Agilent AdvanceBio Gly-X InstantPC

Quick N-glycan Sample Preparation

Agilent AdvanceBio Gly-X InstantPC reagents and consumables let you prepare samples for analysis including N-glycan release, labeling and cleanup, in as little as 1 hour using this simple step by step process.

You will need these additional items to prepare your samples:

<table>
<thead>
<tr>
<th>Lab supplies and equipment</th>
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<tr>
<td>Pipettes and pipette tips</td>
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<tr>
<td>Eppendorf tubes</td>
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<td>Graduated cylinder (250 mL or larger)</td>
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<tr>
<td>Glass storage vessel (250 mL or larger)</td>
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<td>Thermocycler</td>
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<table>
<thead>
<tr>
<th>Additional reagents</th>
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<tr>
<td>Formic acid</td>
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<td>Acetonitrile</td>
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1. Prepare N-glycanase working solution. Pipette 1.2 µL of N-glycanase to one Eppendorf tube per sample. Make sure to change the pipette tip between transfer steps.
   Duration: 1 min  
   Total time: 1 min

2. Add 2 µL of Digestion Buffer to each tube. Return the N-glycanase and Digestion Buffer to 4 °C.
   Duration: 1 min  
   Total time: 2 mins

3. Prepare InstantPC Dye solution. Add 150 µL of InstantPC Dye Solvent (green cap) to the InstantPC Dye (orange cap). Vortex until dissolved. The dye is a dry solid that goes into solution after solvent is added. Return the InstantPC Dye Solvent to -20 °C.
   Duration: 5 mins  
   Total time: 7 mins

4. Prepare Load/Wash solution. Add 6 mL of formic acid to a 6 mL glass graduated cylinder.
   Duration: 3 mins  
   Total time: 10 mins

5. Bring the volume up to 240 mL with acetonitrile.
   Duration: 3 mins  
   Total time: 13 mins
6. Transfer the solution in the graduated cylinder to a glass storage vessel. Cap tightly and swirl to mix.  
Duration: 1 min  Total time: 14 mins

7. Gly-X deglycosylation. Pipette 2 µL of Gly-X Denaturant into wells of a Gly-X Deglycosylation Plate for each sample you are preparing.  
Duration: 1 min  Total time: 15 mins

8. Add 20 µL of glycoprotein sample to each well. Mix well using the pipette. Make sure to change the pipette tip between transfer steps.  
Duration: 3 mins  Total time: 18 mins

9. Tap plate on the benchtop to collect samples at bottom of wells. Put the Gly-X Deglycosylation Plate on heater and incubate uncovered at 90 °C for 3 min.  
Duration: 5 mins  Total time: 23 mins

10. Remove plate and let sit on the bench at room temp for 2 min. Add 2 µL N-Glycanase working solution to each sample well on the plate. Mix well using the pipette.  
Duration: 3 mins  Total time: 26 mins

11. Tap plate on the benchtop to collect samples at bottom of wells. Put the Gly-X Deglycosylation Plate on heater and incubate uncovered at 50 °C for 3 min.  
Duration: 3 mins  Total time: 29 mins

12. Instant PC labeling. Add 5 µL InstantPC Dye solution to each sample well on the plate. Mix well using the pipette.  
Duration: 3 mins  Total time: 32 mins

13. Tap plate on benchtop to collect samples at bottom of wells. Put Gly-X Deglycosylation Plate on heater and incubate uncovered at 50 °C for 1 min.  
Duration: 2 mins  Total time: 34 mins

Duration: 1 min  Total time: 35 mins

15. Install the waste tray in the vacuum manifold.  
Duration: 1 min  Total time: 36 mins

16. Put the Gly-X Cleanup Plate on top of the vacuum manifold.  
Duration: 1 min  Total time: 37 mins

17. Add 400 µL of Load/Wash solution into wells of the Gly-X Cleanup Plate for every sample in your Gly-X Deglycosylation Plate.  
Duration: 3 mins  Total time: 40 mins
18. Add 150 µL of Load/Wash solution to the first sample well on the Gly-X Deglycosylation Plate. Mix well using the pipette.
Duration: 3 mins  Total time: 43 mins

19. Transfer the entire sample (~172 µL) from the Gly-X Deglycosylation Plate into the corresponding well of the Gly-X Cleanup Plate. Mix well using the pipette. Repeat steps 18 and 19 until each sample has been transferred to the Gly-X Cleanup Plate.
Duration: 3 mins  Total time: 46 mins

20. Apply vacuum (~5 in Hg) using a pump to draw the liquid through the well into the waste plate.
Duration: 3 mins  Total time: 49 mins

21. Add 600 µL of Load/Wash Solution to each well. Apply 2 in Hg vacuum again, let the solution pass through. Repeat this step two more times for a total of three washes.
Duration: 10 mins  Total time: 59 mins

22. Set the Gly-X Cleanup Plate aside. Install the Gly-X vacuum manifold spacer (black) and the Collection Plate (PCR plate).
Duration: 1 min  Total time: 60 mins

23. Place the Gly-X Cleanup Plate back on the vacuum manifold. Add 100 µL Gly-X InstantPC Eluent to each well of the Cleanup Plate containing sample. Apply <2 in Hg vacuum.
Duration: 3 mins  Total time: 63 mins

24. Take out the collection plate (PCR plate). Mix each sample prior to analysis. Cover plate with foil.
Duration: 3 mins  Total time: 66 mins

25. Put the collection plate inside the Multi Sampler and run analysis.
Duration: 1 min  Total time: 67 mins
Complete Suite of Glycan Sample Preparation and Analysis Consumables

Everything you need is now available from Agilent:
- Fast, easy-to-use sample prep kits with a variety of N-glycan labels
- Labeled N-glycan standards and libraries
- Unlabeled N-glycan standards and libraries
- Endo- and exoglycosidases for structural characterization of N-glycans
- HILIC columns and other LC supplies for every level of analysis from intact glycoprotein to released glycans or monosaccharides

To learn more, visit www.agilent.com/chem/glycananalysis