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Quick Start Guide

# Agilent AdvanceBio Gly-X InstantPC

## Quick N-glycan Sample Preparation

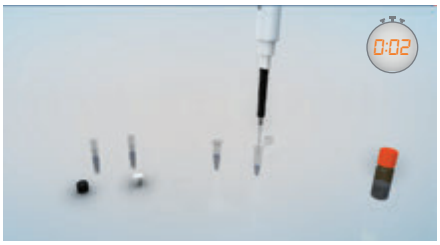
Agilent AdvanceBio Gly-X InstantPC reagents and consumables let you prepare samples for analysis including N-glycan release, labeling and cleanup, in as little as 1 hour using this simple step by step process.

You will need these additional items to prepare your samples:

| Lab supplies and equipment |
|---|
| Pipettes and pipette tips |
| Eppendorf tubes |
| Graduated cylinder (250 mL or larger) |
| Glass storage vessel (250 mL or larger) |
| Thermocycler |

| Additional reagents |
|---|
| Formic acid |
| Acetonitrile |


**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



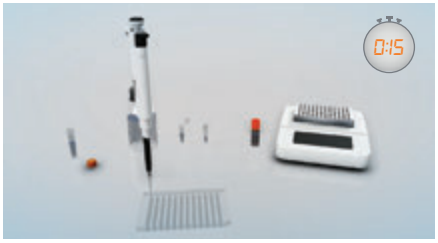

**2.** Add 2 µL of Digestion Buffer to each tube. **Make sure to change the pipette tip between transfer steps.** Return the N-glycanase and Digestion Buffer to 4 °C.
Duration: 1 min Total time: 2 mins


**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

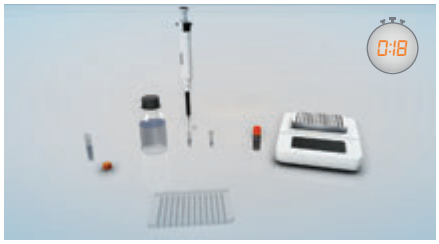

**4. Prepare Load/Wash solution.** Add 6 mL of formic acid to a 6 mL glass graduated cylinder.
Duration: 3 mins Total time: 10 mins


**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



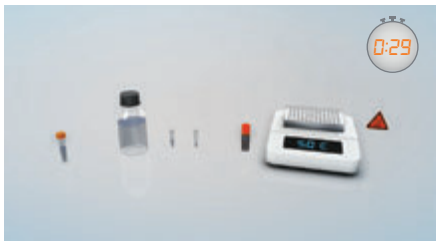

**7. Gly-X deglycosylation.** Pipette 2 µL of Gly-X Denaturant into wells of a Gly-X Deglycosylation Plate for each sample you are preparing.
Duration: 1 min Total time: 15 mins



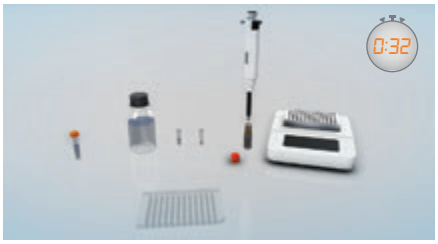

**8.** Add 20 µL of glycoprotein sample to each well. Mix well using the pipette. **Make sure to change the pipette tip between transfer steps.**
Duration: 3 mins Total time: 18 mins


**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

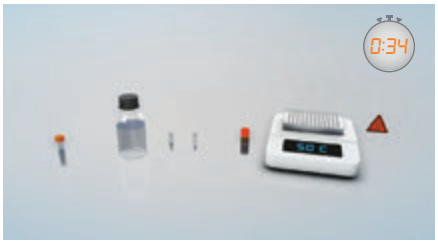

**10.** Remove plate and let sit on the bench at room temp for 2 min. Add 2 µL N-Glycanase working solution to each sample well on the plate. Mix well using the pipette.
Duration: 3 mins Total time: 26 mins



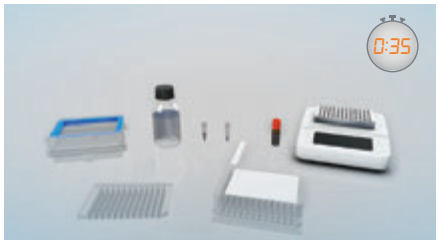

**11. Tap plate on the benchtop to collect samples at bottom of wells.** Put the Gly-X Deglycosylation Plate on heater and incubate uncovered at 50 °C for 3 min.
Duration: 3 mins Total time: 29 mins



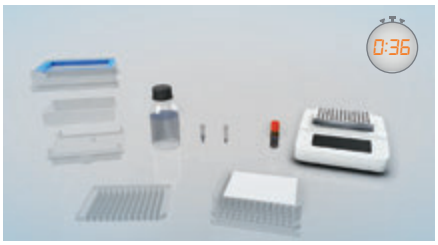

**12. Instant PC labeling.** Add 5 µL InstantPC Dye solution to each sample well on the plate. Mix well using the pipette.
Duration: 3 mins total time: 32 mins



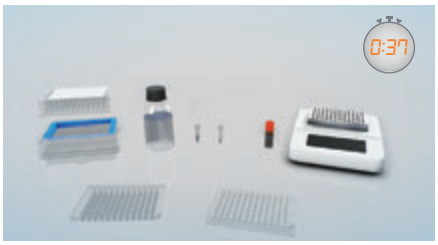

**13.** Tap plate on benchtop to collect samples at bottom of wells. Put Gly-X Deglycosylation Plate on heater and incubate uncovered at 50 °C for 1 min.
Duration: 2 mins Total time: 34 mins




**14. InstantPC cleanup.** Carefully remove the white caps on the Gly-X Cleanup Plate.
Duration: 1 min Total time: 35 mins



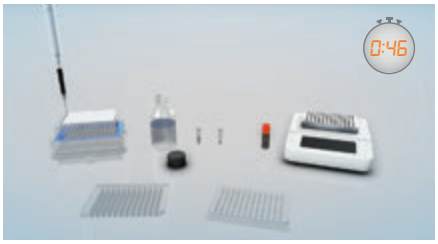

**15.** Install the waste tray in the vacuum manifold.
Duration: 1 min Total time: 36 mins




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

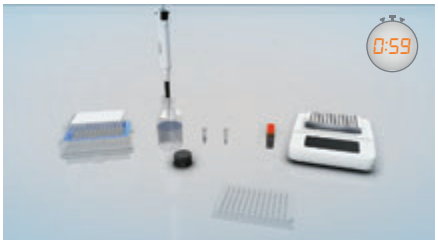

**17.** Add 400 µL of Load/Wash solution into wells of the Gly-X Cleanup Plate for every sample in your Gly-X Deglycosylation Plate.
Duration: 3 mins Total time: 40 mins


**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

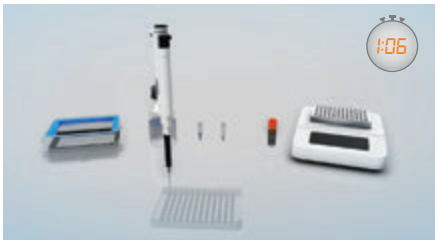

**20.** Apply vacuum (<5 in Hg) using a pump to draw the liquid through the well into the waste plate.
Duration: 3 mins Total time: 49 mins




**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**

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