

GlycanAssure™ HyPerformance APTS Kit

N-glycan purification and APTS labeling of glycoproteins

Catalog Numbers A33953, A33952, A38927, and A38928

Pub. No. MAN0016960 **Rev.** C.0

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *GlycanAssure™ HyPerformance APTS Kit User Guide* (Pub. No. MAN0016959). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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.

About this Quick Reference

This Quick Reference provides brief procedures for preparing glycoprotein samples with the GlycanAssure™ HyPerformance APTS Kit. See the *GlycanAssure™ HyPerformance APTS Kit User Guide* (Pub. No. MAN0016959) for detailed sample preparation procedures, and for materials preparation, liquid chromatography (LC), and capillary electrophoresis (CE) procedures.

Prepare samples using tubes

Perform rapid deglycosylation

1 Denature

a. In a 1.5-mL microcentrifuge tube, combine the 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

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

2 Digest

- a. Remove the 1.5-mL microcentrifuge tubes from incubation, add 3 μL of Digestion Buffer to each tube, then let cool for 2 minutes.
- b. Add 1.5 μL of PNGase F to each microcentrifuge tube, then thoroughly pipet to mix.
- c. 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.

Label

3 Label

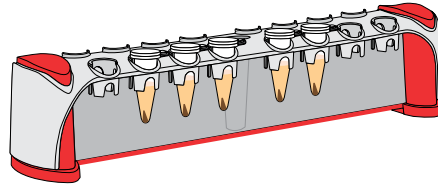
- a. Remove the 1.5-mL microcentrifuge tubes from incubation.
- b. Vortex the APTS Reagent Mix and Reductant tubes, then centrifuge each tube to bring the contents to the bottom.
- c. Add 10.0 μL of APTS Reagent Mix to each microcentrifuge tube, then thoroughly pipet to mix.
- d. Add 2.0 μL of Reductant to each microcentrifuge tube, then thoroughly pipet to mix.
- e. Leave the microcentrifuge tube caps open, place the tubes in a tube rack, then incubate for 1 hour.

IMPORTANT! Keep the tubes uncapped.

Clean up

4 Bind beads and wash

- a. Remove the 1.5-mL microcentrifuge tubes from incubation, then position the tubes in the tube rack.



- b. Add 50 μL of the bead suspension to each tube, then cap the tubes.
- c. 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.



- d. 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.
- e. Cap the tubes, then vortex as described in step 4c for at least 10 seconds.
- f. Remove the tubes from the tube rack, then briefly centrifuge to bring the contents to the bottom.

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- 4** Bind beads and wash
(continued)
- g. Place the tubes in the tube rack, then place the tube rack on the DynaMag™-2 Magnet for at least 30 seconds.
 - h. Using a 1-mL pipette, transfer the clear supernatant to a waste bottle to discard.
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- 5** Wash twice
- a. Remove the tube rack from the DynaMag™-2 Magnet, then place the rack on the lab bench.
 - b. Add 200 µL of prepared Wash Buffer to each 1.5-mL microcentrifuge tube, then cap the tubes.
 - c. 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.
 - d. Remove the tubes from the tube rack, then centrifuge to bring the contents to the bottom.
 - e. Place the tubes in the tube rack, then place the tube rack on the DynaMag™-2 Magnet for at least 30 seconds.
 - f. Transfer all supernatant to a waste bottle to discard. Do not leave residual supernatant in the tubes.
 - g. Remove the tube rack from the DynaMag™-2 Magnet, then place the rack on the lab bench.
 - h. Repeat step 5b to step 5g for a second wash.

Immediately proceed to the appropriate procedure for your application:

- “Elute glycans and prepare for LC” on page 3
- “Elute glycans and prepare for CE” on page 3

Elute glycans and prepare for LC or CE

- 6** Elute glycans and prepare for LC
- a. Add 20 µL of Elution Buffer to each 1.5-mL microcentrifuge tube, then cap the tubes.
 - b. Vortex the tubes, then gently centrifuge for 2 seconds.
 - c. Let the tubes sit in the tube rack for 10 minutes.
 - d. Centrifuge the tubes for ≥1 minute to separate the Magnetic Beads from the solution.
 - e. Gently place the tubes on the DynaMag™-2 Magnet for at least 30 seconds to capture the beads.

