tube rack on the vortexer and hold the tubes in place with the palm of your hand, then vortex the tubes until the solution turns a homogeneous brown color.

5. Tap the tube rack on the lab bench to bring the contents to the bottom, then add 370 µL of 100% acetonitrile to each tube.
6. Cap the tubes, then vortex as described in step 4 for at least 10 seconds.
7. Remove the tubes from the tube rack, then briefly centrifuge to bring the contents to the bottom.

8. Place the tubes in the tube rack, then place the tube rack on the DynaMag™-2 Magnet for at least 30 seconds.
   Ensure that the solution is clear (not light brown/brown with beads).



9. Using a 1-mL pipette, transfer the clear supernatant to a waste bottle to discard.

IMPORTANT! Remove any drops of dye that are left on the tube walls or caps. Residual dye can negatively affect the level of free dye.

Wash twice

1. Remove the tube rack from the DynaMag™-2 Magnet, then place the rack on the lab bench.
2. Add 200 µL of prepared Wash Buffer to each 1.5-mL microcentrifuge tube, then cap the tubes.

   Note: Prepare the Wash Buffer as described on page 9.
3. Center the bottom of the tube rack on the vortexer and hold the tubes in place with the palm of your hand, then vortex for at least 30 seconds.
4. Remove the tubes from the tube rack, then centrifuge to bring the contents to the bottom.
5. Place the tubes in the tube rack, then place the tube rack on the DynaMag™-2 Magnet for at least 30 seconds.
6. Transfer all supernatant to a waste bottle to discard. Do not leave residual supernatant in the tubes.
7. Remove the tube rack from the DynaMag™-2 Magnet, then place the rack on the lab bench.
8. Repeat step 2 to step 7 for a second wash.

Immediately proceed to the appropriate procedure for your application:

- “Elute glycans and prepare for LC“ on page 19
- “Elute glycans and prepare for CE“ on page 19

Elute glycans and prepare for LC

1. Add 20 µL of Elution Buffer to each 1.5-mL microcentrifuge tube, then cap the tubes.
2. Vortex the tubes, then gently centrifuge for 2 seconds.

   Note: Centrifuge only to bring the contents to the bottom, not to separate the Magnetic Beads from the solution.
3. Let the tubes sit in the tube rack for 10 minutes.
4. Centrifuge the tubes for ≥1 minute to separate the Magnetic Beads from the solution.
5. Gently place the tubes on the DynaMag™-2 Magnet for at least 30 seconds to capture the beads.

- Proceed immediately to "Liquid chromatography (LC)" on page 26.
  Or
- Store the labeled glycan samples at 4°C for same-day use, or at –20°C for up to 3 months.

Elute glycans and prepare for CE

1. Add 600 µL of Elution Buffer to each 1.5-mL microcentrifuge tube, then cap the tubes.

   Note: If the CE analysis results show truncated peaks, increase the volume of Elution Buffer as needed.
2. Vortex the tubes, then gently centrifuge for 2 seconds.

