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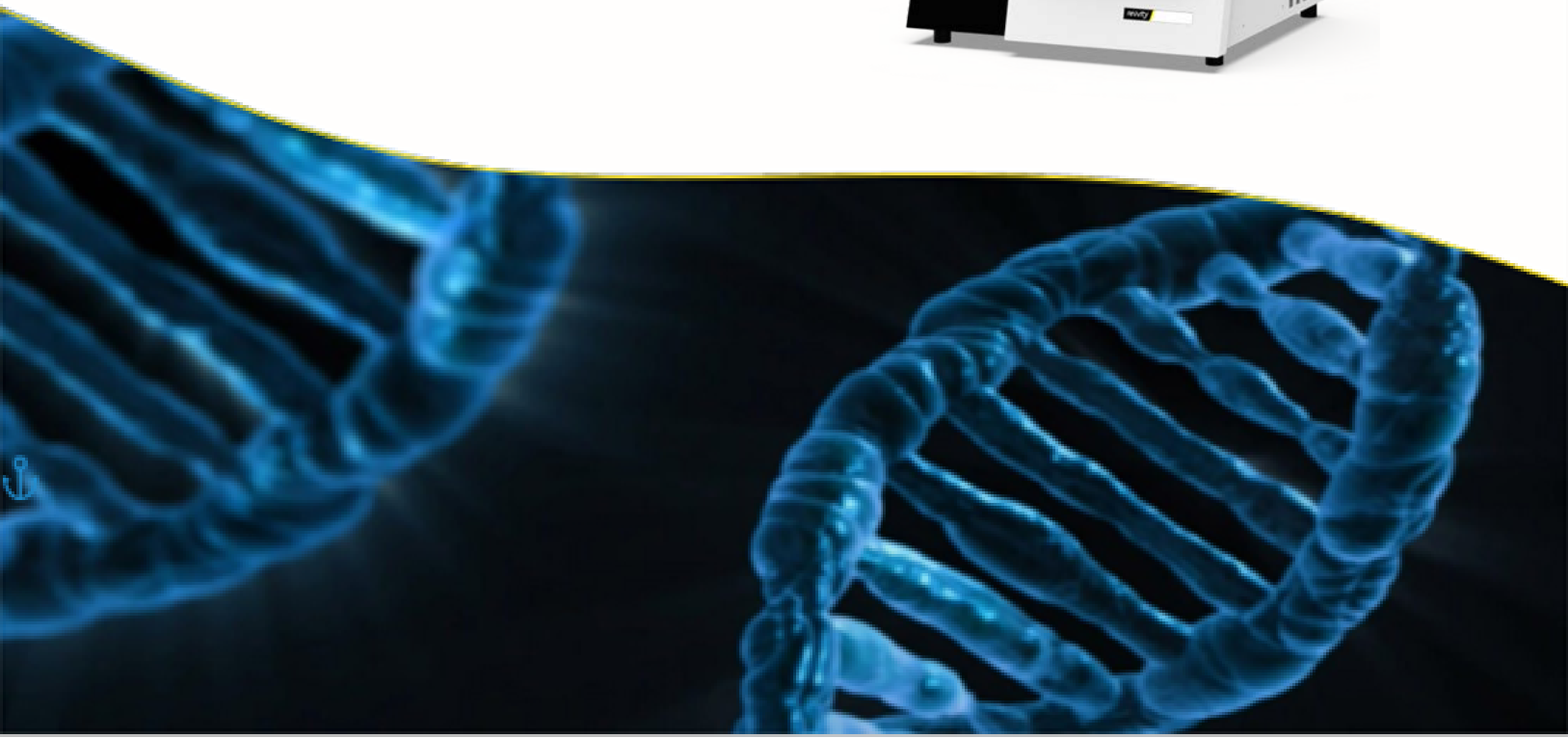
## LabChip User Guide

# Glycan Profiling Assay User Guide

For LabChip® GXII Touch

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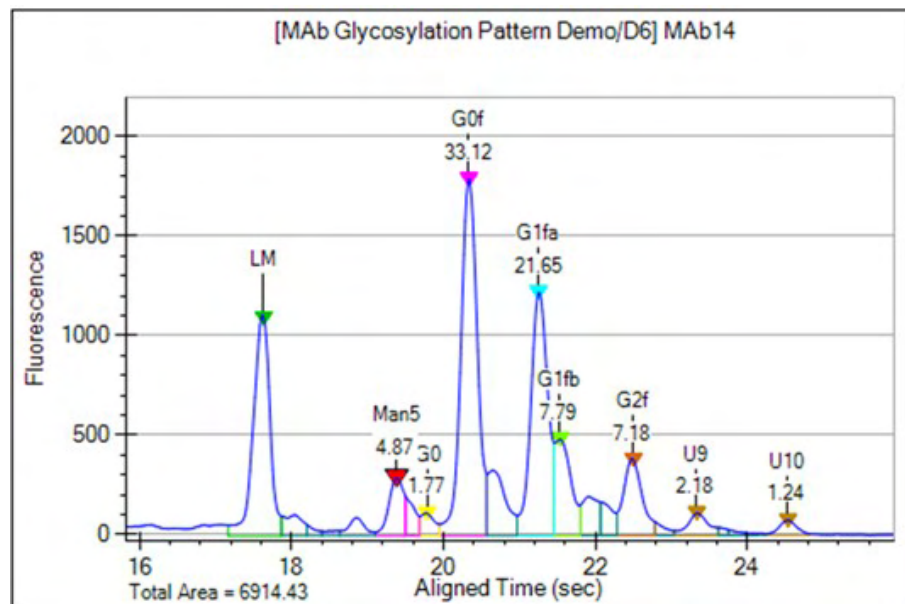
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# Introduction

## Applications

The Glycan Profiling system provides a high throughput method for analysis of the glycosylation patterns of monoclonal antibodies (MAb) and other glycosylated proteins. The system provides methods for: 1) deglycosylation, where N-linked glycans are released enzymatically using PNGase F; 2) fluorescent labeling of the released glycans in presence of released proteins; 3) separation by microchip based capillary electrophoresis (CE); and 4) detection and analysis using the LabChip GXII Touch instrument. Fast separation time - less than 45 seconds per sample - makes this method valuable for use in high throughput screening of antibody glycosylation patterns in early stage development.

The microchip-based CE analysis gives resolution sufficient to determine the relative abundance of the major N-glycan types found on antibodies, which contain primarily neutral N-linked glycans consisting of complex, hybrids, and high-mannose type oligosaccharides.



**Figure 1. Glycosylation profile of a therapeutic MAb. Relative abundance of the major glycan types can be reliably quantified.**

## Features

- Fast separation time - less than 45 seconds per sample, about 1.5 hours for 96 samples.
- Software determines the relative amounts of each glycan present.
- All-included reagent set provides predictable, reproducible results.
- Reagents provided in reagent vials.
- Automatic sampling from a 96-well plate.

# Specifications

## Assay Specifications

**Table 1. Assay Specifications**

Amount of Sample Required	8 $\mu$ L with concentration range of 1.25-7.5 mg/mL (10-60 $\mu$ g of MAb total)
Reproducibility of %Area	<b>HT Glycan assay:</b> CV < 10% for a peak $\geq$ 2.5% of total glycans <b>HT Glycan Extended Range assay:</b> CV < 10% for a peak $\geq$ 2.5% of total glycans and at a concentration $\geq$ 2.5 ng/ $\mu$ L CV < 25% for peak $\geq$ 2.5% of total glycans and concentration 1.0 - 2.5 ng/ $\mu$ L
Limit of Detection	<b>HT Glycan assay:</b> 1 ng of G0f standard (smallest amount of labeled G0f standard that can be detected) <b>HT Glycan Extended Range assay:</b> 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	<b>HT Glycan assay:</b> Appropriate for neutral glycans found on MAbs, some charged glycans may run outside of our usable range <b>HT Glycan Extended Range assay:</b> 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

## Storage Conditions

**Release and Labeling Kit Storage:** Store reagent vials at -20°C.

**Chip Storage:** Prior to use, store chips at 2 - 8°C. After first use, store chips at room temperature (20 - 25°C) for up to 30 days.

**Reagent Kit Storage:** Store all reagents at 2 - 8°C.

**CRITICAL:**

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

## Release and Labeling Kit Contents

**Glycan Release and Labeling Kit, P/N CLS155434**






**Table 2. Reagent Vials**

Item	Vial Contents	Quantity
Denaturing Reagent	Glycoprotein denaturing reagent	4 vials, 0.15 mL each
PNGase F Reagent	Oligosaccharides releasing reagent	4 vials, 0.15 mL each
Dye	Oligosaccharides labeling reagent	4 vials, 0.15 mL each

## Reagent Kit Contents

**Glycan LabChip Reagent Kit, P/N 760525**

**Table 3. Reagents**

Reagent	Vial	Quantity
Glycan Gel Matrix	Red 	2 vials, 1.8 mL each
Glycan Marker (dry)	Green 	4 vials
Glycan Marker Diluent	White 	1 vial, 0.65 mL
Glycan Ladder (dry)	Yellow 	4 vials
Glycan Ladder Diluent	Purple 	1 vial, 0.75 mL

**Table 4. Consumables**

Item	Supplier and Catalog Number	Quantity
Detection Window Cleaning Cloth	VWR <sup>®</sup> , Cat. # 21912-046	1
Swab	ITW Texwipe <sup>®</sup> , Cat. # TX758B	3
Ladder Tubes 0.2 mL	(Not sold separately)	10
Buffer Tubes 0.75 mL	(Not sold separately)	10

## High Resolution LabChips

**Table 5. High Resolution LabChips**

Item	Part Number
HT High Resolution LabChip (for use with LabChip GXII Touch HT)	760524
24 High Resolution LabChip (for use with LabChip GXII Touch 24 Or HT)	CLS138951

## Additional Items Required

- Revvity Hard-Shell thin-wall 96-well skirted PCR plate (blue), P/N 6008870 (recommended).
- Heating device: a PCR thermocycler. (A heat block that accepts a 96-well PCR plate will also work. An incubator may work but is less desirable.)
- Adhesive plate seals (PCR type with a minimum thermal range of -20°C to 80°C. For example, Beckman Coulter Cat. # 538619 or Axygen Cat. # PCR-AS-200).
- Centrifuge with rotor that accepts a 96-well PCR plate.
- Optional: Plate shaker (for example, VarioMag Magnetic Shaker, Cat. # 51110, 2000 rpm, amplitude = 2 mm).

