LabChip User Guide

Low MW Protein Express Assay User Guide

For LabChip® GXII Touch

 PN CLS140163 Rev. F
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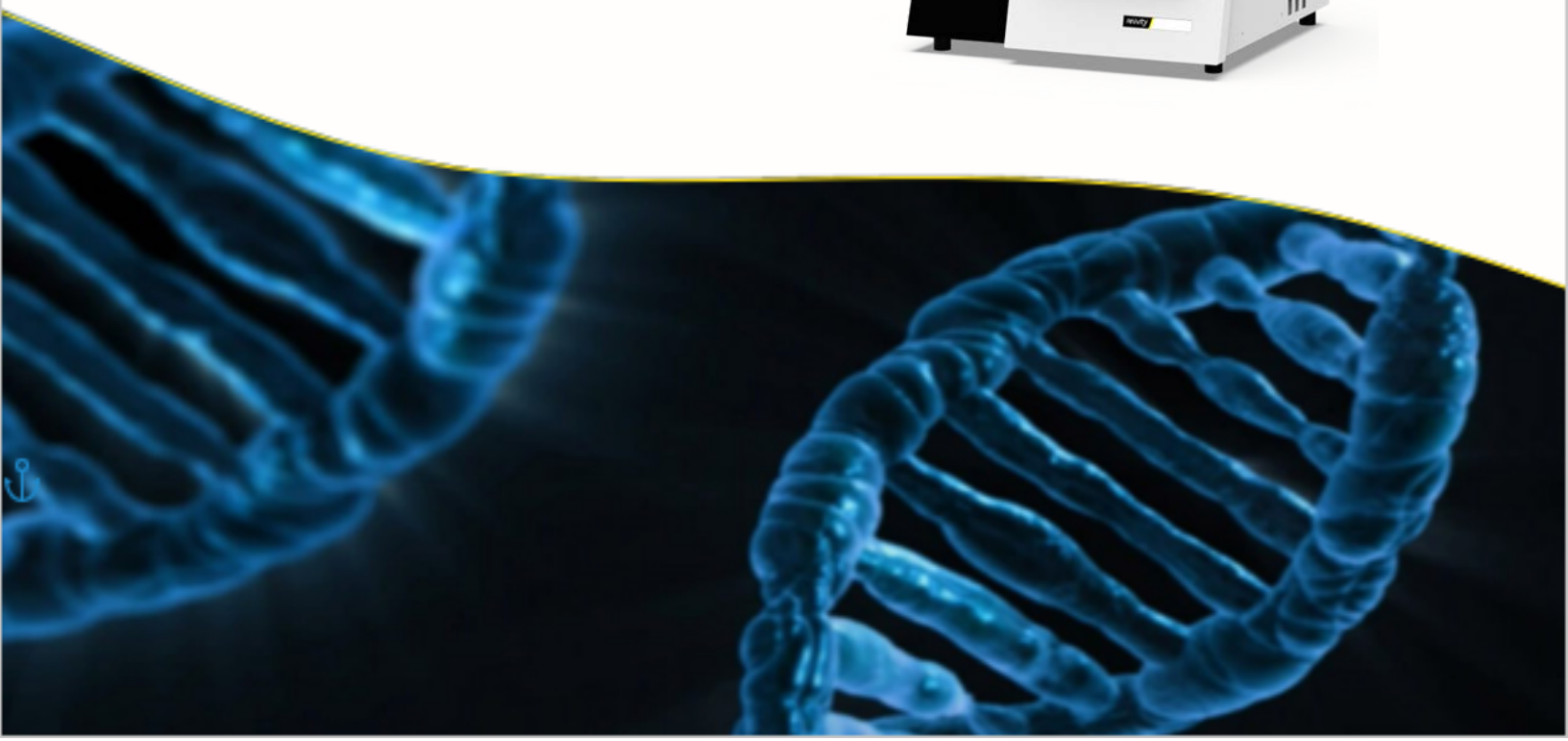

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Specifications

Assay Specifications

Table 1. Assay Specifications

Sizing Range	5 kDa - 80 kDa
Sizing Resolution[a]	± 10% 14 - 80 kDa, ± 20% <14 kDa
Sizing Accuracy	± 20% up to 80 kDa ± 10% (CAII, BLG)[b]
Sizing Reproducibility	3% CV (CAII, BLG)[b]
Linear Concentration Range	30 - 2000 ng/µL (BLG, CAII in PBS)[b]
Maximum Total Protein Concentration	10 mg/mL
Quantitation Reproducibility	30% CV up to 80 kDa. Above 80 kDa, quantitation is not specified.
Chip Lifetime	HT: 400 samples 24: 200 samples
Samples per Chip Prep	HT: up to 384 samples LT: up to 48 samples
Chip Preps per Reagent Kit	HT: 4 chip preps LT: 4 chip preps

a. Resolution is defined as the height of the valley between two peaks to be no more than 50% of the maximum peak height. Actual separation performance can depend on the sample and application.

b. CAII = Carbonic Anhydrase, BLG = beta-Lactoglobulin

Sample Conditions

Table 2. Sample Conditions

Buffers, Salts and Additives	Refer to Buffer, Salt and Additive Compatibility on page 32 for compatibility with specific buffers, salts and additives. If your conditions are not listed, contact Revvity Technical Support (see page 34) for more information on compatibility.
Particulates	Sample plates should be spun down prior to analysis. All buffers should be filtered with a 0.22 µm cellulose acetate filter.
Salt Concentration	Total salt concentration must not exceed 0.5 M

Storage Conditions

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

Reagent Storage: Store the Low MW Protein Express Ladder (yellow cap) at -20°C when not in use. Store all other reagents at 2 - 8°C when not in use. Store the LMW Protein Express Dye and LMW Protein Express Lower Marker in the dark when not in use.

Store prepared Gel-Dye solution in the dark at 2 - 8°C for up to 3 weeks.

