

Glycan Profiling Quick Guide

LabChip® GXII Touch

Sample Preparation

Denature

1. Thaw and spin Denaturing Plate at 1200g for 1 minute.
2. Carefully remove plate seal.
3. Add 8µL of sample (monoclonal antibody) with concentration range of 1.25 to 7.5mg/mL (10µg to 60µg total protein) to Denaturing Plate. Mix by pipetting up and down or with a plate shaker.
4. Seal plate carefully with an adhesive plate seal.
5. Spin plate at 1200g for 1 minute.
6. Incubate for 10 minutes at 70°C using a PCR machine or heat block.

Digestion

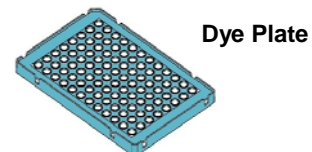
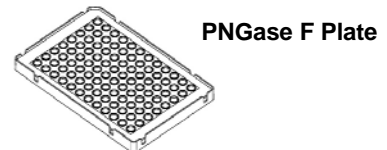
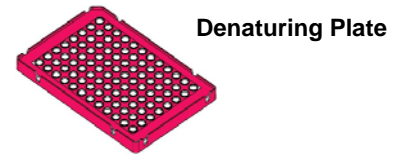
1. Thaw PNGase F Plate.
2. Spin both PNGase F Plate and Denaturing Plate at 1200g for 1 minute.
3. Carefully remove plate seals.
4. Transfer all denatured sample to PNGase F Plate. Mix by pipetting up and down or with a plate shaker.
5. Seal plate carefully with an adhesive plate seal.
6. Spin PNGase F Plate at 1200g for 1 minute.
7. Incubate for 1 hour at 37°C using a PCR machine or heat block.

Labeling

1. Thaw Dye Plate.
2. Spin both Dye Plate and PNGase F Plate at 1200g for 1 minute.
3. Carefully remove plate seals.
4. Transfer 8µL of digested sample to the Dye Plate. Mix by pipetting up and down or with a plate shaker.
5. Spin Dye Plate at 1200g for 1 minute.
6. Incubate the unsealed plate for 2 hours at 55°C, or until dry, using a PCR machine (lid open) or heat block.

Reconstitution

1. Add 100µL water (Milli®-Q or equivalent) to dried samples.
2. Seal plate carefully with an adhesive plate seal.
3. Mix samples on a plate shaker at maximum speed for at least 1 minute.
4. Spin plate at 1200g for 1 minute.
5. Carefully remove plate seal.
6. Run plate on LabChip GXII Touch.



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Chip Preparation Procedures

Note: The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.

Preparing the Buffer Tube

1. Add **750µL** water (Milli®-Q or equivalent) to the 0.75mL Buffer Tube.
2. Insert the Buffer Tube into the buffer slot on the LabChip GXII Touch instrument.



Buffer Tube

Preparing the Ladder Tube

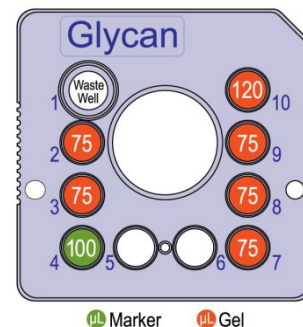
1. Add **145µL** of Ladder Diluent (purple circle) to one of the Ladder (yellow circle) tubes.
2. Vortex at highest speed for about 30 seconds and spin down.
3. Transfer **120µL** of prepared ladder to the 0.2mL Ladder Tube.
4. Insert the Ladder Tube into the ladder slot on the LabChip GXII Touch.



Ladder Tube

Preparing the Chip

1. Remove reagents from all wells of the chip using a vacuum.
2. Rinse and completely aspirate all wells twice with water (Milli®-Q or equivalent).
3. Aspirate any water that may have spilled onto the outside of the chip.
4. Add **75µL** of Gel Matrix (red circle) to chip wells 2, 3, 7, 8, and 9 using a Reverse Pipetting Technique.
5. Add **120µL** of Gel Matrix (red circle) to well 10 using a Reverse Pipetting Technique.
6. **For HT Glycan assay:**
 - Prepare Marker Solution by adding **125µL** of Marker Diluent (white circle) to one of the Marker (green circle) tubes. Vortex at highest speed for 30 seconds and spin down.
 - Transfer **100µL** of prepared marker solution to chip well 4. (*Note: Prepare marker solution just before loading the chip in the LabChip GXII Touch and starting the assay. Do not prepare marker solution in advance as the marker signal degrades over time.*)
7. **For HT Glycan Extended Range assay:**
 - Add **100µL** of Marker Diluent (white circle) to chip well 4.
8. Place the chip in the LabChip GXII Touch instrument to begin the assay.



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Chip Cleaning and Storage

After use, clean and store the chip in the chip storage container. The cleaning procedure can be conducted the following day, when running overnight.

1. Remove reagents from each well using a vacuum.
2. Rinse and completely aspirate each active well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli®-Q or equivalent).
3. Add **120 µL** water (Milli®-Q or equivalent) to each active well.
4. Cover all wells with Parafilm® and store at room temperature (20 - 25°C).

Assay Specifications

Amount of Sample Required	8 µL with concentration range of 1.25-7.5 mg/mL (10 - 60 µg of MAb total)
Reproducibility of %Area	HT Glycan assay: CV < 10% for a peak \geq 2.5% of total glycans HT Glycan Extended Range assay: CV < 10% for a peak \geq 2.5% of total glycans and at a concentration \geq 2.5 ng/µL CV < 25% for a peak \geq 2.5% of total glycans and at a concentration 1.0 - 2.5 ng/µL
Limit of Detection	HT Glycan assay: 1 ng of G0f standard (smallest amount of labeled G0f standard that can be detected) HT Glycan Extended Range assay: 1 ng of Man3, G1f, G2, and G2S2 standards (smallest amount of labeled glycan standard that can be detected)
Deglycosylation	>95% of all N-linked glycans will be released from MAb
Usable Size Range	HT Glycan assay: Appropriate for neutral glycans found on MAbs, some charged glycans may run outside of our usable range HT Glycan Extended Range assay: Appropriate for neutral and charged glycans found on MAbs
Sizing Reproducibility	CV < 2.5%
Sample Prep, Chip Prep, and Analysis Time	< 8 hours for one 96-well plate
Chip Lifetime	400 samples
Samples per Chip Prep	Up to 192 samples
Chip Preps per Reagent Kit	7 chip preps

Contact PerkinElmer

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LabChip Chip QC test data portal: <https://www.perkinelmer.com/tools/LabChipQCSearch>

LabChip Reagent CoA: <https://www.perkinelmer.com/tools/COASearch>

For the complete Glycan Profiling Assay User Guide, go to: <http://www.perkinelmer.com>

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Publication Date: January 23, 2020.

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