# Safety and Usage

## Safety Warnings and Precautions

### CAUTION

*We recommend that this product and components be handled only by those who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. All chemicals should be considered potentially hazardous. Wear suitable protective clothing, such as laboratory overalls, safety glasses, and gloves when handling chemical reagents. Avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.*

### WARNING!



*Denaturing Buffer, Ladder, and Gel Matrix contain SDS. Avoid inhalation and contact with skin and eyes.*

## Usage

The Glycan Profiling Assay is for use with the LabChip GXII Touch instrument. LabChip GXII Touch instruments are for research use only and not for use in diagnostic procedures.



# Sample Preparation Procedures

To prepare the samples, follow the procedures below:

- 1 [“Denature” on page 9](#)
- 2 [“Digestion” on page 10](#)
- 3 [“Labeling” on page 11](#)
- 4 [“Reconstitution” on page 12](#)
- 5 See [“Chip Preparation Procedures” on page 13](#)

## Denature

### Hints

- **Spin-down:** Spin down all reagent vials before removing the cap to collect any reagent that may be attached to the vial cap. Keep reagents frozen at all times. Remove reagents from freezer only prior to use.
- **Aliquoting the reagents:** The Denaturing, PNGase- F, and Dye reagents can be aliquoted into Eppendorf tubes if running fewer than 72 samples. For best results, aliquot reagents into pre-cooled tubes and freeze immediately after aliquoting.
- **Storage:** When aliquoting Denaturing, PNGase F, or Dye reagents, freeze the vials immediately after aliquoting.
- **Plate Seals:** Use plate seals with a minimum thermal range of -20°C to 80°C. For all plate sealing, use of a rubber roller is recommended.

## Procedure

- 1 Thaw and spin Denaturing reagent at 1200 g for 1 minute.
- 2 Add 8  $\mu$ L of sample (monoclonal antibody, concentration range of 1.25-7.5 mg/mL, 10  $\mu$ g to 60  $\mu$ g total protein) into a PCR vial or a 96-well plate. Add 4  $\mu$ L of Denaturing Reagent. Mix by pipetting up and down or with a plate shaker. Spin down at 1200g for 1 minute.
  - a Samples should be at least one-step purified. Crude sample media or buffers that contain sugars may interfere with the labeling efficiency of the desired glycans.
  - b For a control blank sample, add 8  $\mu$ L of 18 Megohm, 0.22- $\mu$ m filtered water (Milli-Q<sup>®</sup> or equivalent) instead of sample and follow all digestion and labeling steps.
- 3 Seal plate carefully with an adhesive plate seal. **Note:** Ensure the plate is completely sealed to prevent evaporation.
- 4 Spin Denaturing samples at 1200 g for 1 minute.
- 5 Incubate Denaturing samples for 10 minutes at 70°C using a PCR machine (with lid temperature at 70°C), heat block, or incubator.

## Digestion

### Hints

- **Break point:** If needed, after digestion, samples can be frozen at -20°C and labeled the next day. Overnight digestion is not recommended.
- **Incubators:** Use of incubators or ovens for incubation is not recommended. If using an incubator for the labeling step, be careful of acetic acid fumes when opening the incubator door.

## Procedure

- 1 Thaw PNGase F reagent.
- 2 Spin both PNGase F reagent and Denaturing samples at 1200 g for 1 minute.
- 3 Carefully remove plate seal.
- 4 Transfer 5 $\mu$ L of PNGase F to the Denaturing samples. Mix by pipetting up and down or with a plate shaker.

- 5 Seal plate carefully with an adhesive plate seal. **Note:** Ensure the plate is completely sealed to prevent evaporation.
- 6 Spin PNGase F samples at 1200 g for 1 minute.
- 7 Incubate for 1 hour at 37°C using a PCR machine (with lid temperature at 37°C), heat block, or incubator.

## Labeling

### Hints

- **Can more than 8  $\mu$ L of digested sample be labeled?** Sample buffer conditions resulting in optimal glycan peak intensity was observed when labeling 8  $\mu$ L of the digested sample. Labeling more or less will result in lower signal intensity.
- **Break point:** If needed, after labeling, dried samples may be sealed and stored frozen at -20°C and reconstituted the next day. Overnight labeling is not recommended.
- **Mixing:** It is best to use a plate shaker with max speed.
- **Incubators:** Carefully inspect samples to ensure they are completely dry. It is likely that using an incubator or oven will increase the drying time to more than 2 hours.

### Procedure

- 1 Thaw Dye reagent.
- 2 Spin both Dye reagent and Digestion samples at 1200 g for 1 minute.
- 3 Carefully remove plate seal.
- 4 Transfer 8 $\mu$ L of Digested sample to a new PCR vial or 96-well plate. Add 5 $\mu$ L of Dye reagent. Mix by pipetting up and down or with a plate shaker.
- 5 Spin Dye Plate at 1200 g for 1 minute.
- 6 Incubate the unsealed plate for 2 hours at 55°C (or until sample is completely dry) using a PCR machine (with lid open) or heat block.

**Caution:** *The dye contains acetic acid. Beware of fumes. Incubating under a fume hood is recommended. Sample must be completely dry to ensure sufficient labeling.*

## Reconstitution

- 1 Add 100µL of water (Milli-Q® or equivalent) to dried samples.
- 2 Seal plate carefully with an adhesive plate seal. **Note:** Ensure the plate is completely sealed to prevent spilling during mixing.
- 3 Mix samples on a plate shaker at maximum speed for at least 1 minute. Verify the pellet has completely dissolved.
- 4 Spin plate at 1200 g for 1 minute.

***Note:** After spinning there may be a white pellet at the bottom of the wells. Be careful not to disturb the pellet as it may cause a chip sipper clog.*

- 5 Carefully remove the plate seal. The plate is ready to run on the LabChip GXII Touch.

# Chip Preparation Procedures

## CRITICAL:

- The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.
- The assay requires exact and consistent adherence to the protocol as shown in this guide, or results may be compromised by increased variability.
- Fresh Milli-Q® water should be obtained the day of the assay.
- Adherence to the full vortex time is important for assay performance.

## Preparing the Buffer Tube

- 1 Add **750 µL** of water (Milli-Q® or equivalent) to the 0.75 mL Buffer Tube. Ensure there are no air bubbles in the Buffer Tube.
- 2 Insert the Buffer Tube into the buffer slot on the LabChip GXII Touch instrument.

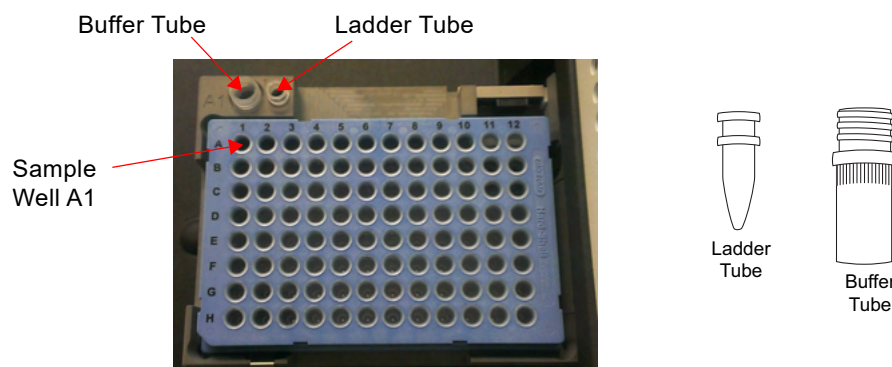


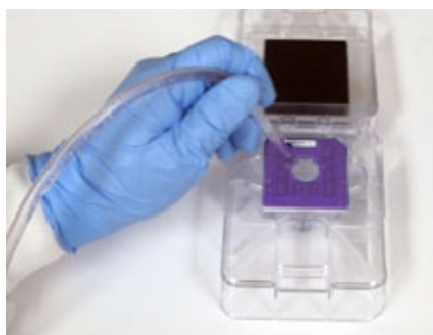
Figure 2. Locations of the Buffer Tube and Ladder Tube in the LabChip GXII Touch instrument

## Preparing the Ladder Tube

- 1 Add **145 µL** of Glycan Ladder Diluent (purple cap ●) to one of the Ladder tubes.
- 2 Vortex at highest speed for about 30 seconds and spin down. Make sure the ladder is completely reconstituted.
- 3 Transfer **120 µL** of prepared ladder to the 0.2 mL Ladder Tube. Ensure there are no air bubbles in the Ladder Tube.
- 4 Insert the Ladder Tube into the ladder slot on the LabChip GXII Touch instrument.

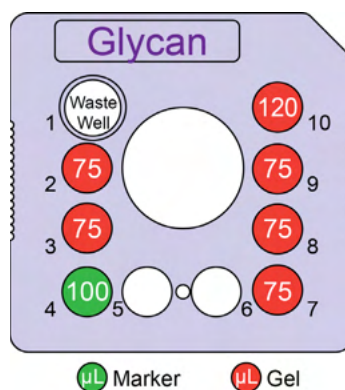
## Preparing the Chip

- 1 Allow the chip to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.
- 2 Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells (see [Figure 3](#)). For details on how to set up a vacuum line, see [page 44](#).
- 3 Rinse and completely aspirate each active chip well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q<sup>®</sup> or equivalent). Do not allow active wells to remain dry.
- 4 If any water spilled onto the top or bottom of the chip surfaces during rinsing, aspirate using a vacuum line. DO NOT move the tip over the central region of the detection window. Use the provided Detection Window Cleaning Cloth to clean the chip detection window.



**Figure 3. Using a vacuum to aspirate the chip wells is more effective than using a pipette. See [page 44](#) for more details.**

- 5 Using a reverse pipetting technique, add 75  $\mu\text{L}$  of Gel Matrix (red cap ●) to chip wells 2, 3, 7, 8, and 9, and 120  $\mu\text{L}$  to well 10 ([Figure 5](#)).