CRITICAL:
The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. Remove the LMW Protein Express Ladder from the padded shipping pack and allow to warm from -20°C to room temperature for 45 minutes.

Reagent Kit Contents

Low Molecular Weight (LMW) Protein Express Reagent Kit, P/N 760573

Table 3. Reagents

Reagent	Vial	Quantity
LMW Protein Express Dye Solution	Blue	1 vial, 0.09 mL
LMW Protein Express Gel Matrix	Red	2 vials, 1.8 mL each
LMW Protein Express Sample Buffer	White	2 vials, 1.4 mL each
Protein Express Wash Buffer	Purple	4 vials, 1.8 mL each
LMW Protein Express Lower Marker	Green	1 vial, 0.5 mL
LMW Protein Express Ladder	Yellow	1 vial, 0.08 mL

Table 4. Consumable Items

Item	Supplier and Catalog Number	Quantity
Spin Filters	Costar®, Cat. # 8160	8
Detection Window Cleaning Cloth	VWR®, Cat. # 21912-046	1
Swab	ITW Texwipe®, Cat. # TX758B	3
Centrifuge Tubes, 2.0 mL	(Not sold separately)	5
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 GXII Touch HT)	760524
24 High Resolution LabChip (for use with GXII Touch 24 or HT)	CLS138951

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. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

WARNING!

- *Dye contains DMSO. Avoid contact with skin and eyes.*
- *Dye contains SDS. Avoid inhalation and contact with skin and eyes.*
- *Wash Buffer and Sample Buffer contain LDS. Avoid inhalation and contact with skin and eyes.*
- *Gel Matrix contains Methyl urea. Avoid contact with skin and eyes.*

Usage

The Low Molecular Weight (Low MW) Protein 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.

Preparation Procedures

CRITICAL:

- *The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. Protect the LMW Protein Express Dye Solution and the LMW Protein Express Lower Marker from light.*
- *Remove the LMW Protein Express Ladder from the padded shipping pack and allow to warm from -20°C to room temperature (20 - 25°C) for 45 minutes.*
- *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.*

Additional Items Required

- 0.6 mL centrifuge tubes and/or 96-well plates for denaturing protein samples.
- Means for heating samples to 100°C — 96-well PCR instrument or heating block.

  Note: *Avoid using non-stick lab consumables. They may induce unexpected or erratic assay results caused by surface treatments leaching into dye or gel components.*
- 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- 70% isopropanol solution in DI water.
- Reducing agents: BME (beta-mercaptoethanol), 1M DTT (dithiothreitol) or 100 mM TCEP.

Preparing the Gel-Dye and Gel Destaining Solutions

Notes: *The LMW Protein Express Dye solution contains DMSO and* ***must be thawed completely*** *before use.*

The dye is light sensitive. ***Do not expose the LMW Protein Express Dye solution or Gel-Dye solution to light for any length of time.*** *Keep the LMW Protein Express Dye solution and the prepared Gel-Dye solution in the dark when not in use.*

Do not exceed 9300 rcf when filtering Gel-Dye solution. Exceeding 9300 rcf will change the properties of the gel.

1. Vortex the thawed LMW Protein Express Dye for 20 seconds before use.
2. Transfer 520 µL of LMW Protein Express Gel Matrix (red cap 🔴) using a reverse pipetting technique to the 2.0 mL centrifuge tube provided with the reagent kit.
3. Add 20 µL of LMW Protein Express Dye solution (blue cap 🔵).
4. Vortex the solution until it is well mixed.
   Note: *Gel matrix is extremely viscous. Make sure the Gel-Dye solution has an even blue color before transferring to the spin filter. Insufficient mixing of gel and dye will cause inconsistent assay results.*
5. Transfer the solution into one of the spin filters provided with the reagent kit.
6. Transfer 250 µL of the LMW Protein Express Gel Matrix (red cap 🔴) into a separate spin filter. This will be used as the Destain solution.
7. Centrifuge the Gel-Dye solution and the Destain solution at 9300 rcf for 8 minutes at RT.
8. Discard the filters, and then label and date the tubes.
9. Store the Gel-Dye solution in the dark at 2 - 8°C. Use within 3 weeks.

The volumes of Gel-Dye and Destain solutions prepared are the amount required for one HT or LT chip prep.

Preparing the Sample Denaturing Solution

1. Pipette 700 µL of LMW Protein Express Sample Buffer (white cap ◯). into a 2.0 mL centrifuge vial.
2. If samples need to be reduced, add 24.5 µL of BME or 1 M DTT or 3.75 µL of 100 mM TCEP.
3. Vortex for 10 seconds. This volume of sample buffer and reducing agent is sufficient to prepare 96 samples. A smaller volume can be prepared if running fewer than 96 samples.

Preparing the Protein Samples and Ladder

Notes: *Samples can be prepared in either a 96-well or 384-well PCR plate or in 0.6 mL microcentrifuge tubes (and subsequently pipetted into a plate). Procedures for both are described here.*

The LMW Protein Express Ladder should be kept frozen. It is recommended that you aliquot the ladder into 12 µL lots for individual use after thawing for the first time. Store the aliquots at -20°C.

1. For each sample to be analyzed, pipette 7 µL of denaturing solution into the wells of a microtiter plate or into individual 0.6 mL microcentrifuge tubes.
2. Pipette 2 µL of each protein sample into the wells of the 96-well plate or microcentrifuge tube. Cover the plate with foil to minimize evaporation.
3. Allow the LMW Protein Express Ladder (yellow cap ●) to thaw completely followed by gentle vortexing for 10 seconds. Briefly spin the ladder vial. Ensure no precipitate is visible in the solution. If precipitate is present, leave the vial at room temperature for a little longer and then repeat the gentle vortex and spin.
4. Pipette 12 µL of LMW Protein Express Ladder into a microcentrifuge tube or into the well of a microtiter plate.