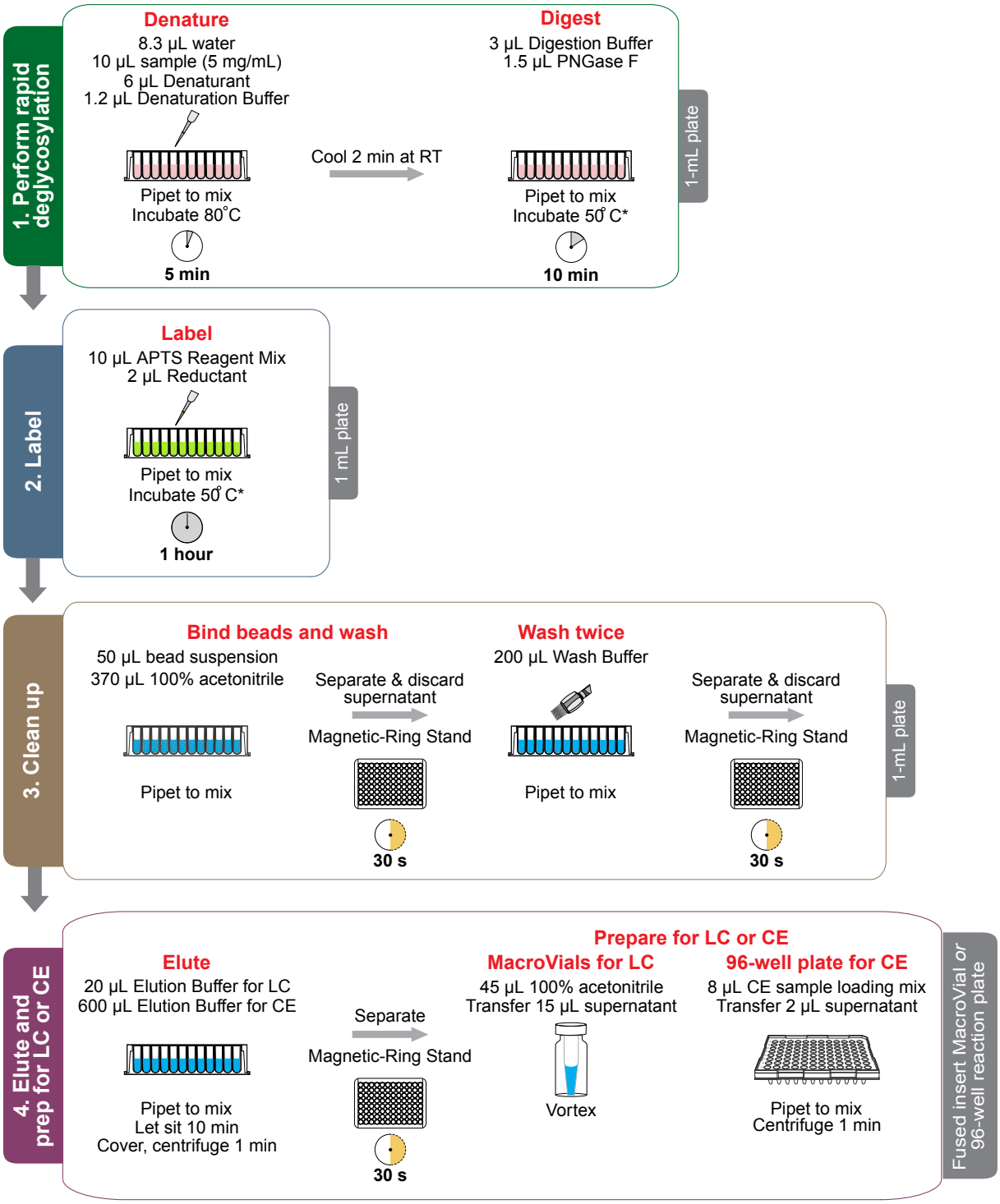
   Note: Centrifuge only to bring the contents to the bottom, not to separate the Magnetic Beads from the solution.
3. Let the tubes sit in the tube rack for 10 minutes.
4. Centrifuge the tubes for ≥1 minute to separate the Magnetic Beads from the solution.
5. Gently place the tubes on the DynaMag™-2 Magnet for at least 30 seconds to capture the beads.

- Proceed immediately to "Capillary electrophoresis (CE)" on page 30.
  Or
- Store the labeled glycan samples at 4°C for same-day use, or at –20°C for up to 3 months.

Prepare *N*-glycan samples using plates

Workflow for 96-well reaction plates

The volumes provided in the figure below are for one reaction at a glycoprotein input amount of 50 µg and a concentration of 5 mg/mL. For other input amounts and concentrations, see Table 7.



* If you are using a convection oven, set the temperature to 50°C. If you are using a heat block, set the temperature to 55°C.

Perform rapid deglycosylation

Denature

1. In a 96-well reaction plate, combine the denaturation components (Table 7):
   a. Add the water (if needed) and glycoprotein sample to each well.
   b. Gently vortex the tube of thawed Denaturant (equilibrated to room temperature), then add the Denaturant to each well.
   c. Vortex the Denaturation Buffer tube, add the buffer to each well, then thoroughly pipet to mix.

Note: Alternatively, you can combine the Denaturant, Denaturation Buffer, and water to prepare a denaturation mixture for multiple samples. Use Table 7 to calculate the total volume required for all samples, then include 20% overage to account for the loss that occurs during pipetting.