**Figure 4. Reagent Placement (HT and LT)**

**Note:** Prepare Marker Solution just before loading the chip into the LabChip GXII Touch and starting the assay. Do not prepare Marker Solution in advance because the marker signal degrades over time.

**6** For **Glycan** assay:

- a** Prepare Marker Solution by adding **125  $\mu$ L** of Marker Diluent (white cap ○) to one of the Marker tubes ●.
- b** Vortex at the highest speed for 30 seconds and spin down. Make sure marker is completely reconstituted.
- c** Transfer **100  $\mu$ L** of prepared Marker Solution to chip well 4.

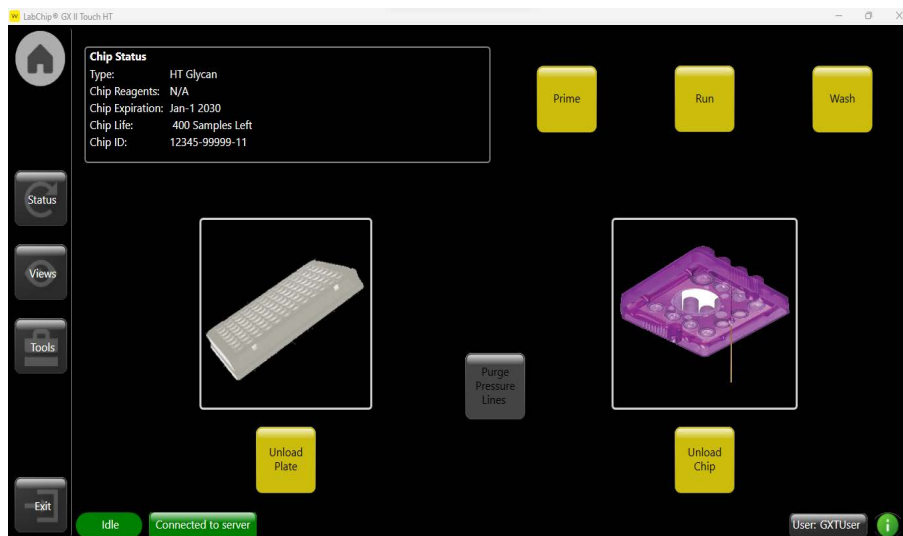
**7** For **Glycan Extended Range** assay:

- Add **100  $\mu$ L** of Marker Diluent (white cap ○) to chip well 4.

## Inserting a Chip into the LabChip GXII Touch Instrument

**Note:** Clean the O-rings on the top plate of the chip interface on the LabChip GXII Touch instrument daily. Use the provided lint-free swab dampened with water (Milli-Q<sup>®</sup> or equivalent) to clean the O-rings using a circular motion. Allow the O-rings to dry before inserting a chip.

- 1 Check that the sample plate, Buffer Tube, and Ladder Tube are placed on the instrument properly.
- 2 Remove the chip from the chip storage container and inspect the chip window. Clean BOTH sides of the chip window with the provided Detection Window Cleaning Cloth dampened with a 70% isopropanol solution in DI water.
- 3 Touch the **Unload Chip** button on the Home screen (Figure 5).

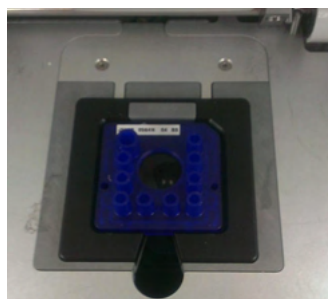


**Figure 5. Home Screen**

- 4 Insert the chip into the LabChip GXII Touch instrument (Figure 6) and close the chip door securely.

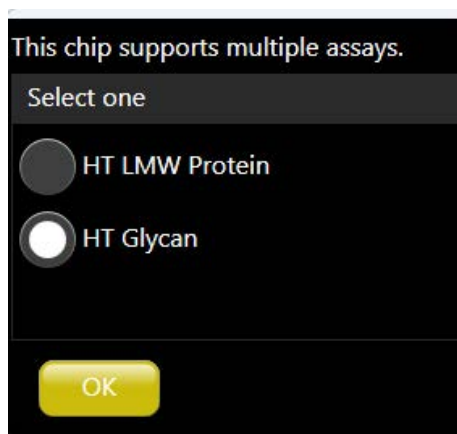
**Note:** Do not keep the chip door open for any length of time. Dye is sensitive to light and can be photobleached.





**Figure 6. Chip in the LabChip GXII Touch Instrument**

- 5 Touch the **Load Plate** button on the Home screen (Figure 5) to retract the sample plate and move the sipper to the Buffer Tube. The Assay Choice screen opens as shown in Figure 7.



**Figure 7. Assay Choice Screen**

## Running the Assay

**Note:** Chips can be primed independently from running assays. Touch the Prime button on the Home screen (Figure 5). Select the desired assay from the Assay drop-down list (see Figure 9). Touch the Prime button on the Chip Priming screen. Make sure the Buffer Tube is placed in the instrument.

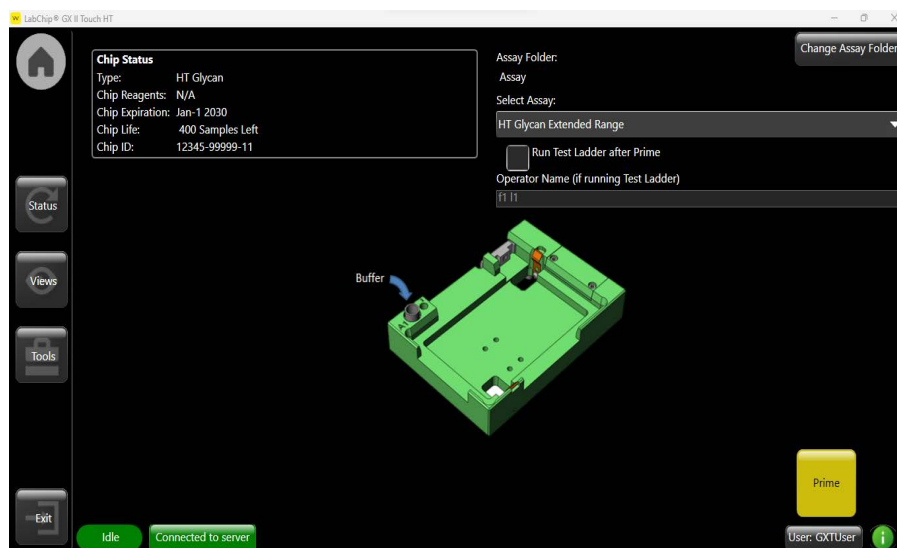


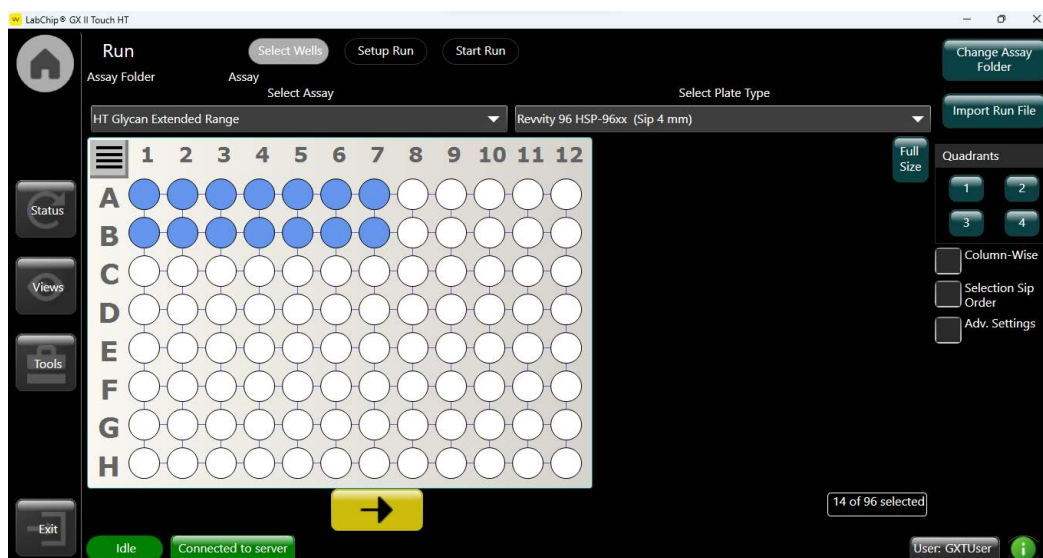
Figure 8. Prime Screen

- 1 Touch the **Run** button on the Home screen (see Figure 5 on page 16).
- 2 On the **Select Wells** tab, select the desired assay type: **HT Glycan Extended Range** or **HT Glycan** in the Select Assay drop-down list (see Figure 9).



Figure 9. Select Assay Drop-Down List

- 3 Specify the plate name, well pattern, and whether to read wells in columns or rows (see [Figure 10](#)). If desired, specify the number of times each well is sampled under **Adv. Settings**. Touch the green arrow.



**Figure 10. Select Wells Tab**

- 4 In the **Setup Run** tab, select the operator name, the option to read barcodes, the destination of the file, the use of sample names, expected peaks, and excluded peaks, and the file name convention. Select **Auto Export** to export results tables automatically (see [Figure 11](#)). Touch the green arrow.

