

Glycan Profiling Quick Guide

LabChip® GXII Touch

Sample Preparation

Denature

1. Thaw the Denaturing reagent.
2. Add 8µL of sample (monoclonal antibody, concentration range of 1.25 to 7.5mg/mL, 10µg to 60µg total protein) into a PCR vial or 96-well plate.
3. Add 4µL of Denaturing solution.
4. Mix by pipetting up and down.
5. Spin down at 1200g for 1 minute.
6. Incubate for 10 minutes at 70°C using a PCR machine or heat block.

Digestion

1. Thaw the PNGase F reagent.
2. Transfer 5µL of PNGase F reagent to the denatured sample.
3. Mix by pipetting up and down.
4. Spin down at 1200g for 1 minute.
5. Incubate for 1 hour at 37°C using a PCR machine or heat block.

Labeling

1. Thaw the Dye.
2. Transfer 8µL of the digested sample into a PCR vial or 96-well plate.
3. Add 5µL of Dye.
4. Mix by pipetting up and down.
5. Spin down at 1200g for 1 minute.
6. Incubate for 2 hours at 55°C, or until dry, using a PCR machine (lid open) or heat block.

Reconstitution

1. Add 100µL of water (Milli®-Q or equivalent) to dried samples.
2. Cap the vials or seal the plate.
3. Mix thoroughly on a plate shaker at maximum speed for 1 minute or until completely dissolved.
4. Spin down at 1200g for 1 minute.
5. Run the plate on the LabChip GXII Touch.



Figure 1. 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

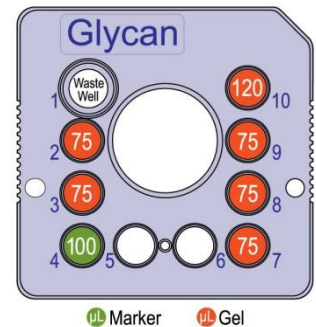
1. Add **145µL** of Ladder Diluent (purple) to one of the Ladder (yellow) tubes.
2. Vortex at highest speed for about 30 seconds and spin down.
3. Transfer **120µL** of prepared ladder to the 0.2mL Ladder Tube.
4. Insert the Ladder Tube into the ladder slot on the LabChip GXII Touch.



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) to chip wells 2, 3, 7, 8, and 9 using a Reverse Pipetting Technique.
5. Add **120µL** of Gel Matrix (red) to well 10 using a Reverse Pipetting Technique.
6. **For HT Glycan assay:**
 - Prepare Marker Solution by adding **125µL** of Marker Diluent (white) to one of the Marker (green) 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) 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 **120µL** 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 | 8µL with concentration range of 1.25-7.5mg/mL (10 - 60µg 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 2.5ng/µL CV < 25% for a peak \geq 2.5% of total glycans and at a concentration 1.0 - 2.5ng/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 |



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

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

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

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