Table 7 Denaturation components

Component	Volume for 1 reaction					
	20-µg input			50-µg input		
	1 mg/mL	2 mg/mL	5 mg/mL	2 mg/mL	3 mg/mL	5 mg/mL
Water (HPLC grade)	0.00 µL	8.30 µL	14.30 µL	0.00 µL	1.63 µL	8.30 µL
Glycoprotein sample	20.00 µL	10.00 µL	4.00 µL	25.00 µL	16.67 µL	10.00 µL
Denaturant	6.50 µL	6.00 µL	6.00 µL	7.80 µL	6.00 µL	6.00 µL
Denaturation Buffer	1.30 µL	1.20 µL	1.20 µL	1.60 µL	1.20 µL	1.20 µL
Total volume	**27.80 µL**	**25.50 µL**	**25.50 µL**	**34.40 µL**	**25.50 µL**	**25.50 µL**

Note: For 80-µg and 100-µg input amounts, see Appendix B, "Additional glycoprotein input amounts".

2. Place the plate in a convection oven, then incubate at 80°C for 5 minutes.

Digest

1. Remove the 96-well reaction plate from incubation, then let cool for 2 minutes.
2. Prepare a mixture of Digestion Buffer and PNGase F (Table 8), then thoroughly pipet to mix.

Table 8 Digestion mixture

Component	Volume for 1 reaction[1]
Digestion Buffer	3.0 µL
PNGase F	1.5 µL
Total volume	**4.5 µL**

[1] Include 20% overage to account for the loss that occurs during pipetting.

3. Using an electronic repeater pipette, add 4.5 µL of digestion mixture to each well, then thoroughly pipet to mix.

4. Place the uncovered plate in a convection oven, then incubate at 50°C for 10 minutes.

Label

IMPORTANT! Ensure that the Magnetic Beads are prepared and ready for use as soon as labeling is complete. See "Prepare the Magnetic Beads" on page 10.

1. Remove the 96-well reaction plate from incubation.

2. Vortex the APTS Reagent Mix and Reductant tubes, then centrifuge each tube to bring the contents to the bottom.

3. Using an electronic repeater pipette, add 10.0 µL of APTS Reagent Mix to each well, then thoroughly pipet to mix.

4. Add 2.0 µL of Reductant to each well, then thoroughly pipet to mix.

5. Tightly cap the APTS Reagent Mix and Reductant tubes, then return these tubes to storage at –25°C to –15°C.

6. Place the uncovered plate in a convection oven, then incubate at 50°C for 1 hour.

   IMPORTANT! Keep the plate uncovered.

Clean up

Bind beads and wash

1. Remove the 96-well reaction plate from incubation.
2. Vortex the bead suspension (prepared as described in "Prepare the Magnetic Beads" on page 10) on medium speed for 10–20 seconds or until the beads are completely resuspended.
3. Using an electronic repeater pipette, add 50 µL of the resuspended beads to each well. After each addition, pipet ≥10 times to mix.
4. Add 100% acetonitrile:
   a. Transfer a volume of 100% acetonitrile to a reservoir.
   b. Using a 1-mL multichannel pipette, add 370 µL of the 100% acetonitrile to each well.
   c. Pipet ≥10 times to mix.

      Note: For effective mixing, slowly move the multichannel pipette tip to the bottom of the well as the liquid is taken up into the pipette.
5. Place the plate on the Magnetic-Ring Stand for 30 seconds.
   The beads should move away from the center and form a ring around the well. If there are beads in the center, gently tap the plate up and down or move the plate slightly back and forth until the center is clear.
6. Using a multichannel pipette, transfer the clear supernatant to a waste bottle to discard.

   Note: Place the pipette tip in the center of the well to avoid transferring any beads.

Wash twice

1. Remove the 96-well reaction plate from the Magnetic-Ring Stand.
2. Using a multichannel pipette, add 200 µL of prepared Wash Buffer to each well, then pipet ≥10 times to mix.

   Note: Prepare the Wash Buffer as described on page 9.
3. Place the plate on the Magnetic-Ring Stand for at least 30 seconds.
4. Transfer all supernatant to a waste bottle to discard. Do not leave residual supernatant in the wells.
5. Repeat step 2 to step 4 for a second wash.

Immediately proceed to the appropriate procedure for your application:

- "Elute glycans and prepare for LC" on page 25
- "Elute glycans and prepare for CE" on page 25

Elute glycans and prepare for LC

1. Using a repeater pipette, add 20 µL of Elution Buffer to each well of the 96-well reaction plate, then pipet ≥10 times to mix until the solution is a homogeneous brown color.

