

ProfilerPro 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

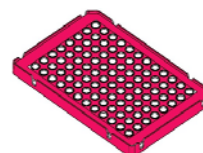
1. Thaw PNGase F Plate.
2. Spin both PNGase F Plate and Denaturing Plate at 1200g for 1 minute.
3. Carefully remove plate seals.
4. Transfer all denatured sample to PNGase F Plate. Mix by pipetting up and down or with a plate shaker.
5. Seal plate carefully with an adhesive plate seal.
6. Spin PNGase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

Labeling

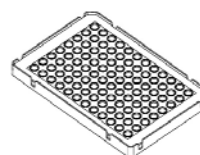
1. Thaw Dye Plate.
2. Spin both Dye Plate and PNGase F Plate at 1200g for 1 minute.
3. Carefully remove plate seals.
4. Transfer 8µL of digested sample to the Dye Plate. Mix by pipetting up and down or with a plate shaker.
5. Spin Dye Plate at 1200g for 1 minute.
6. Incubate the unsealed plate for 2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

Reconstitution

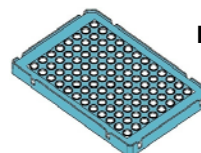
1. Add 100µL water (Milli®-Q or equivalent) to dried samples.
2. Seal plate carefully with an adhesive plate seal.
3. Mix samples on a plate shaker at maximum speed for at least 1 minute.
4. Spin plate at 1200g for 1 minute.
5. Carefully remove plate seal.
6. Run plate on LabChip GXII Touch.



Denaturing Plate



PNGase F Plate



Dye Plate



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Chip Preparation Procedures

Note: Allow the chip and all reagent kit components to equilibrate to room temperature for about 20-30 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

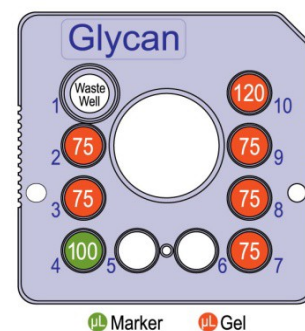
1. Add 145µL of Ladder Diluent (purple circle) to one of the Ladder (yellow circle) tubes.
2. Vortex at highest speed for about 30 seconds and spin down.
3. Transfer 120µL of prepared ladder to the 0.2mL Ladder Tube.
4. Insert the Ladder Tube into the ladder slot on the LabChip GXII Touch instrument.



Ladder Tube

Preparing the Chip

1. Remove reagents from all wells of the chip using a vacuum.
2. Rinse and aspirate all wells with water (Milli®-Q or equivalent). Repeat, and be careful to aspirate all the water in the wells and any water that may have spilled onto the outside of the chip.
3. Add 75µL of Gel Matrix (red circle) to chip wells 2, 3, 7, 8, and 9 and 120µL to well 10 using a Reverse Pipetting Technique.
4. Prepare marker solution by adding 125µL of Marker Diluent (white circle) to one of the Marker (green circle) tubes. Vortex at highest speed for about 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.*)
5. Place the chip in the LabChip GXII Touch instrument to begin the assay.



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Chip Cleaning and Storage

After use, the chip must be cleaned and stored in the chip 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 thoroughly 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. Make sure to 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	CV < 10% for a peak \geq 2.5% of total glycan
Limit of Detection	1 ng of G0f standard (smallest amount of labeled G0f standard that can be detected)
Deglycosylation	>95% of all N-linked glycans will be released from MAb
Usable Size Range	Appropriate for neutral glycans found on MAbs, some charged glycans may run outside of our usable range
Sizing Reproducibility	CV < 2.5%
Sample Prep, Chip Prep, and Analysis Time	< 8 hours for a 96-well plate
Chip Lifetime	HT: 400 samples 24: 400 samples
Samples per Chip Prep	HT: up to 192 samples LT: up to 48 samples
Chip Preps per Reagent Kit	HT: 4 chip preps LT: 4 chip preps

Contact PerkinElmer

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

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