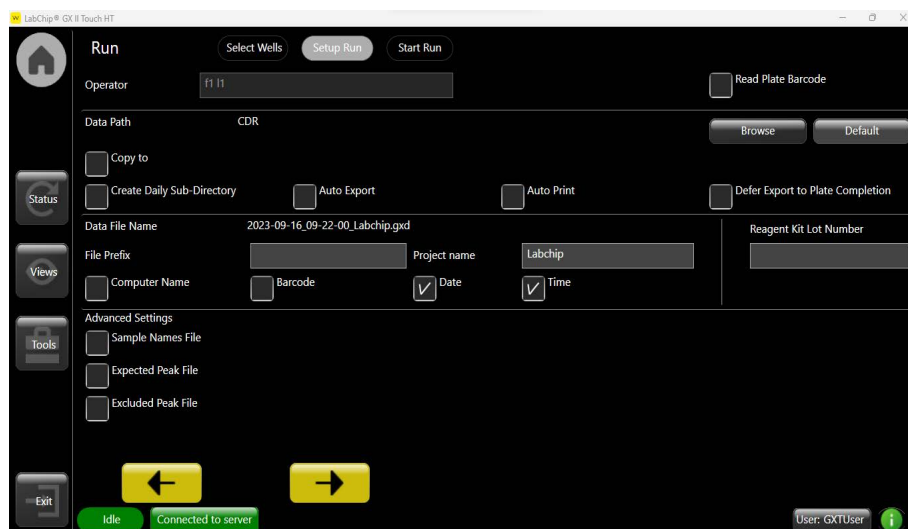


Figure 11. Setup Run Tab

5 Touch **Start** to begin the run.

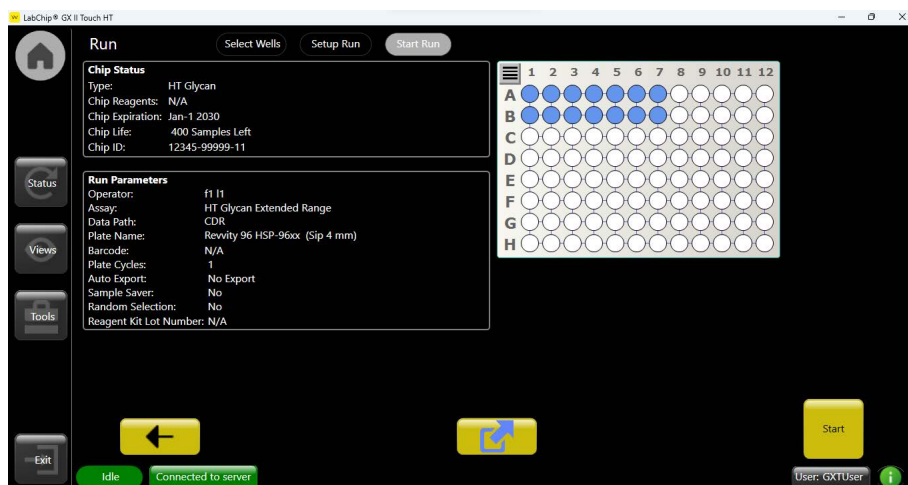


Figure 12. Start Run Tab

## Cleaning and Storing the Chip

After use, clean and store the chip in the chip storage container.

- 1 Place the chip into the chip storage container. Verify the sipper is submerged in the fluid reservoir.
- 2 Remove the reagents from each well of the chip using vacuum.
- 3 Rinse and completely aspirate each active well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q<sup>®</sup> or equivalent).
- 4 Add **120 µL** water (Milli-Q<sup>®</sup> or equivalent) to the active wells.
- 5 Cover the wells with Parafilm<sup>®</sup> to prevent evaporation and store the chip at room temperature (20 - 25°C) until next use. Allowing chip wells to dry may lead to changes in chip performance. The chip must be used to its lifetime (to the total number of samples) within 30 days of analyzing the first plate of samples. See [“Assay Specifications” on page 5](#) for Chip Lifetime.

## Chip Cartridge Cleaning

### 1 Daily

- a Inspect the inside of the chip cartridge and O-rings for debris.
- b Use the provided lint-free swab dampened with water (Milli-Q<sup>®</sup> or equivalent) to clean the O-rings using a circular motion. If the O-rings stick to the chip or a pressure leak is detected, perform the more extensive monthly cleaning procedure.

### 2 Monthly

- a To reduce pressure leaks at the chip interface, clean the O-rings frequently. Remove the O-rings from the top plate of the chip interface on the LabChip GXII Touch instrument. Soak O-rings in water (Milli-Q<sup>®</sup> or equivalent) for a few minutes. Clean the O-ring faces by rubbing between two fingers. Wear gloves.
- b To reduce the occurrence of current leaks, clean the chip interface frequently. Clean the top plate of the chip interface using the provided lint free swab dampened with water (Milli-Q<sup>®</sup> or equivalent).
- c Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

# Analyzing Data

## Peak Identification and Size in the Electropherogram

The LabChip GX Touch software analyzes the electropherograms, assigning a size (among other things) to each observed peak. It may not be intuitively obvious what this size means given that glycan molecules with the same mass can sometimes have very different apparent electrophoretic mobility. During the labeling process, the glycans are derivatized with a charged fluorophore. The electrophoretic mobility of each glycan in our specific separation medium is dependent on its charge, mass, and structure - where structural elements such as monosaccharide composition (mannose, glucose, galactose), glycosidic linkage position (C2, C3, C4, C6), and anomericity ( $\alpha$ - vs  $\beta$ -) have been shown to affect electrophoretic mobility. Considering these complexities, the size that the software assigns does not have a direct physical meaning, like the size of a DNA molecule in base pairs or the size of a protein in Daltons. Rather, the sizes assigned to glycan peaks by the LabChip GX Touch software relate the migration time of each peak to the migration time of the glucose peaks in the glycan ladder. It is a way of normalizing the migration time of each peak so that results can be more easily compared across different runs, different instruments, different reagent lots, etc.

The Ladder is a mixture of  $\alpha(1-6)$ -linked glucose oligosaccharides with a various number of monomeric glucose units. The Lower Marker is maltohexaose which runs with a size of 6.6 RGU.

Figure 13 shows the Lower Marker (LM) and ladder peaks (L) annotated with RGU units.

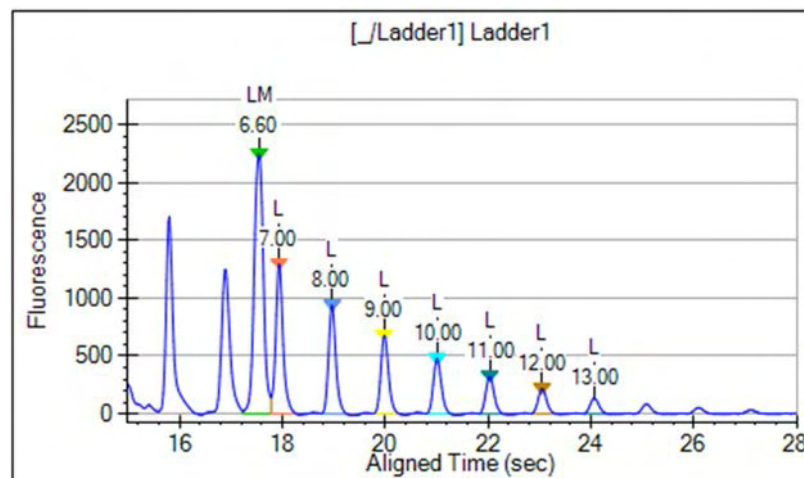
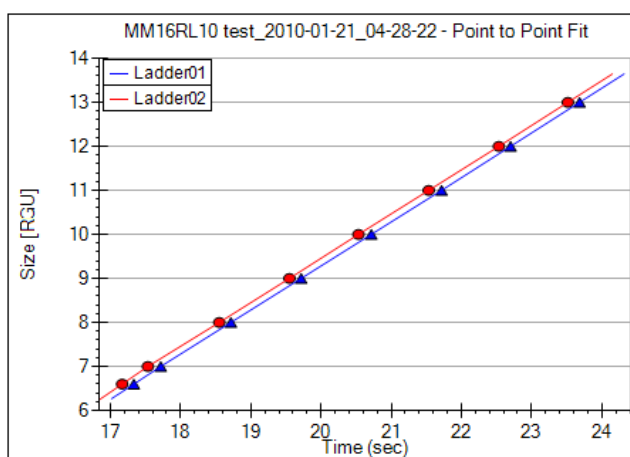


Figure 13. Ladder peaks (L) Lower Marker (LM) annotated with RGU units

For example, if a peak has a migration time half way between the 9-mer and 10-mer  $\alpha(1-6)$ -linked glucose oligosaccharides in the ladder, that peak will be assigned a size of 9.5 Relative Glucose Units (RGU). These units are specific to the particular Gel Matrix and the fluorophore that are provided in the Glycan Profiling kits. They may not correspond to Glucose Units assigned by other analysis methods.



**Figure 14. Example of two standard curves that relate migration time to size in Relative Glucose Units (RGU)**

The LabChip GX Touch software allows the user to assign names to peaks based on their size. If you observe that the G0f peak in your sample has a size of 9.3 RGU, you can name the peak “G0f” using the Expected Glycans tab on the Analysis Settings window.

## Identifying Peaks by Co-migration with Known Glycan Standards

It is sometimes useful to compare the migration time of peaks in the samples to the migration time of known glycan standards, to help identify the peaks in the samples. When running glycan standards, it is important that the buffer the standards are presented in is closely matched to the buffer the samples are presented in. If the buffers are not well matched, the migration time of the standards may not correspond to the migration time of the sample. A simple method to achieve matched buffers is to label the standards using the reagents from the Glycan Release and Labeling kit. Prepare the standards the same way as you prepared the test samples. Any dilutions of the standards prior to addition in the Denaturing Plate should be done with water (Milli-Q<sup>®</sup> or equivalent).

Figure 15 shows a mix of glycan standards compared to an IgG sample, run in well-matched buffers. Blue = IgG, Red = Mix of six glycan standards (Man5, G0, G0f, G1f, G2, G2f). The standards were run at about 50 ng/ $\mu$ L concentration.