   Do not add denaturing solution to the ladder.
5. Denature samples and ladder at 100°C for 5 minutes. Optimum denaturing conditions may vary by sample type.
6. Tap or spin the sample plate to move the fluid to the bottom of the wells.

7 Spin the LMW Protein Express Ladder (and sample tubes if used) for 15 seconds using a mini-centrifuge.

8 Add 35 µL of water (Milli-Q® or equivalent) to each sample well or sample tube and mix by pipetting up and down a few times. Avoid creating air bubbles. This step should not be done more than an hour before starting the assay. Vortex the sample tubes (if used) for a few seconds. If using a plate, a pipettor or plate shaker can be used to mix the water with the samples.

9 Add 120 µL of water (Milli-Q® or equivalent) to the LMW Protein Express Ladder. Vortex the ladder solution for a few seconds to achieve good mixing.

10 If the samples are prepared in tubes, transfer 44 µL of each sample onto a 96-well plate.

11 Spin the sample plate to eliminate bubbles and move the fluid to the bottom of the wells.

12 Place the sample plate onto the LabChip GXII Touch plate holder.

13 Transfer 120 µL of prepared ladder solution to the provided 0.2 mL Ladder Tube. Ensure there are no air bubbles in the Ladder Tube.

14 Insert the Ladder Tube into the ladder slot on the LabChip GXII Touch instrument.

Preparing the Buffer Tube

1 Add 750 µL of Protein Express Wash Buffer (purple cap ●) to the 0.75 mL Buffer Tube provided with the reagent kit. 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.

Note: *Replace the Buffer Tube with a freshly prepared tube every 8 hours when the chip and instrument are in use.*

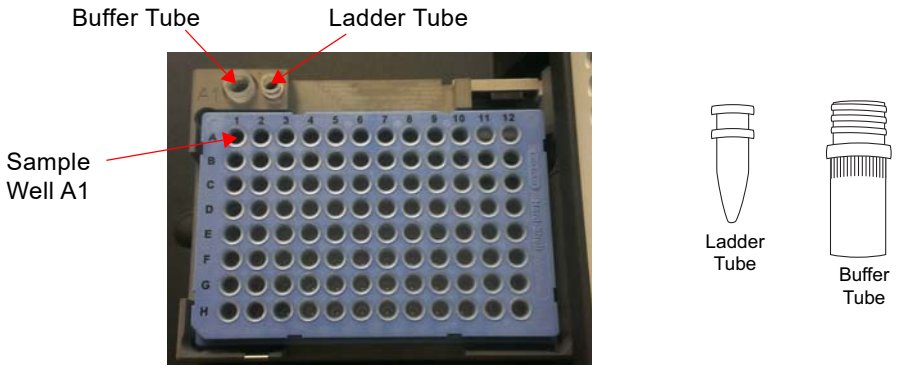

Figure 1. Buffer Tube and Ladder Tube in the GXII Touch instrument

Preparing the Chip

Note: *Use the High Resolution Chip for either Low MW Protein Express or Glycan Profiling, but not both assays on the same 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 2). For more details on how to set up a vacuum line see page 33.

3 Rinse and completely aspirate each active chip well (1, 2, 3, 4, 7, 8, 9 and 10) twice with water (Milli-Q® 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 the vacuum line. DO NOT run the tip over the central region of the detection window. Use the provided Detection Window Cleaning Cloth to clean the chip detection window.

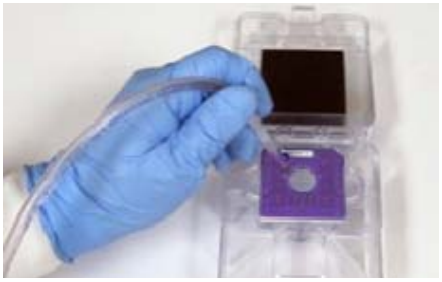

Figure 2. Using a vacuum to aspirate the chip wells is more effective than using a pipette. See page 33 for more details.

5 Using a reverse pipetting technique, add Gel-Dye solution to chip wells 3, 7, 8 and 10 as shown in Figure 3

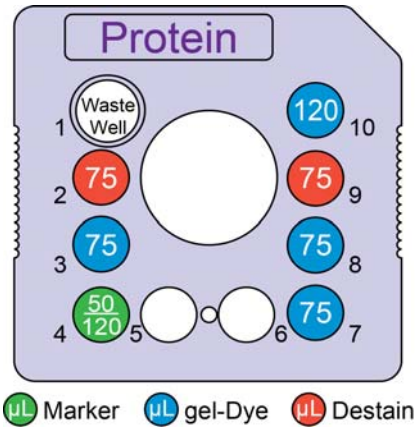

Figure 3. Reagent placement

6 Using a reverse pipetting technique, add Destain solution to chip wells 2 and 9 as shown in Figure 3.

7 If the chip will be used to analyze multiple 96-well plates or will be in use for up to 8 hours, add 120 µL of LMW Protein Express Lower Marker (green cap ●) to chip well 4. If the chip will only be used to analyze one 96-well plate or a partial plate and then stored for future use, the marker volume can be reduced to 50 µL. Make sure the marker volume is pipetted accurately. If there is not enough marker in chip well 4, the marker will deplete and will not be added to subsequent samples on-chip. Data collected without marker peaks cannot be analyzed by the software.

8 Make sure the rims of the chip wells are clean and dry.

9 **IMPORTANT:** Ensure chip well 1 (waste well) is empty before placing the chip into the instrument.

Inserting a Chip into the LabChip GXII Touch Instrument

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 Revvity-supplied clean-room cloth dampened with a 70% isopropanol solution in DI water.
3. Touch the *Unload Chip* button on the *Home* screen.

