LabChip User Guide

Small RNA Assay User Guide

For LabChip® GXII Touch

 PN CLS153470 Rev D
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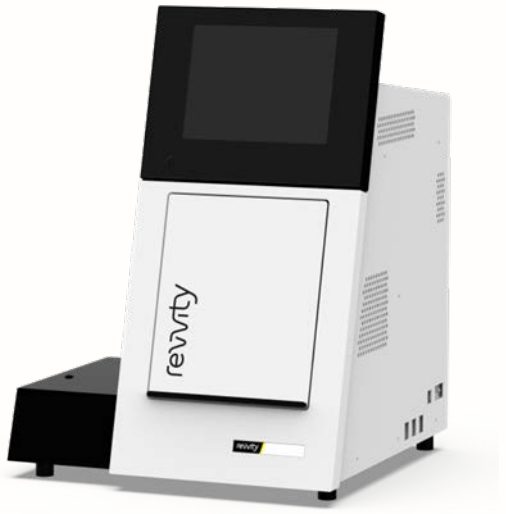

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Specifications

Assay Specifications

The Small RNA Assay is for use with the LabChip GX Touch/GXII Touch instruments. The LabChip GX Touch/GXII Touch instruments are for research use only and not for use in diagnostic procedures.

Table 1. Assay Specifications

Size Range	20 - 150 nucleotides
Linear Concentration Range	100 - 10,000 pg/µL
Sensitivity[a]	50 pg/µL
Sizing Reproducibility	CV < 5.0% or 4 nt
RNA Sample Volume	2 µL
Maximum Salt Concentration	10 mM Tris
Run Time	60 seconds per sample (~2.5 hours for 96 samples)
Compatible Plate Types	96-well or 384-well
Samples per Chip Prep	Up to 96 samples per HT chip prep (divided into 2 runs of 48 samples) Up to 48 samples per LT chip prep
Chip Preps per Reagent Kit	5 HT chip preps or 10 LT chip preps
For Research Use Only	

a. Maximum sensitivity is 50 pg/µL for samples (in TE buffer, 10 mM Tris, pH 8.0) run on-plate undiluted in sample buffer.

Sample Conditions

Table 2. Sample Conditions

Additives	Revvity recommends that BSA and detergents exceeding 0.05 mg/mL and 0.01% (v/v) respectively in concentration not be used. Higher concentrations can result in chip failure. In addition, non-aqueous solvents are not compatible with LabChip protocols.
Particulates	Spin down all sample plates prior to analysis. Filter all buffers with a 0.22 µm cellulose acetate filter.

Table 2. Sample Conditions(Continued)

Salt Concentration	Total salt concentration must not exceed 10 mM Tris. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.

Small RNA Reagent Kit Contents

The Small RNA Reagent Kit (P/N CLS153530) contains the reagents and consumables listed in the tables below.

Note: Use only consumables that are within their expiration date.

Storage: When not in use, store reagents at the temperatures specified in Table 3.

Table 3. Reagents

Reagent	Vial	Quantity	Storage Temperature
RNA Dye Concentrate	Blue	1 vial, 0.5 mL	2-8°C
RNA Chip Storage Buffer	White	5 vials, 1.8 mL each	2-8°C
Small RNA Gel Matrix	Red	5 vials, 0.510 mL each	2-8°C
Small RNA Marker	Green	1 vial, 0.8 mL	2-8°C
10X RNA Sample Buffer Concentrate	Purple	3 vials, 2.0 mL each	2-8°C
Small RNA Ladder[a]	Yellow	2 vials, 0.05 mL	-70°C

a. Additional Small RNA Ladder can be ordered separately using Part Number CLS153594.

Table 4. Consumable Items

Item	Supplier and Catalog Number	Quantity
Spin Filters	Costar®, Cat. # 8160	10
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

DNA 5K/RNA/CZE LabChips

Storage: When not in use, store chips at 2-8°C. If using a prepared chip again within 24 hours, the chip can be stored at room temperature.

Table 5. DNA 5K/RNA/CZE LabChips

Item	Part Number	Samples per Chip
DNA 5K/RNA/CZE HT Chip (GX Touch/GXII Touch HT)	760435	2000
DNA 5K/RNA/CZE 24 Chip (GX Touch/GXII Touch HT or 24)	CLS138949	750

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 products are used in accordance with the principles of good laboratory practice. All chemicals should be considered potentially hazardous. When handling chemical reagents, wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

WARNING!