   Note: Ensure that all Magnetic Beads are expelled from the pipette tip.

2. Let the plate sit for 10 minutes.

3. Cover the plate, then centrifuge for 1 minute to separate the Magnetic Beads from the solution.

4. Place the plate on the Magnetic-Ring Stand for at least 30 seconds to capture the beads.

- Proceed immediately to "Liquid chromatography (LC)" on page 26.
  Or
- Store the labeled glycan samples at 4°C for same-day use, or at –20°C for up to 3 months.

Elute glycans and prepare for CE

1. Using a repeater pipette, add 600 µL of Elution Buffer to each well of the 96-well reaction plate, then pipet ≥10 times to mix until the solution is a homogeneous brown color.

   Note: If the CE analysis results show truncated peaks, increase the volume of Elution Buffer as needed. Switch to a Nunc™ 1.3-mL DeepWell™ Plate (Cat. No. 260252) for volumes >600 µL.

2. Let the plate sit for 10 minutes.

3. Cover the plate, then centrifuge for 1 minute to separate the Magnetic Beads from the solution.

4. Place the plate on the Magnetic-Ring Stand for at least 30 seconds to capture the beads.

- Proceed immediately to "Capillary electrophoresis (CE)" on page 30.
  Or
- Store the labeled glycan samples at 4°C for same-day use, or at –20°C for up to 3 months.

A LC and CE recommendations

Liquid chromatography (LC)

Materials for LC

Table 9 Liquid chromatography system

Item	Source
Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software	CHROMELEON7 or higher
Thermo Scientific™ Vanquish™ Fluorescence Detector F (Micro flow cell: 2-µL, biocompatible)	VF-D50-A
One of the following instruments, equipped with the Vanquish™ Fluorescence Detector F:	
Thermo Scientific™ Vanquish™ Horizon UHPLC System (1,500 bar binary)	IQLAAAGABHFAPUMZZZ
Thermo Scientific™ Vanquish™ Flex Binary UHPLC System (1,000 bar binary)	IQLAAAGABHFAPUMBJC
Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC System (1,000 bar quaternary)	IQLAAAGABHFAPUMBHV

Table 10 Equipment and consumables

Item	Source
Thermo Scientific™ 9-mm MS-Certified Clear Screw Thread Kit (100 LC vials and 100 caps with pre-assembled septa)	MSCERT5000-31LVW
100% acetonitrile, HPLC grade	ACROS Organics™ AC610010040
Ammonium formate, 99%	ACROS Organics™ AC401152500
Thermo Scientific™ Accucore™ 150 Amide HILIC LC Column (2.6-µm, 150 Å, 2.1 × 150 mm)	16726-152130

Prepare mobile phase

1. Add 6.31 g of ammonium formate to 0.9 L of deionized water, then mix thoroughly until all salts are dissolved.
2. Filter the solution through a 0.2-µm HPLC-certified nylon filter, then transfer to a glass mobile phase bottle.
3. Carefully add formic acid to adjust the pH to 4.4.
4. Bring the total volume to 1 L with deionized water.

Prepare the glycan sample for LC

If frozen, thaw the labeled glycan sample. Protect from light.

1. *(If needed)* Place the labeled glycan sample on a magnet to capture the beads:
   - **Tubes**—Place the tubes on the DynaMag™-2 Magnet for at least 30 seconds.
   - **Plates**—Place the plate on the Magnetic-Ring Stand for at least 30 seconds.
2. Add the following components to each LC vial:

Component	Volume
100% acetonitrile	45 µL
Labeled glycan supernatant **Note:** Avoid bead carryover.	15 µL

3. Tightly cap the vial, then vortex for 10 seconds at high speed to mix thoroughly.
4. Place the vials on the instrument.