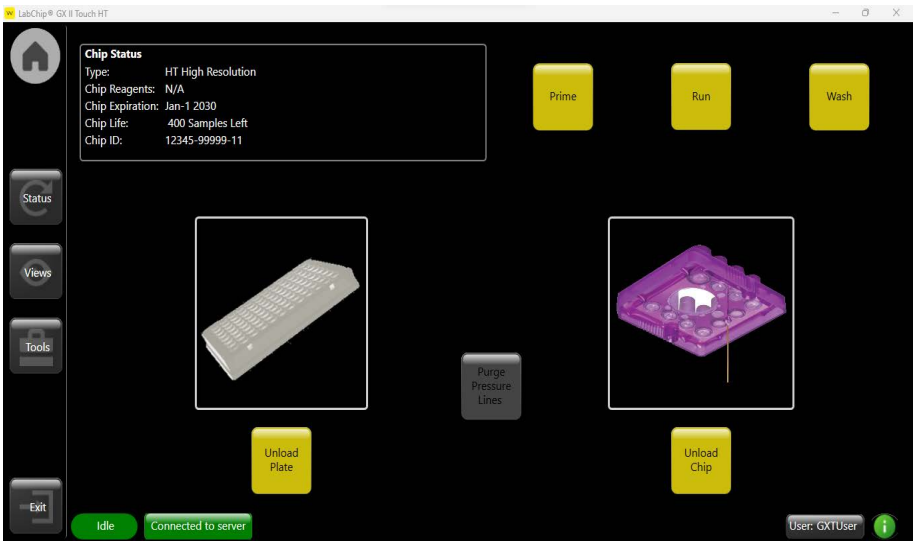

Figure 4. *Home* screen

4. Insert the chip into the LabChip GXII Touch instrument (Figure 5) and close the chip door securely.

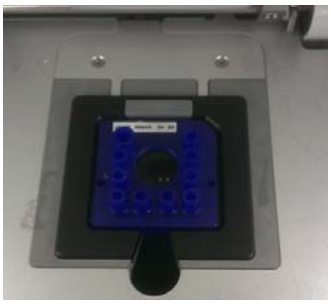

Figure 5. Chip in the LabChip GXII Touch instrument

5 Touch the *Load Plate* button on the *Home* screen (Figure 4) to retract the sample plate and send the sipper to the Buffer Tube.

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

Running the Assay

Note: *Chips can be primed independently from running assays. Select the assay of choice from the insert (see Figure 7).Touch the Prime button on the Home screen. Make sure the Buffer Tube is placed on the instrument.*

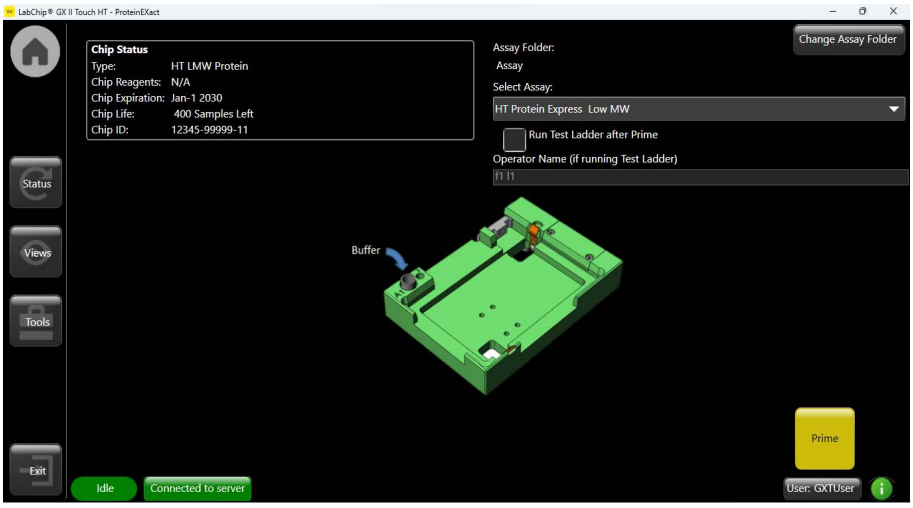

Figure 6. Chip priming screen

1 Touch the *Run* button (see Figure 6).

2 Select the appropriate assay type (see Figure 7), plate name, well pattern, and whether to read wells in columns or rows. Select number of times each well is sampled under *Adv. Settings* (Figure 8). Touch the green arrow.

Figure 7. The *Assay Choices* menu

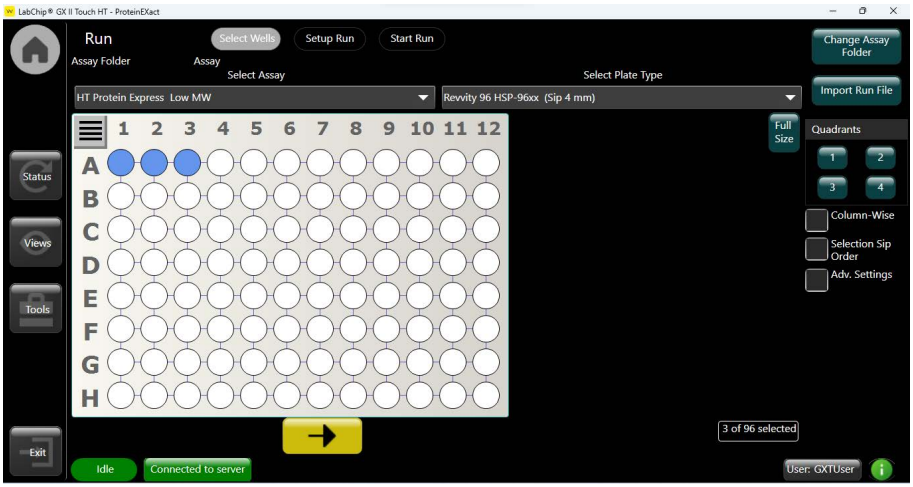

Figure 8. Selecting wells

3 In the *Setup Run* tab, select the operator name, the option to read barcode, the destination of the file, the inclusion of sample names, expected peaks, and excluded peaks and the filename convention. Select *Auto Export* to export results tables automatically (Figure 9). Touch the green arrow.