You can proceed immediately to liquid chromatography or store the labeled glycan samples.

- 7** Elute glycans and prepare for CE
- a. Add 600 µL of Elution Buffer to each 1.5-mL microcentrifuge tube, then cap the tubes.
 - b. Vortex the tubes, then gently centrifuge for 2 seconds.
 - c. Let the tubes sit in the tube rack for 10 minutes.
 - d. Centrifuge the tubes for ≥1 minute to separate the Magnetic Beads from the solution.
 - e. Gently place the tubes on the DynaMag™-2 Magnet for at least 30 seconds to capture the beads.

You can proceed immediately to capillary electrophoresis or store the labeled glycan samples.

Prepare samples using plates

Perform rapid deglycosylation

1 Denature

- a. In a 96-well reaction plate, combine the denaturation components:

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

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

2 Digest

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

Table 1 Digestion mixture

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

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

- c. Using an electronic repeater pipette, add 4.5 μ L of digestion mixture to each well, then thoroughly pipet to mix.
d. Place the uncovered plate in a convection oven, then incubate at 50°C for 10 minutes.

Label

3 Label

- a. Remove the 96-well reaction plate from incubation.
b. Vortex the APTS Reagent Mix and Reductant tubes, then centrifuge each tube to bring the contents to the bottom.
c. Using an electronic repeater pipette, add 10.0 μ L of APTS Reagent Mix to each well, then thoroughly pipet to mix.
d. Add 2.0 μ L of Reductant to each well, then thoroughly pipet to mix.
e. Place the uncovered plate in a convection oven, then incubate at 50°C for 1 hour.

IMPORTANT! Keep the plate uncovered.

Clean up

- 4 Bind beads and wash**
- Remove the 96-well reaction plate from incubation.
 - Vortex the bead suspension on medium speed for 10–20 seconds or until the beads are completely resuspended.
 - Using an electronic repeater pipette, add 50 μL of the resuspended beads to each well. After each addition, pipet ≥ 10 times to mix.
 - Using a 1-mL multichannel pipette, add 370 μL of 100% acetonitrile to each well, then pipet ≥ 10 times to mix.
 - Place the plate on the Magnetic-Ring Stand for 30 seconds.
 - Using a multichannel pipette, transfer the clear supernatant to a waste bottle to discard.
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- 5 Wash twice**
- Remove the 96-well reaction plate from the Magnetic-Ring Stand.
 - Using a multichannel pipette, add 200 μL of prepared Wash Buffer to each well, then pipet ≥ 10 times to mix.
 - Place the plate on the Magnetic-Ring Stand for at least 30 seconds.
 - Transfer all supernatant to a waste bottle to discard. Do not leave residual supernatant in the wells.
 - Repeat step 5b to step 5d for a second wash.

Immediately proceed to the appropriate procedure for your application:

- “Elute glycans and prepare for LC” on page 5
- “Elute glycans and prepare for CE” on page 5

Elute glycans and prepare for LC or CE

- 6 Elute glycans and prepare for LC**
- 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.
 - Let the plate sit for 10 minutes.
 - Cover the plate, then centrifuge for 1 minute to separate the Magnetic Beads from the solution.
 - Place the plate on the Magnetic-Ring Stand for at least 30 seconds to capture the beads.

You can proceed immediately to liquid chromatography or store the labeled glycan samples.

- 7 Elute glycans and prepare for CE**
- 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.
 - Let the plate sit for 10 minutes.
 - Cover the plate, then centrifuge for 1 minute to separate the Magnetic Beads from the solution.
 - Place the plate on the Magnetic-Ring Stand for at least 30 seconds to capture the beads.

You can proceed immediately to capillary electrophoresis or store the labeled glycan samples.

Limited product warranty

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Revision history: Pub. No. MAN0016960

Revision	Date	Description
C.0	2 May 2018	Added new kits, Cat. Nos. A38927 and A38928.
B.0	21 March 2018	Clarified the recommended input amounts. Aligned text and formatting with related documentation.
A.0	24 January 2018	New document.

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