RNA Dye contains DMSO. S24/25: Avoid contact with skin and eyes.

Usage

The Small RNA Assay is for use with LabChip GX Touch/GXII Touch instruments. LabChip GX Touch/GXII Touch instruments are for research use only. Not for use in diagnostic procedures.

Preparation Procedures

Additional Items Required

- 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent)
- 70% isopropanol solution in DI water
- DEPC-treated water (nuclease-free)
- PCR cap strips
- Revvity Hard-Shell thin-wall 384-well PCR plate (blue), 6008910 (recommended)
- Revvity Hard-Shell thin-wall 96-well skirted PCR plate (blue), 6008870 (recommended)

Preparing the Ladder Aliquots

The first time a vial of the Small RNA Ladder is thawed, denature the ladder. Aliquot the Small RNA Ladder to five 10 µL aliquots for individual use after thawing a vial for the first time.

Note: *Store the Small RNA Ladder (yellow cap) at* -70°C*. Keep thawed Small RNA Ladder on ice. Avoid multiple freeze-thaws.*

1. Thaw the Small RNA Ladder (yellow cap) on ice.
2. Spin down the Small RNA Ladder.
3. Heat-denature at 70°C for 2 minutes.
4. Immediately snap cool on ice for 5 minutes.
5. Pipette 6 µL of Small RNA Ladder into the provided 0.2 mL Ladder Tube.
6. With the remainder of the Small RNA Ladder, prepare four 10 µL aliquots in DNAse/RNAse-free tubes.
7. Store unused aliquots at -70°C.
8. When using frozen Small RNA Ladder aliquots, do not heat-denature again.

Preparing the Gel-Dye Solution

Note: *The prepared volume of Gel-Dye solution is enough for one HT (High-Throughput) or two LT (Low-Throughput) chip preps.*

Warning: *The dye is light sensitive.* ***Do not expose the Dye or Gel-Dye solution to light for any length of time.*** *Keep the prepared Gel-Dye solution in the dark.*

1. Allow the chip and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use.

   Note: *The RNA Dye contains DMSO and* ***must be thawed completely*** *before use.*

2. Vortex the thawed **RNA Dye Concentrate** (blue cap 🔵) for 10 - 15 seconds before use.

3. Add **70 µL** of **RNA Dye Concentrate** (blue cap 🔵) to **one vial, 510 µL,** of Small RNA Gel Matrix (red cap 🔴).

4. Vortex and invert the tube several times until the Gel-Dye solution is well mixed and spin down for a few seconds.

5. Transfer the Gel-Dye solution to a spin filter. Use a centrifuge tube filled with 580 µL of water to balance the centrifuge.

6. Centrifuge at 9300 rcf for 10 minutes at room temperature.

7. Discard the filter.

8. Label and date the tube.

9. Store in the dark at 2-8°C. Use within 5 days.

Preparing the Chip

1. Allow the chip to equilibrate to room temperature 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 1). For details on how to set up a vacuum line, see page 34.

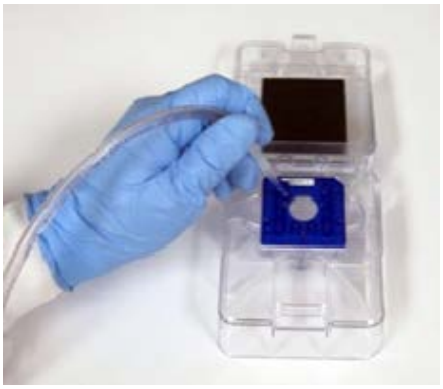

Figure 1. Using a vacuum to aspirate the chip wells is more effective than using a pipette

3. Rinse and completely aspirate each active chip well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
4. If any water spills onto the top or bottom chip surfaces during rinsing, aspirate using the vacuum line. DO NOT run the tip over the detection window. Use the provided Detection Window Cleaning Cloth dampened with water (Milli-Q® or equivalent) or alcohol to clean the detection window as needed.
5. Using a reverse pipetting technique, add **Gel-Dye solution** to chip wells 3, 7, 8, and 10 as shown in Figure 2 (Low-throughput) or Figure 3 (High-throughput).