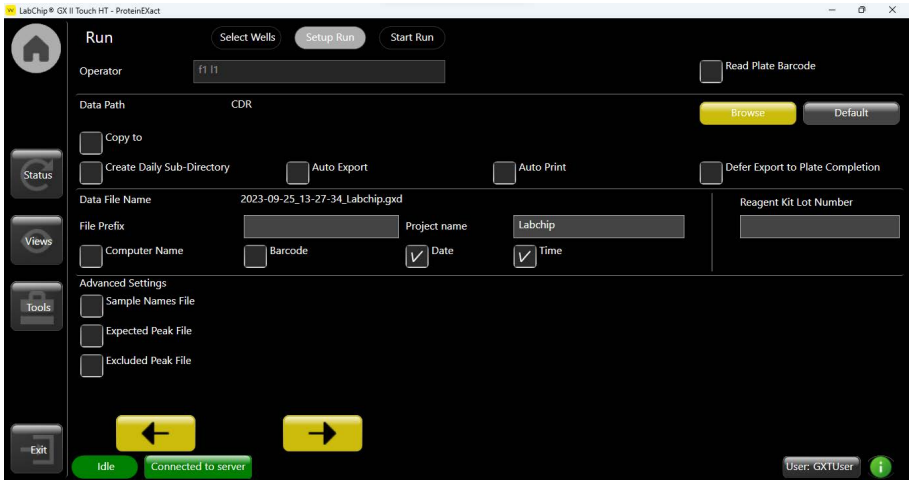

Figure 9. Run setup screen

4 Touch *Start* to begin the run.

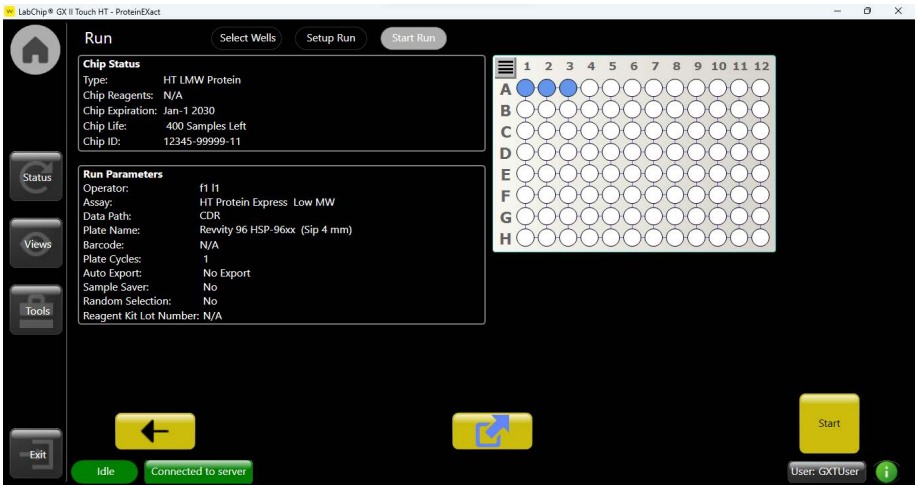

Figure 10. Starting a run

Cleaning and Storing the Chip

After use, the chip must be cleaned and stored in the chip container.

1. Place the chip into the chip storage container. The sipper should be 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® or equivalent).
4. Add 120 µL of water (Milli-Q® or equivalent) to the active wells.
5. Cover the wells with Parafilm® 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. Use within 30 days of analyzing the first sample. See Assay Specifications on page 3 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® 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® 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® or equivalent).
   - **c** Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

Results

LMW Protein Express Ladder Result

The electropherogram of a typical LMW Protein Express ladder is shown in Figure 11. Peaks to the right of the lower marker and system peaks in order of increasing migration time correspond to proteins of increasing size i.e. 6.5 kDa, 15.9 kDa, 20.4 kDa, 28.9 kDa, 48.4 kDa, and 68.4 kDa respectively.

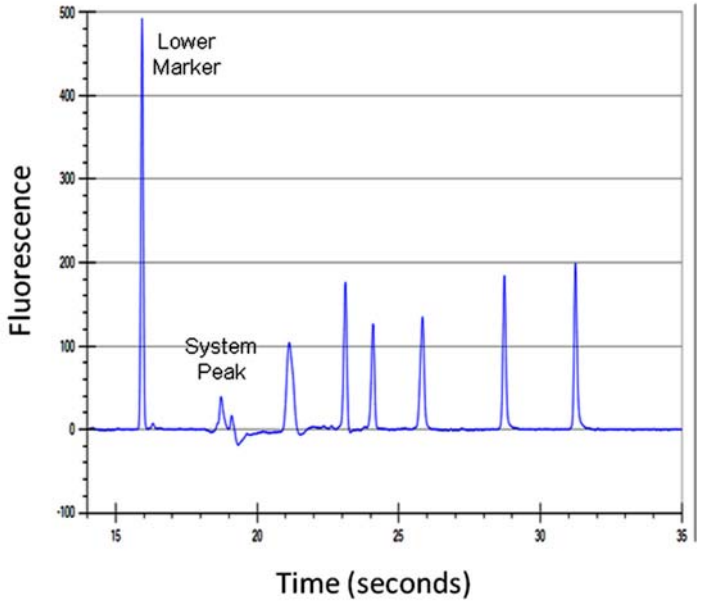

Figure 11. LMW Protein Express ladder electropherogram

Troubleshooting

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

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:

1 Air bubble in sipper introduced during chip priming.

What to do:

1 Reprime the chip. See LabChip Kit Essential Practices on page 26 for instructions on how to reprime the chip.

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

Possible causes:

1 Clog in sipper or marker channel of chip.

What to do:

1 Reprime the chip. See LabChip Kit Essential Practices on page 26 for instructions on how to reprime the chip.

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.