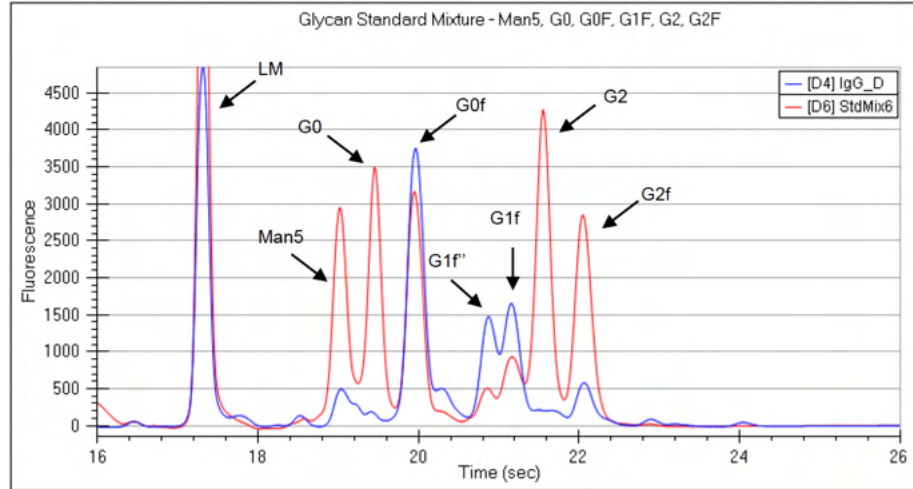


Figure 15. Standards and an IgG Sample



## Results

In the Glycan Ladder, the Lower Marker should be the tallest peak. Following the Lower Marker peak are 7 ladder peaks with Relative Glucose Units (RGUs) of 7 to 13.

Figure 16 shows the Glycan Ladder: Lower Marker (LM) and ladder peaks (L) annotated with RGU units. For monoclonal antibody samples the Lower Marker should migrate before the major N-glycan peaks (Man5, G0, G0f, G1f', G1f, and G2f).

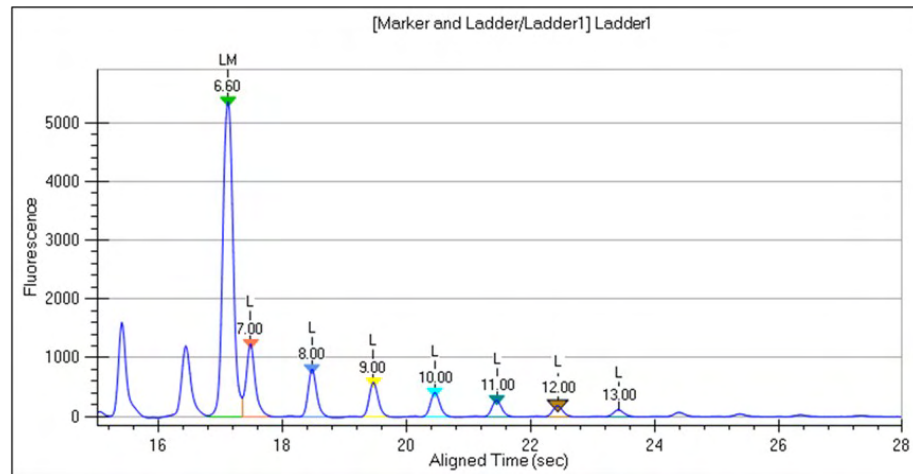


Figure 16. Glycan Ladder

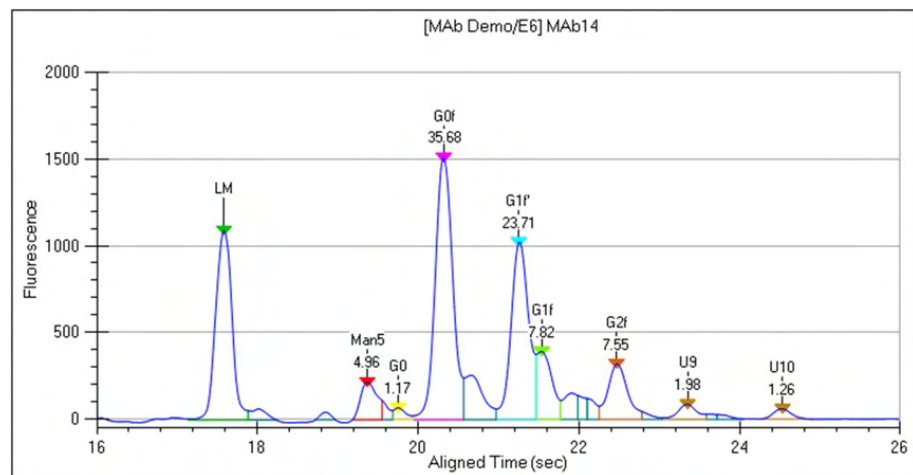
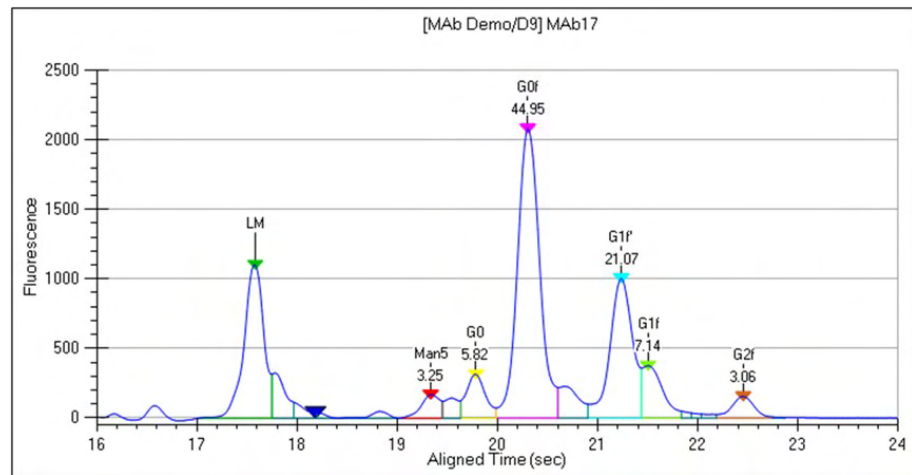


Figure 17. MAb14: Major N-glycan peaks are annotated with percent area



**Figure 18. MAb17: Major N-glycan peaks are annotated with percent area**

# Troubleshooting

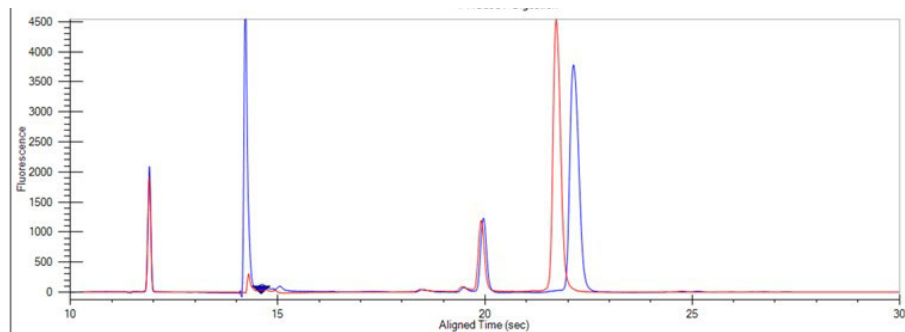
*Note: Some of the data examples shown in this section were generated with assays other than the assay described in this user guide.*

## How to Test for Complete Digestion

Revvity recommends testing for complete deglycosylation by PNGase F using the Protein Express assay. Compare the digested sample (unlabeled) to the undigested sample. Follow the protocol for the Protein Express Kit for reduced conditions.

*Note: It may be necessary to dilute the digested sample depending on the starting concentration. A starting concentration of 2 mg/mL or below is recommended for the Protein Express assay.*

Under reduced conditions you will see a shift from the heavy chain (HC) to the non-glycosylated heavy chain (NGHC) for the digested sample.



**Figure 19. Blue: control (undigested), Red: Digested MAb**

### Symptom: Digestion is incomplete.

#### Possible causes:

- 1 The samples were incompletely denatured.
- 2 There was an insufficient amount of PNGase F in the reaction.

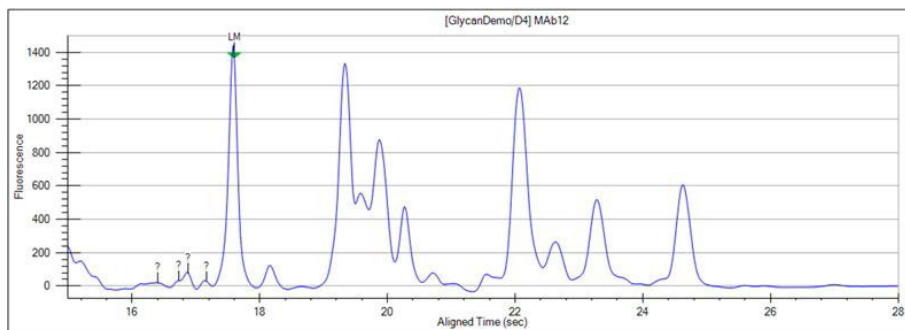
#### What to do:

- 1 Repeat the sample preparation, being careful to mix the Denaturing solution. Small deviations in the amount of denaturing buffer added to the sample may significantly affect the denaturing reaction. Additionally, verify that the samples are being denatured at 70°C for 10 minutes.

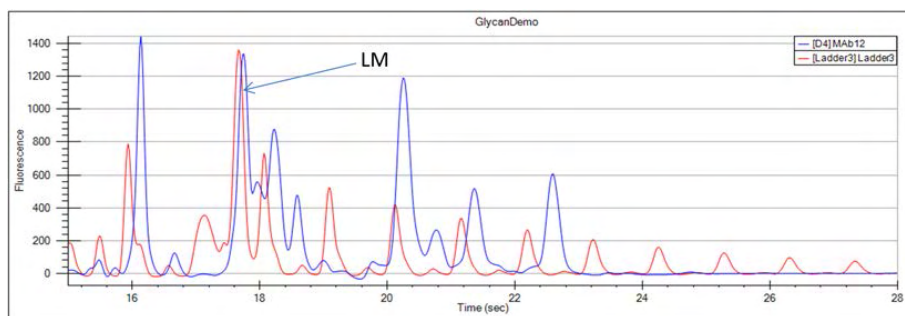
- 2 If the sample tested was on the high end of the Glycan assay concentration range, then dilute the sample down to 5 mg/mL and repeat sample preparation. Some MABs may be more difficult to deglycosylate than others. In this event, a greater amount of PNGase F to MAb and longer incubation time may be needed to completely deglycosylate samples.
- 3 Repeat the sample preparation, being careful to mix the PNGase F solution. Small deviations in the amount of PNGase F solution added to the reaction mix may significantly affect activity of the PNGase F enzyme. Verify that the digestion plate is incubated at 37°C for 1 hour or longer.