LC analysis parameters

Table 11 Instrument parameters

Parameter	Value
Column	Accucore™ 150 Amide HILIC LC Column (2.1 × 150 mm, 2.6-µm, 150 Å)
Column temperature	50°C
Injection volume	15-µL injection (or lower, as appropriate for your sample)
FLD	λ_{ex} 455 nm, λ_{em} 500 nm
Mobile phase B	100% acetonitrile
Mobile phase A	100 mM ammonium formate, pH 4.4 (see page 27)
Data collection rate	10 Hertz
Sensitivity	7 or lower, as appropriate for your samples
PMT gain & Filter Wheel	Auto & 435 nm
Lamp mode	High power

Table 12 50-minute method

Time	Flow rate	Acetonitrile	100 mM ammonium formate, pH 4.4
0.0 minutes	0.45 mL/minute	68%	32%
45.0 minutes	0.45 mL/minute	55%	45%
45.5 minutes	0.45 mL/minute	40%	60%
47.0 minutes	0.45 mL/minute	40%	60%
47.5 minutes	0.45 mL/minute	68%	32%
50.0 minutes	0.45 mL/minute	68%	32%

Example LC data (from the Accucore™ 150 Amide HILIC LC Column)

Figure 1 and Figure 2 provide representative LC data from the Accucore™ 150 Amide HILIC LC Column (with Vanquish™ Fluorescence Detector F).

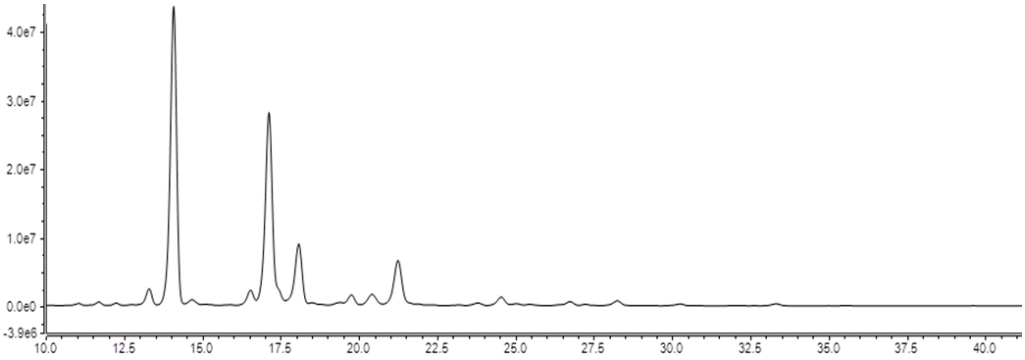


Figure 1 *N*-Glycan profile of NIST mAb #8671 (lot# 14HB-D-001)—Zoomed-out

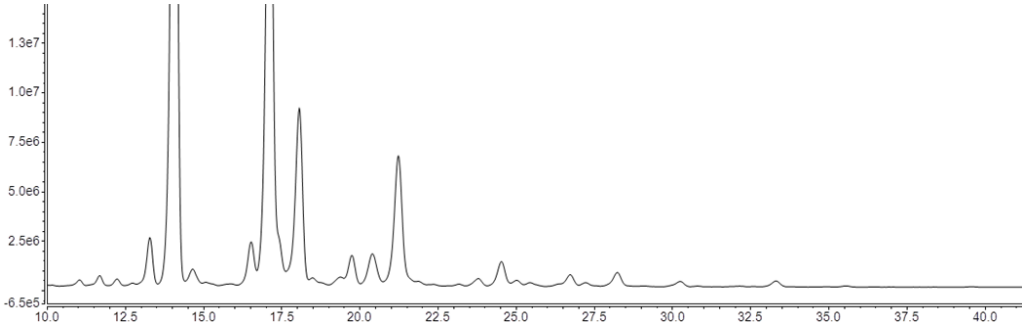


Figure 2 *N*-Glycan profile of NIST mAb #8671 (lot# 14HB-D-001)—Zoomed-in

Capillary electrophoresis (CE)

Materials for CE

Table 13 Capillary electrophoresis system

Item	Source
3500 Genetic Analyzer for Protein Quality Analysis, with software	A30886 Contact your local sales office.
3500xL Genetic Analyzer for Protein Quality Analysis, with software	A30887 Contact your local sales office.

Table 14 Equipment and consumables

Item	Source
GlycanAssure™ CE Module	A38263[1]
MicroAmp™ Optical 96-Well Reaction Plate	4306737
Septa for 3500/3500xL Genetic Analyzers, 96 well	4412614
Retainer & Base Set (Standard) for 3500/3500xL Genetic Analyzers, 96 well	4410228
Heat block or oven	MLS

[1] Cat. No. A38263 is included in the GlycanAssure™ HyPerformance APTS Kit (Cat. Nos. A38927 and A38928). See "Contents and storage" on page 6.