What to do:

1 Add more sample to the well.

2 Manually insert a larger volume pipette tip (~100 µL) into the sample well and 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 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 LabChip Kit Essential Practices on page 26 for instructions on how to reprime the chip.

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

Possible causes:

1 Low or no ladder volume in the Ladder Tube.

What to do:

1 Add more ladder solution to the Ladder Tube and restart the run. Recommended standard ladder volume is 120 µL (minimum volume is 100 µL).

Symptom: No marker peaks but sample peaks are present.

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 marker well or chip remaining idle on instrument for extended period of time. Add or replenish the marker solution in the chip using the following procedure:

- Touch the *Unload Chip* button on the Home screen to open the chip door.
- Return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from chip well 4 using a vacuum line.
- Rinse and completely aspirate chip well 4 twice with water (Milli-Q® or equivalent).

- Add LMW Protein Express Marker Solution (green cap) to chip well 4.
- Reinsert the chip back into the instrument.
- Restart the run.

2 Perform a marker channel unclogging procedure by repriming the chip. See Repriming Chips on page 28 for instructions on how to reprime the chip.

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

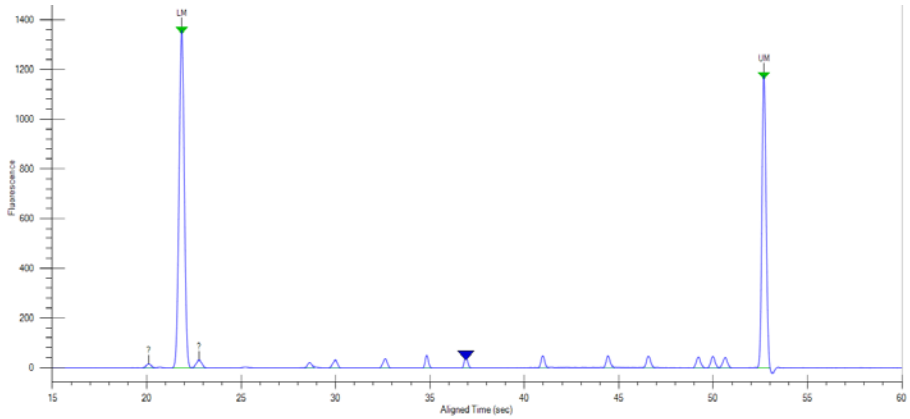

Figure 12. Small ladder peaks in sample well caused by delayed sip

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 28 for instructions on how to reprime the chip.

Symptom: Unexpected sharp peaks.

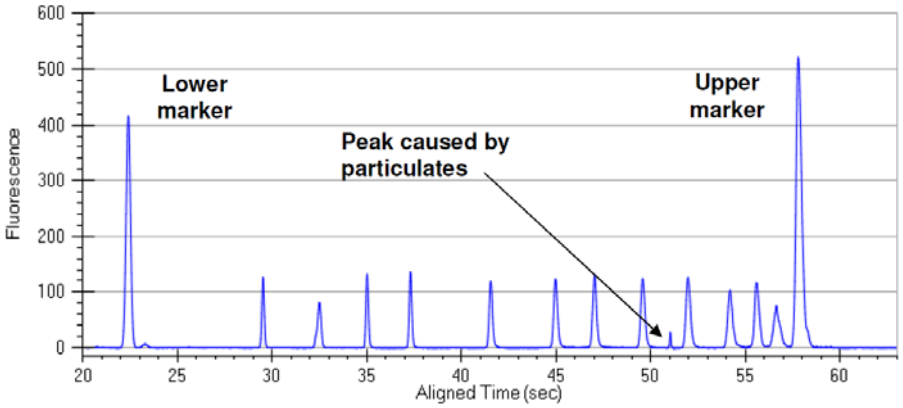

Figure 13. Unexpected sharp peak

Possible causes:

- Dust or other particulates introduced through sample or reagents.

What to do:

1 Do one or all of the following:

- Replace the 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent) used for chip preparation.
- Replace the buffer used for sample and reagent preparation.
- Use a 0.22-micron 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.

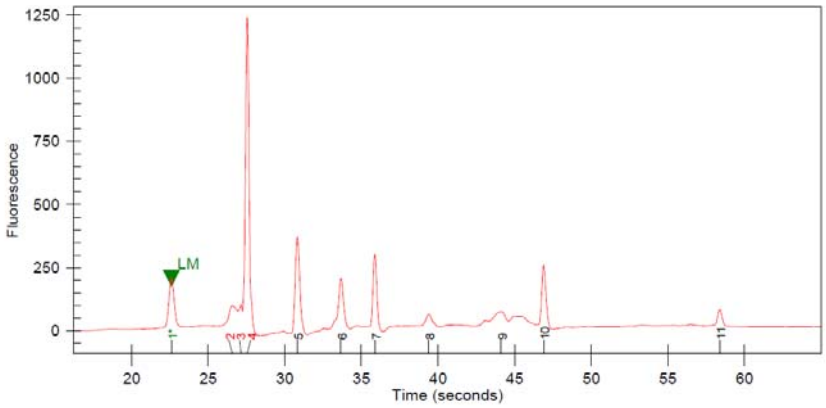

Figure 14. Humps in several electropherograms

Possible causes:

1 Electrode 7 is dirty and has contaminated the Gel-Dye solution 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 (see Buffer, Salt and Additive Compatibility on page 32).

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.*

Normal migration time windows for the markers are:

- *LMW Protein Express Assay Lower Marker: 15 - 17.5 seconds*
- *Upper Ladder Protein on the first plate: 31.5 - 34 seconds*
- *Upper Ladder Protein on the third plate: 29.5 - 32 seconds*

Possible causes:

1 Incorrect Gel to Dye ratio. Migration time is sensitive to dye concentration and peaks will migrate too fast or too slow if the dye concentration in the gel is too low or too high, respectively.