**Symptom: Peaks ahead of the marker, so Lower Marker is misidentified.**

If the sample contains peaks that run faster than the lower marker, the software may incorrectly identify a sample peak as the lower marker. This can be corrected by manually setting the correct lower marker. Hover over the lower marker peak with the mouse, right click, and select **Force Lower Marker**. To identify the correct peak as the lower marker, click Turn Off Analysis in the Analysis menu and overlay the sample with a ladder. Look for the peak in the sample that aligns with the lower marker in the ladder (should be the tallest peak). Turn the analysis back on and select the lower marker peak.



**Figure 20. Incorrect Lower Marker**



**Figure 21. Sample and ladder overlaid with analysis turned off**

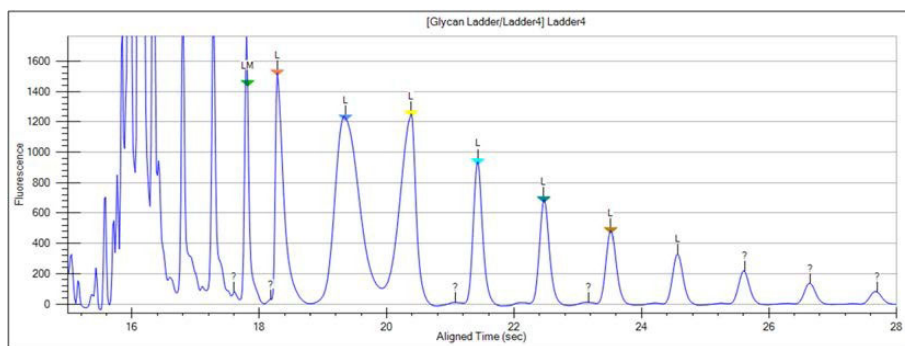
### How to include peaks ahead of the Lower Marker in the analysis:

By default, the software only includes peaks that migrate after the lower marker in the analysis. If there are peaks before the lower marker that should be included in the analysis, you can manually include the peaks. First, be sure that the lower marker is assigned to the proper peak (see previous section). Peaks to the left of the marker are labeled with “?” by the software. Right-click on the peak you want to include and select Include Peak. After the peak is included in the analysis, all analysis features can be applied to the peak.

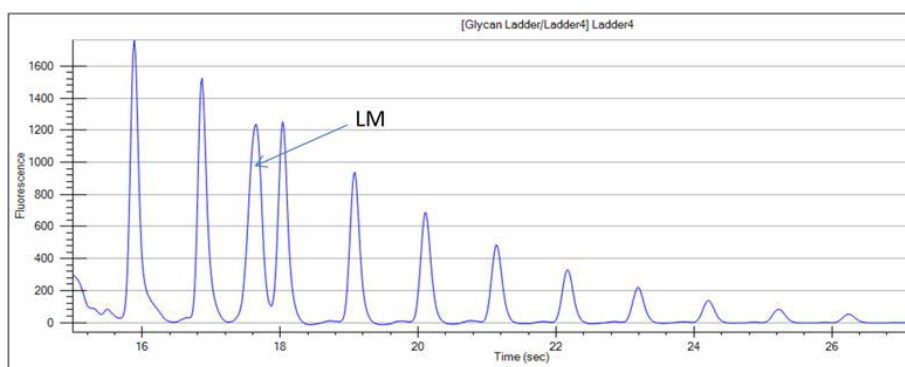
### Symptom: Lower Marker is misidentified in the ladder

If the ladder peaks are compressed and/or broad, the software may be miscalling the marker peak. There are two ways to correct this:

- 1 Manually select the proper marker peak by hovering over the peak with the mouse, right click, and select Force Lower Marker. It is often helpful to click Turn Off Analysis to identify the proper peak.
- 2 If the lower marker peak height is lower than the ladder peaks, the software often misidentifies the lower marker peak. In this case, remove the chip from the instrument, clean out well 4, and replace with fresh marker.



**Figure 22. Compressed and broadened ladder peaks due to misidentified lower marker**



**Figure 23. Example of a degraded lower marker peak with analysis turned off**

**Symptom: No ladder or sample peaks but marker peaks detected.**

*Note: The lower marker peak height will most likely be greater than normal height.*

**Possible causes:**

- Air bubble in sipper introduced during chip priming.

**What to do:**

- Reprime the chip (see [“Repriming Chips”](#) on page 39).

**Symptom: Missing sample, ladder and marker peaks.**

**Possible causes:**

- Clog in sipper or marker channel of chip.

**What to do:**

- Reprime the chip (see [“Repriming Chips”](#) on page 39).

**Symptom: Ladder detected but no sample peaks.****Possible causes:**

- 1 The sipper is not reaching the sample due to low sample volume in the well of the plate.
- 2 If the missing sample peaks occurred only in a few wells of the plate, check those wells for air bubbles.
- 3 The sipper is not reaching the sample due to an incorrect capillary height setting or incorrect plate definition.
- 4 If the plate has been uncovered for some time, sample evaporation might have occurred.
- 5 Debris from the sample or sample prep is clogging the sipper.
- 6 The samples were not labeled completely due to incomplete drying.

**What to do:**

- 1 Add more sample to the well.
- 2 Manually insert a larger volume pipette tip (~100  $\mu$ L) into the sample well to dislodge the bubble. Rerun these sample wells.
- 3 Check the plate definitions.
- 4 Check the sample wells, especially around the edge of the plate where evaporation is fastest, and make a fresh plate if volumes are low.
- 5 If you suspect there may be debris in the samples, spin the sample plate down in a centrifuge (e.g., 3000 rpm for 5 minutes). Unclog the sipper by repriming the chip (see [“Repriming Chips” on page 39](#)).
- 6 To ensure complete drying, repeat the sample preparation procedures allowing the drying reaction to extend beyond 2 hours until the samples are completely dry.

**Symptom: No ladder peaks but sample peaks and marker peaks are present.****Possible causes:**

- Low or no ladder volume in the Ladder Tube.

**What to do:**

- Add more ladder to the Ladder Tube and restart the run. Recommended standard ladder volume is 120  $\mu\text{L}$  (minimum volume is 100  $\mu\text{L}$ ).

**Symptom: No marker peaks but sample peaks are present for Glycan assay.****Possible causes:**

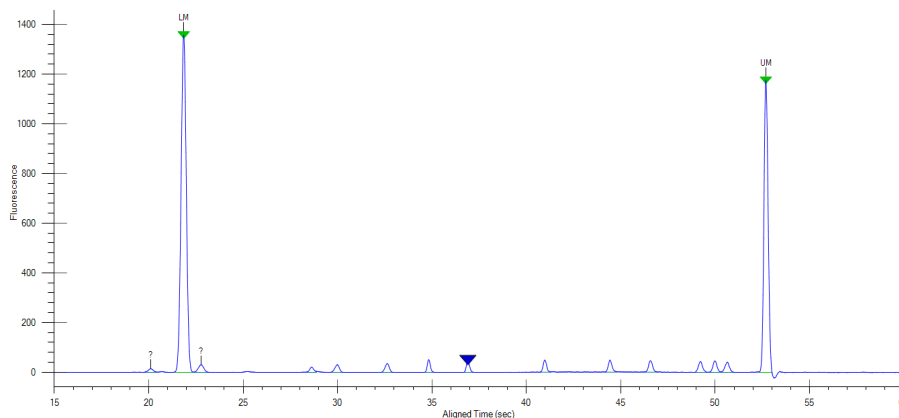
- 1 No marker added to chip well 4.
- 2 If there is marker solution in chip well 4, the problem may be due to a marker channel clog.

**What to do:**

- 1 This may be due to not filling the marker well or the chip remaining idle on the instrument for an extended period of time. Add or replenish the marker solution in the chip using the following procedure:
  - a Touch the **Unload Chip** button on the Home screen to open the chip door.
  - b Return the chip to the chip storage container ensuring the sipper is submerged in the fluid.
  - c Thoroughly aspirate all fluid from chip well 4 using a vacuum.
  - d Rinse and completely aspirate chip well 4 twice with water (Milli-Q<sup>®</sup> or equivalent).
  - e Add Marker Solution to chip well 4. See [“Preparing the Chip” on page 14](#) for instructions on preparing the Marker Solution.
  - f Reinsert the chip back into the instrument.
  - g Restart the run.
- 2 Perform a marker channel unclogging procedure by repriming the chip (see [“Repriming Chips” on page 39](#)).



**Symptom: Ladder traces show up in the lanes following the ladders (delayed sip).**



**Figure 24. Small ladder peaks in sample well caused by delayed sip**

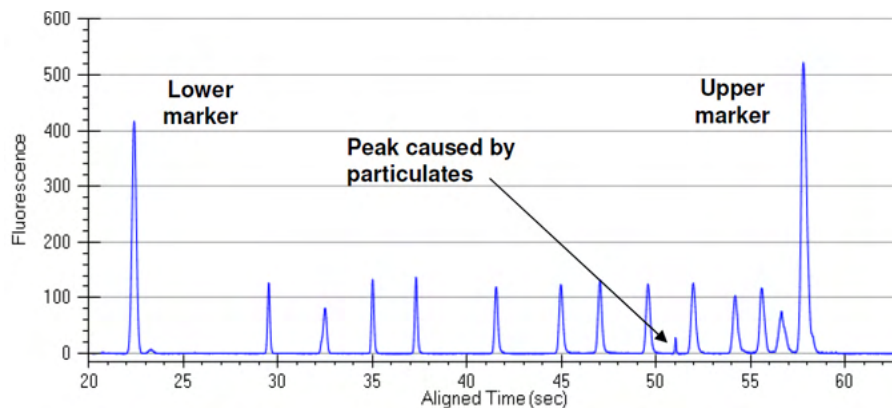
**Possible causes:**

- 1 Separation channel overloaded with sample.
- 2 Partial clog in the separation channel.