Prepare the glycan sample for CE

If frozen, thaw the labeled glycan sample. Protect from light.

(If needed) Place the labeled glycan sample on a magnet to capture the beads:

- **Tubes**—Place the tubes on the DynaMag™-2 Magnet for at least 30 seconds.
- **Plates**—Place the plate on the Magnetic-Ring Stand for at least 30 seconds.

Perform CE separation

1. Prepare the CE sample loading mixture:
   a. Combine the following components in a 1.5-mL microcentrifuge tube:

GlycanAssure™ CE Module Component	Volume for 24 wells
GlycanAssure™ LIZ™ Dye Size Standard	2.0 µL
GlycanAssure™ Loading Buffer	200.0 µL
GlycanAssure™ Landmark Red	2.0 µL[1]

[1] Use 2.0 µL as a starting point and adjust as required.

   b. Cap the tube, mix well, then centrifuge to bring the contents to the bottom.

   c. Incubate the tube at 60°C for 5 minutes in a heat block or oven, then cool to room temperature.

2. Load a 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 diluted labeled glycans to the wells that contain CE sample loading mixture. Avoid bead carryover.
3. Pipet ≥5 times to mix.
4. Place a Septa for 3500/3500xL Genetic Analyzers on the plate.
5. Centrifuge the plate at 1,000 rpm for 1 minute to bring the contents to the bottom.
6. Load the plate into the Retainer & Base Set (Standard).
7. Load the plate/retainer onto the 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 more information on performing the run, see the *3500/3500xL Genetic Analyzer with GlycanAssure™ Data Acquisition Software User Guide* (Pub. No. 100036372).

Perform data analysis

For information on performing data analysis, see the *GlycanAssure™ Data Analysis Software v1.0 User Guide* (Pub. No. 100036373).

Example CE data

Figure 3 provides representative CE data from the 3500xL Genetic Analyzer (24 capillary).

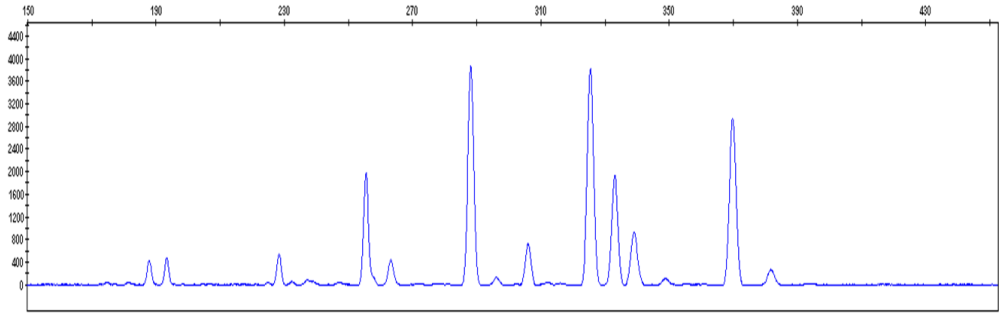


Figure 3 *N*-Glycan profile of human IgG

B Additional glycoprotein input amounts

For glycoprotein samples at concentrations ≥5 mg/mL

These volumes are appropriate for tubes or plates.

Table 15 Denaturation components

Component	Volume for 1 reaction	
	80-µg input	**100-µg input**
Water (HPLC Grade)	2.3 µL	0.0 µL
Glycoprotein sample	16.0 µL	20.0 µL
Denaturant	6.0 µL	6.5 µL
Denaturation Buffer	1.2 µL	1.3 µL
Total volume	**25.5 µL**	**27.8 µL**

C

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, and so on). 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. 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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also 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/bmbl5/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

Documentation and support

Related documentation

Document	Publication number
GlycanAssure™ HyPerformance APTS Kit Quick Reference	MAN0016960
3500/3500xL Genetic Analyzer with GlycanAssure™ Data Acquisition Software User Guide	100036372
GlycanAssure™ Data Analysis Software v1.1 User Guide	100036373
Documentation for the Vanquish™ Horizon UHPLC System	See **thermofisher.com/vanquish**
Related product document	**Publication number**
GlycanAssure™ AutoXpress Kit User Guide	MAN0017457
AutoMate Express™ Instrument User Guide	4441982

Portable document format (PDF) versions of 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**.

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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