Note: *Excess dye within the separation channel will slow down migration, and less dye in the separation channel will make peaks migrate faster.*

2 Particulates from the samples may be clogging the separation channel (this will slow down migration).

3 Gel-Dye solution was not primed properly into the chip.

What to do:

1 Prepare a fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye solution. See Repriming Chips on page 28 for instructions on how to wash and reprime the chip.

2 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.

3 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 28 for instructions on how to wash and reprime the chip.

4 Check the O-rings on the top surface of the chip interface and clean if necessary.

Symptom: High baseline fluorescence (e.g., greater than 1000 counts).

Possible causes:

1 The destain wells (2 and 9) do not contain Destain solution (gel matrix with no dye).

2 The destain wells (2 and 9) may have been contaminated with dye either because the well was improperly flushed after priming or because dye was mistakenly pipetted into the well.

What to do:

1 Prepare a fresh Destain solution. Wash and reprime the chip with the new Destain solution. See Repriming Chips on page 28 for instructions on how to wash and reprime the chip.

Symptom: Lower than expected signal for ladders and samples.

Possible causes:

1 Improper SDS concentration in Gel-Dye solution.

What to do:

1 Ensure that the Dye Concentrate is completely thawed and mixed. Prepare fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye solution. See Repriming Chips on page 28 for instructions on how to wash and reprime the chip.

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.[1]
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

- Allow the chip, sample plate, and all refrigerated reagents to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. Protect the LMW Protein Express Dye Solution and the LMW Protein Express Lower Marker from light.
- Remove the LMW Protein Express Ladder from the padded shipping pack and allowed to warm from -20°C to room temperature (20 - 25°C) for 45 minutes.
- 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 Revvity-supplied clean room cloth can be used on the chip to clean the detection window.
- Water used for chip preparation procedures must be 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- Using the “Reverse Pipetting Technique” (described next) will help avoid introducing bubbles into the chip when pipetting the gel.

1. Revvity 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

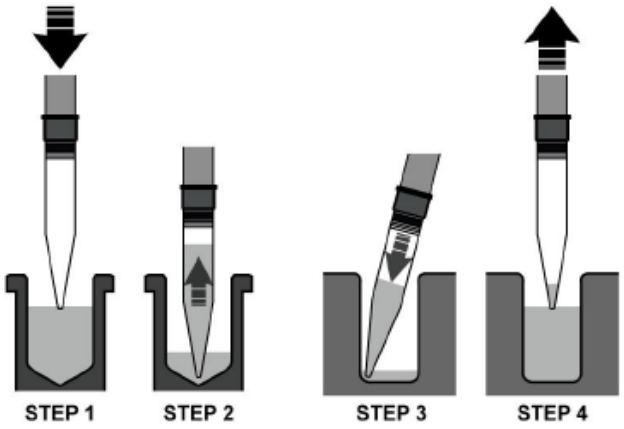

Figure 15. Reverse pipetting

1. Depress the pipette plunger to the second stop.
2. Aspirate the selected volume plus an excess amount from the tube.
3. Dispense the selected volume into the corner of the well by depressing plunger to the first stop.
4. Withdraw the pipette from the well.

Reagents

- Store the reagents as specified in Storage Conditions on page 4.
- Protect the LMW Protein Express Dye, Gel-Dye solution, and LMW Protein Express Lower Marker from light.
- The Gel-Dye solution expires 3 weeks after preparation.
- For optimal performance, use one reagent kit per chip. The Low MW Protein Express Reagent Kit contains the reagents to run four 96-well plates or four chip preparations, whichever comes first.

Chips

Repriming Chips

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

Washing Chips

Important Note: *Wash chips only with water (Milli-Q® 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 protocol that is required is to rinse and aspirate the active wells twice with water (Milli-Q® or equivalent), and store the chip with 120 µL of water (Milli-Q® or equivalent) in each active well.

1. Thoroughly aspirate all fluid from the chip wells using a vacuum line.
2. Rinse and completely aspirate each active well (1, 2, 3, 4, 7, 8, 9 and 10) twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
3. Add 120 µL of water (Milli-Q® 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 µL of water (Milli-Q® or equivalent) to the Buffer Tube. Install into the instrument.

7 Touch the *Wash* button (Figure 16).

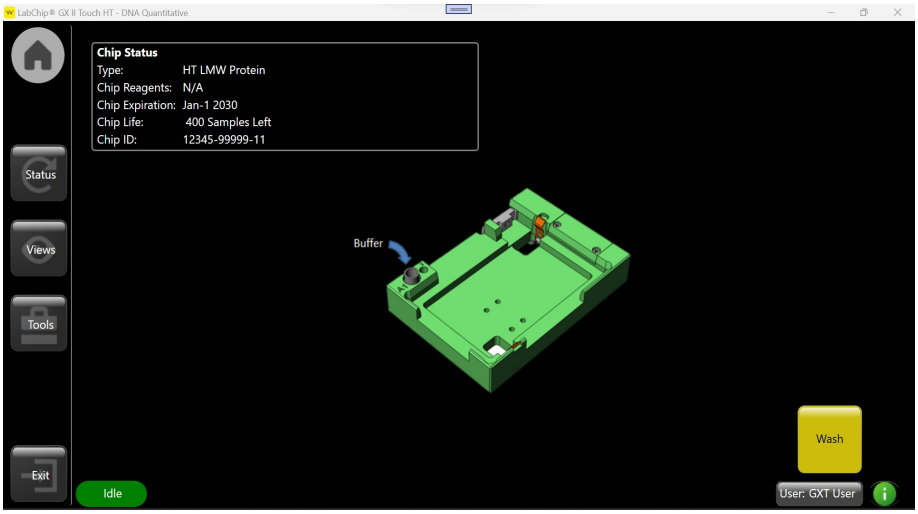

Figure 16. Wash screen

8 After completion of the wash cycle, open the chip cartridge and return the chip to the chip container ensuring the sipper is immersed in fluid.