**What to do:**

- 1 Lower the starting sample concentration.
- 2 Reprime the chip (see [“Repriming Chips” on page 39](#)).

**Symptom: Unexpected sharp peaks.**



**Figure 25. Unexpected sharp peak**

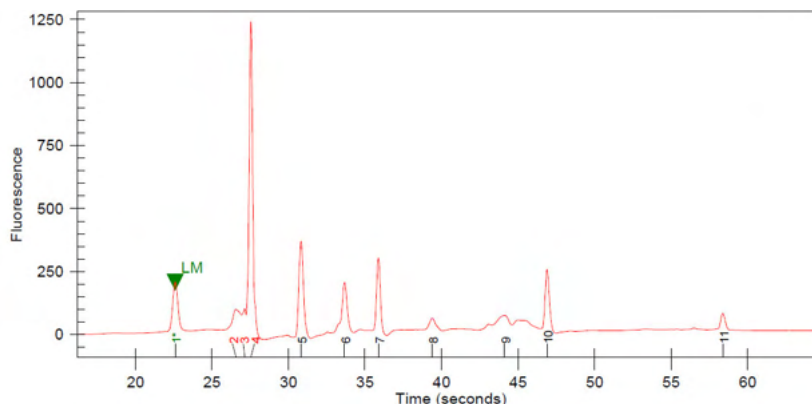
**Possible causes:**

- Dust or other particulates introduced through sample or reagents.

**What to do:**

- Do one or all of the following:
  - Replace the 18 megohm, 0.22- $\mu\text{m}$  filtered water (Milli-Q<sup>®</sup> or equivalent) water used for chip preparation.
  - Replace the buffer used for sample and reagent preparation.
  - Use a 0.22- $\mu\text{m}$  filter for all water and buffers used for chip, sample, and reagent preparation.
  - Spin down sample plate to pellet any particulates.

**Symptom: Humps in several electropherograms which do not correspond to sample data.**



**Figure 26. Humps in several electropherograms**

**Possible causes:**

- 1 Electrode 7 is dirty and has contaminated the Gel Matrix in well 7.
- 2 High concentrations of detergent in the sample buffer can sometimes cause humps in the electropherogram.

**What to do:**

- 1 Before restarting the run, clean electrode 7. Remove the chip and follow the electrode cleaning procedure. We recommend using the provided swab and isopropanol to manually clean electrode 7.
- 2 Lower the detergent concentration in the sample.

**Symptom: Peaks migrating much faster or slower than expected.**

**Note:** *Some migration time variance between chips or within a plate is considered normal chip performance. All chips are QC tested at Revvity prior to shipment.*

**Possible causes:**

- 1 Particulates from the samples may be clogging the separation channel (this will slow down migration).
- 2 Gel Matrix was not primed properly into the chip.

**What to do:**

- 1 If fast or slow migration is observed repeatedly on a new chip, contact technical support to arrange return of the chip to Revvity. Please send a data file showing the failure along with the return request.
- 2 Minimize the loading of particulates in the sample by performing a centrifuge spin of the sample plate (e.g. 3000 rpm for 5 minutes) and/or ensuring the Sip 4 mm plate type is selected in the **Select Wells** screen before starting a new run. The debris may be flushed out of the chip by washing and re-priming the chip (see [“Repriming Chips” on page 39](#)).
- 3 Check the O-rings on the top surface of the chip interface and clean if necessary.

# Frequently Asked Questions

## Kit Specification

- 1 What is in the Lower Marker?

*For HT Glycan assays, the Lower Marker consists of dye-labeled maltohexaose. For HT Glycan Extended Range, the Lower Marker is the free dye.*

- 2 What is in the Ladder?

*The ladder is a dye-labeled glucose homopolymer standard consisting of  $\alpha$ (1-6)-linked glucose oligosaccharides with variable number of monomeric glucose units (1 - 23 or more).*

## Assay Preparation

- 1 Can samples be digested overnight at 37°C using this assay?

*No, overnight digestion of samples may lead to poor percent area reproducibility and sample resolution.*

- 2 Can samples be labeled overnight at 55°C using this assay?

*No, samples should be labeled until dry. Extended incubation of dry labeled samples at 55°C may lead to glycan degradation.*

- 3 Can an evaporator be used to label the glycans instead of incubating at 55°C for 2 hours?

*No, the labeling efficiency using an evaporator is much lower and will result in low sample signal.*

- 4 Can labeled samples be reconstituted in less or more than 100  $\mu$ L of water?

*No, reconstituting samples in 100  $\mu$ L results in a buffer composition optimal for glycan migration in this assay. Reconstituting in less or more than 100  $\mu$ L will change this buffer composition and potentially alter migration and data resolution.*

- 5 After labeling standards using this assay can they be frozen and used later?

*Yes, labeled standards may be frozen for several weeks and tested again at a later time. Keep the thawed labeled standards on ice when not in use.*

- 6 Can samples be prepared using this assay and then analyzed using HPLC or CE- LIF?
  - *HPLC: No, the reagent chemistry of the Glycan assay is not compatible with the HPLC method.*
  - *CE-LIF: Yes, provided the optics system is compatible or adjusted accordingly.*

## Assay Performance

- 1 Can concentrations lower than 1.25 mg/mL be tested using this assay?

*Yes, concentrations as low as 0.5 mg/mL can be tested, however, the percent area of peaks for samples < 1.25 mg/mL are less reproducible (CV > 10%) than samples tested at  $\geq$  1.25 mg/mL.*

- 2 What other glycoproteins in addition to monoclonal antibodies can be tested using this assay?

*Other N-linked glycoproteins can be tested using this assay. However, this assay was optimized to provide a glycosylation pattern for 5 major glycans: Man5, G0, G0f, G1f, and G2F. Other glycans or oligosaccharides may not be detected according to the assay performance specifications.*

- 3 If I am using this kit for the first time, how do I confirm that the kit is working properly?

*Deglycosylation can be confirmed by testing unlabeled digested samples running Protein Express Kit for reduced conditions. Sample labeling can be confirmed by labeling glycan standards, see [“Analyzing Data” on page 22](#).*

- 4 Can charged N-glycans be detected using this assay?

*Some charged glycans will migrate faster than the lower marker. If this is the case, and the peak is within the baseline window then they can be manually included in the analysis. For instruction on how to perform this, see [“Troubleshooting” on page 27](#).*

- 5 How does this assay compare to HPLC and CGE-LIF?

*The Glycan assay is a high throughput assay in which 96 samples from sample preparation to data analysis can be completed in approximately 8 hours. Using HPLC or CGE-LIF, a few samples from sample preparation to data analysis are completed on the order of days.*

# LabChip Kit Essential Practices

To ensure proper assay performance, please follow the important handling practices described below. Failure to observe these guidelines may void the LabChip Kit product warranty.<sup>1</sup>

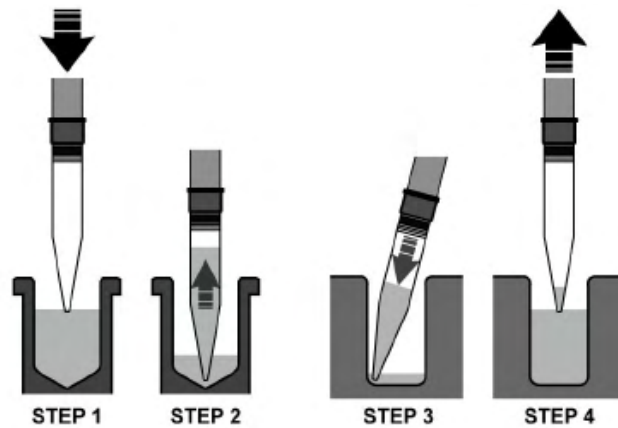
**Note:** *It is important to keep particulates out of the chip wells, channels and capillary. Many of the following guidelines are designed to keep the chips particulate-free.*

For assay and instrument troubleshooting, refer to the LabChip GX Touch software Help file or call Revvity Technical Support at 1-800-762-4000.

## General

- The assay requires exact and consistent adherence to the protocol as shown in this guide or results may be compromised by increased variability.
  - Allow the chip and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use.
  - Store chips, plates, and reagents as specified in [“Storage Conditions” on page 6](#).
  - Clean the O-rings in the chip interface weekly and the electrodes daily. Refer to the Instrument Users Guide Maintenance and Service section for procedures.
  - Avoid use of powdered gloves. Use only non-powdered gloves when handling chips, reagents, sample plates, and when cleaning the instrument electrodes and electrode block.
  - Calibrate laboratory pipettes regularly to ensure proper reagent dispensing.
  - Only the provided Detection Window Cleaning Cloth can be used on the chip to clean the detection window.
  - Water used for chip preparation procedures must be 18 megohm, 0.22- $\mu\text{m}$  filtered water (Milli-Q<sup>®</sup> or equivalent).
  - Using the “Reverse Pipetting Technique” (see [page 39](#)) will help avoid introducing bubbles into the chip when pipetting the Gel Matrix.
1. Revvity, Inc. warrants that the LabChip Kit meets specification at the time of shipment, and is free from defects in material and workmanship. LabChip Kits are warranted for 60 days from the date of shipment. All claims under this warranty must be made within thirty days of the discovery of the defect.

## Reverse Pipetting Technique



**Figure 27. Reverse pipetting**

- 1 Depress the pipette plunger to the second stop.
- 2 Aspirate the selected volume plus an excess amount from the tube. Leave the pipette tip in the solution for 5-10 seconds to ensure that the aspiration is complete.
- 3 Dispense the selected volume into the corner of the well by depressing plunger to the first stop. Hold the pipette for 10 seconds with the plunger at the first stop to ensure that the dispense is complete.
- 4 Withdraw the pipette from the well.
- 5 Retain the excess volume in the pipette tip if performing an additional Reverse Pipetting transfer, or return the excess volume to the source container for later use.

## Chips

### Repriming Chips

- 1 Touch the **Unload Chip** button on the Home screen to open the instrument door. Place the chip into the instrument.
- 2 Close the chip door securely and choose the corresponding assay.
- 3 Touch the **Prime** button on the Home screen.
- 4 Touch the **Prime** button on the Chip Priming screen to reprime the chip.

## Washing Chips

**Important Note:** Wash chips only with water (Milli-Q<sup>®</sup> or equivalent). Use of any other reagents (including Wash Buffer) is likely to cause even more artifacts in subsequent data.

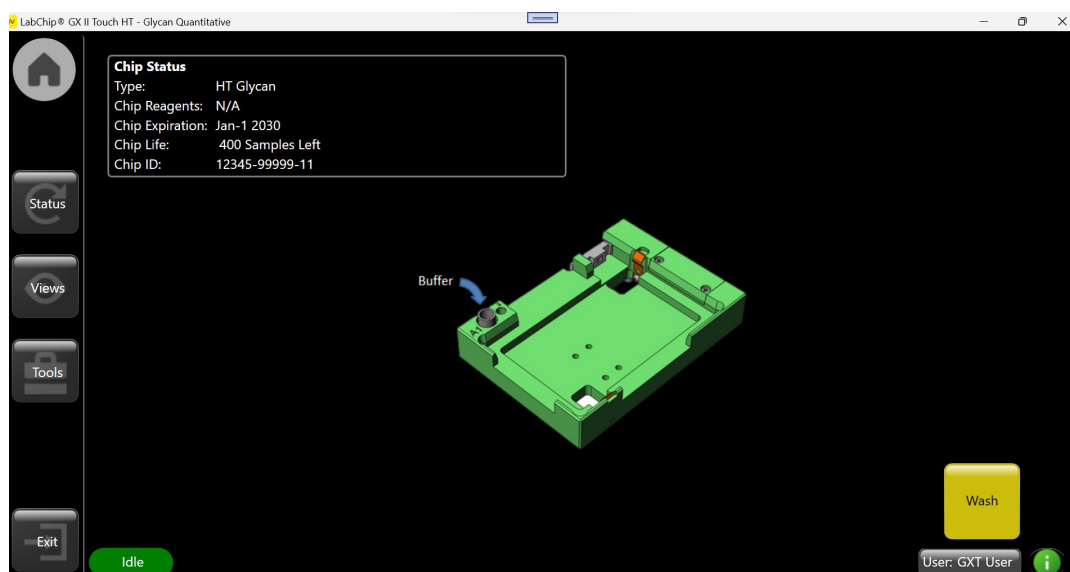
**Notes:** Some protein samples may have components which produce data with extra peaks, spikes or other artifacts. When these artifacts are present, washing chips on the LabChip GXII Touch immediately before the next use can often restore data quality.

Chips should only be washed on the LabChip GXII Touch immediately before they are prepared with fresh reagents and primed on the instrument. Chips should not be washed and left with water in the chip channels for any extended period of time.

For most protein samples, the only chip cleaning required is to rinse and aspirate the active wells twice with water (Milli-Q<sup>®</sup> or equivalent), and store the chip with 120  $\mu$ L of water in each active well.

- 1 Thoroughly aspirate all fluid from the chip wells 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<sup>®</sup> or equivalent). Do not allow active wells to remain dry.
- 3 Add 120  $\mu$ L of water (Milli-Q<sup>®</sup> or equivalent) to each active well (1, 2, 3, 4, 7, 8, 9, and 10).
- 4 Touch the **Unload Chip** button on the Home screen and place the chip into the instrument.
- 5 Close the chip door securely.
- 6 Transfer 750  $\mu$ L of water (Milli-Q<sup>®</sup> or equivalent) into the Buffer Tube. Install into the instrument.
- 7 Touch the **Wash** button on the Home screen, and then touch the **Wash** button on the Wash screen (Figure 28).

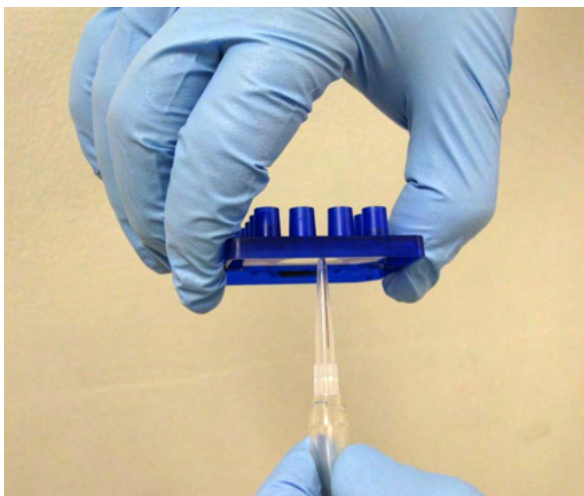




**Figure 28. Wash screen**

- 8 After completion of the wash cycle, open the chip cartridge and return the chip to the chip storage container, ensuring the sipper is submerged in the fluid.
- 9 Thoroughly aspirate all fluid from the chip wells using a vacuum.
- 10 Replace fluid in the wells with freshly made reagents as described in [“Preparing the Chip” on page 14](#). Do not let wells remain dry.
- 11 Transfer 750  $\mu\text{L}$  of water (Milli-Q<sup>®</sup> or equivalent) into a clean Buffer Tube. Install into the instrument.
- 12 Install the Ladder Tube, sample plate, and chip into the instrument and run the assay.

If air bubbles are not dislodged after a reprime, apply a vacuum to the sipper. Perform this by filling all active wells with 100  $\mu\text{L}$  water (Milli-Q<sup>®</sup> or equivalent). Then suction the sipper with a vacuum line, as shown in [Figure 29](#), until droplets of fluid flow out from the sipper. When suctioning the sipper, be careful not to bend or break the sipper. To facilitate this, cut the end of the pipette tip attached to the vacuum line to widen the mouth.



**Figure 29. Removing an air bubble or clog by suctioning the sipper with a vacuum line**

### Other Considerations

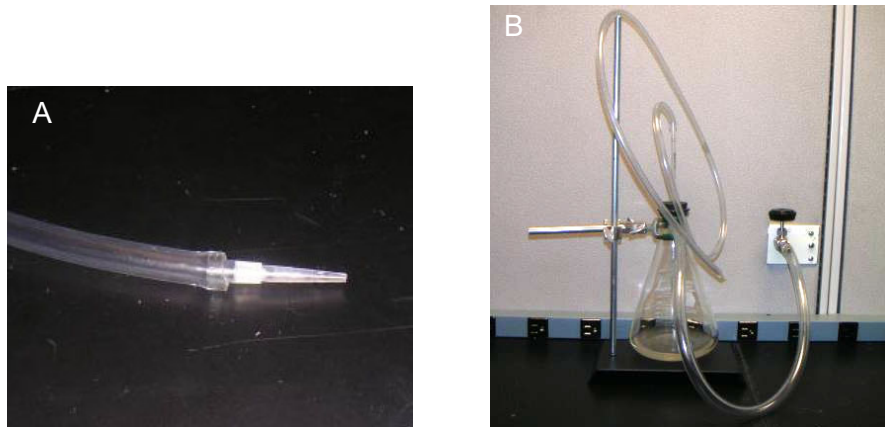
- Store chips as specified in [“Storage Conditions” on page 6](#).
- Do not allow the liquid in the chip storage container to freeze, as this may lead to poor chip performance. Do not submerge the chip in any solution.
- The entire chip surface must be thoroughly dry before use.
- The sipper must be kept submerged in fluid at all times and should not be exposed to an open environment for long periods of time.
- Use care in chip handling to prevent sipper damage. Damage to the sipper can result in inconsistent sampling.
- Avoid exposing the chips to dust by keeping them in a closed environment such as in the chip storage container or in the instrument before and after chip preparation.
- Chips can be prepared and left in the instrument for extended periods of time so that samples can be run as needed throughout the day. Revvity recommends the chip be re-prepared after it has been idle for 8 hours, but the chip can be used continually over an 8-hour work day as long as the maximum recommended idle time of 8 hours and total chip lifetime of 400 samples are not exceeded.

## Samples

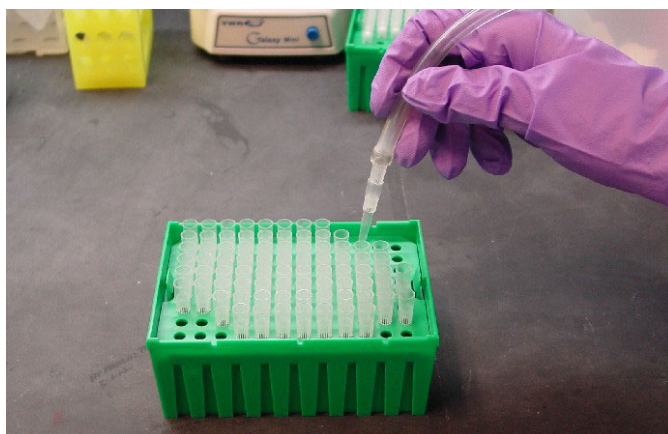
- Prepared sample plates should be free of gas bubbles and particulate debris, both of which may inhibit sipper flow.
- Spin down sample plates containing gas bubbles and/or particulate debris at 3000 rpm (1250 rcf) for 5 minutes prior to analysis.
- Up to 192 samples can be run with a single chip preparation when running the LabChip GXII Touch HT instrument. Up to 48 samples can be run with a single chip preparation when running the LabChip GXII Touch 24 instrument.

## Chip Well Aspiration Using a Vacuum

Aspirating with a pipette can leave used reagents in the chip wells. For this reason, Revvity recommends vacuuming the wells instead. This can be accomplished by attaching a permanent pipette tip to a house vacuum line with trap (Figure 30). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 31).



**Figure 30. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap**



**Figure 31. Replacing the Disposable Pipette Tip**

# Customer Technical Support

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Hopkinton, MA 01748-1668

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**Fax:** +1 203-944-4904  
**Email:** [L3LabChip@Revvity.com](mailto:L3LabChip@Revvity.com)

**Internet:** [www.Revvity.com](http://www.Revvity.com)

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

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

For additional assay and instrument troubleshooting, refer to the LabChip GX Touch Software Help file.

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