9 Thoroughly aspirate all liquid from the chip wells using a vacuum line.

10 Prepare the chip with freshly made reagents as described in Preparing the Chip on page 12. Do not let wells remain dry.

11 Transfer 750 µL of Wash Buffer (purple cap ●) 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 µL water (Milli-Q® or equivalent). Then suction the sipper with a vacuum line, as shown in Figure 17, 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.

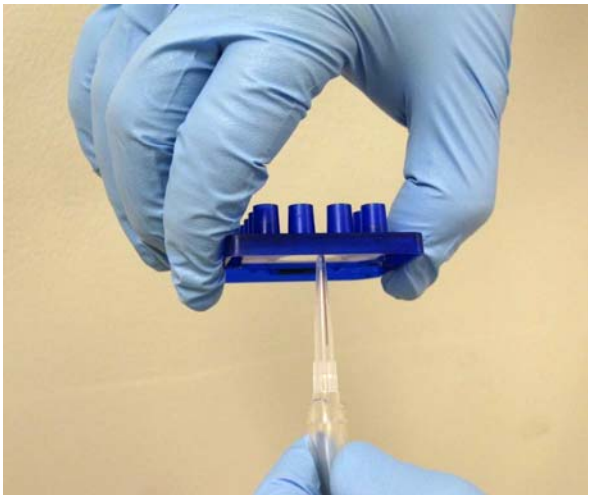

Figure 17. Removing an air bubble or clog by suctioning the sipper with a vacuum line

Other Considerations:

- Store chips as specified in Storage Conditions on page 4.
- Do not allow the liquid in the chip 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 immersed 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 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 number of 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) prior to analysis.
- Up to four 96-well plates (400 samples) can be run with a single chip preparation when running the GXII Touch HT instrument. Up to 48 samples can be run with a single chip preparation when running the GXII Touch 24 instrument.

Buffer, Salt and Additive Compatibility

Table 6. Compatible Buffers, Salts and Additives

Buffer & Salts	Concentration Limit	Additives	Concentration Limit
Tris Chloride	250 mM	Octyl Glucoside	2.5%
Tris Glycine	250 mM	Pluronic F68	0.1%
HEPES	500 mM	Sarcosyl	1.25%
PBS	2 X	CHAPS	0.25%
Sodium Citrate	150 mM	Tween 20	0.4%
Sodium Phosphate	250 mM	Triton X-100	0.6%
Sodium Acetate	600 mM	SDS	2%
Sodium Chloride	500 mM	Zwittergent 3-14	0.4%
Sodium Azide	1.5%	PEG 3350	1%
Sodium Hydroxide	125 mM	Glycerol	30%
Potassium Chloride	225 mM	Urea	8 M
Ammonium Bicarbonate	1000 mM	Sucrose	1 M
Magnesium Chloride	37.5 mM	DMSO	25%
Imidazole	900 mM	EDTA	50 mM
PhosphoSafe		Ethanol	50%
BugBuster	1 X		
BPER			
POP Culture			
Insect POP Culture	0.25 X		

Table 7. Incompatible Buffers, Salts and Additives

Buffer & Salts	Concentration Limit	Additives	Concentration Limit
RIPA	All		

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 18). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 19).

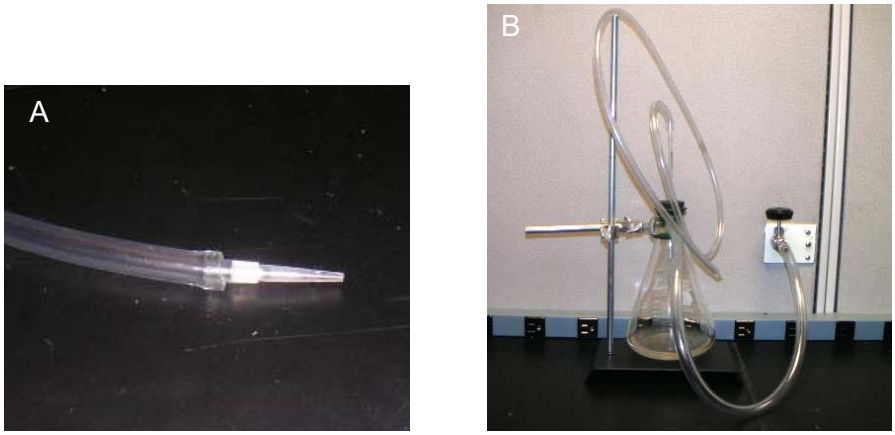

Figure 18. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap

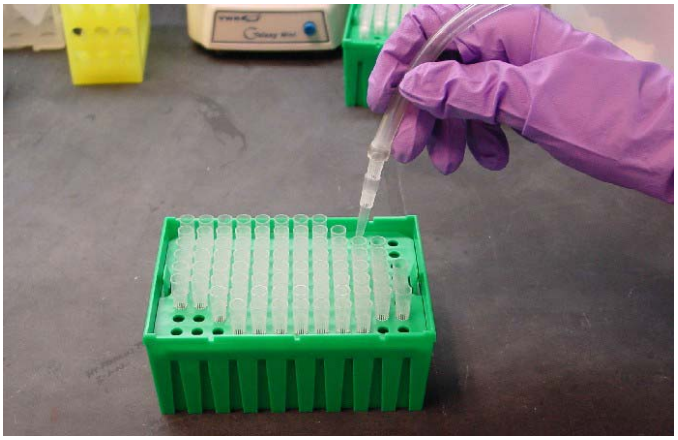

Figure 19. Replacing the disposable pipette tip

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

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