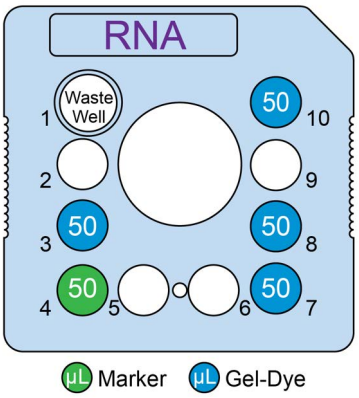

Figure 2. Low-throughput chip preparation

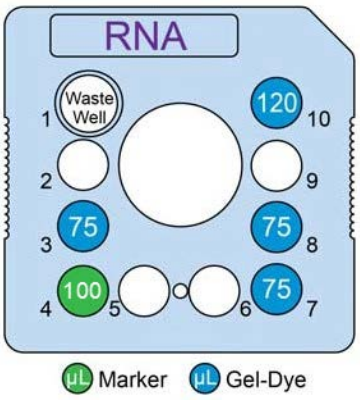

Figure 3. High-throughput chip preparation

6 Add **50 µL** (Low-throughput) or **100 µL** (High-throughput) of **Small RNA Marker** (green cap ●) to **chip well 4** as shown in Figure 2 or Figure 3.

Note: *The marker well may need to be replenished if the chip is in idle mode on the instrument for an extended period of time.*

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

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

Note: *Use the Low-throughput protocol when running the LabChip GX Touch/GXII Touch 24 instrument.*

Preparing the RNA Samples, Ladder and Sample Buffer

Notes: *To minimize sample evaporation, test no more than 48 samples per run. For example, if analyzing 96 samples, test samples in two runs.*

The total salt concentration of samples must not exceed 10 mM Tris. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.

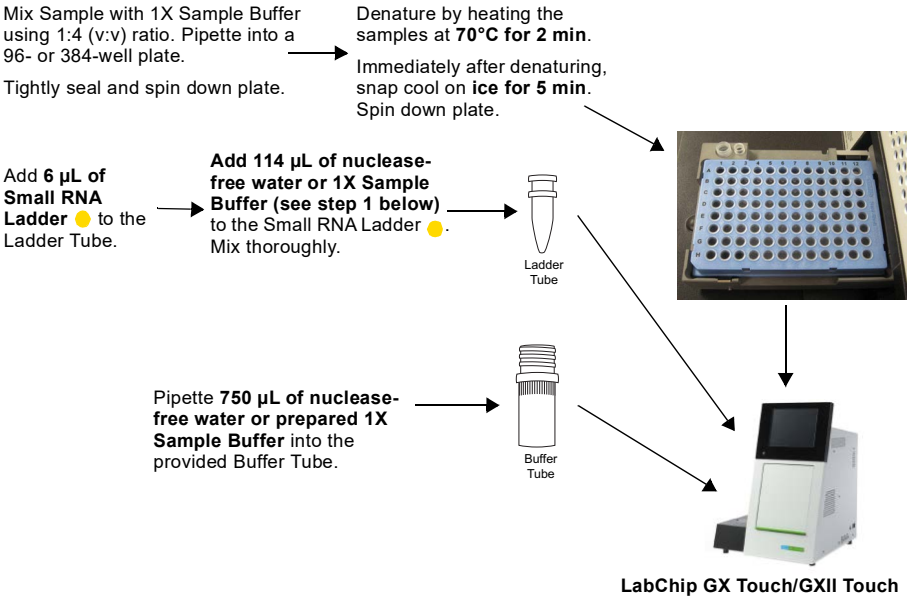

Figure 4. Sample, Ladder Tube, and Buffer Tube Preparation

1 Prepare 1X Sample Buffer by adding **200 µL RNA Sample Buffer Concentrate** (purple cap ●) to **1800 µL DEPC-treated or nuclease-free water**. Nuclease-free water can be used in place of sample buffer for highest sensitivity. Samples can also be run undiluted.

Note: *The RNA Sample Buffer Concentrate is a 10X solution. Sample Buffer is stable after dilution, but to avoid RNase contamination, sample buffer should be prepared fresh.*

2 Mix Sample with 1X Sample Buffer using 1:4 (v:v) ratio. Pipette into a 96- or 384-well plate (up to 48 samples per run.)

3 Tightly seal and spin down plate.

4 Cover samples in microtiter plates with PCR cap strips. Foil is not recommended because the adhesive may contaminate the samples. If diluting the samples, use nuclease-free water.

5 Denature samples by heating at 70°C for 2 minutes.

Note: *For sample heat denature, if a 384-well thermocycler or heat block is not available, the sample plate can be heated by placing the plate on top of one heat block, and then placing another heat block on top of the plate.*

6 Snap cool the samples by immediately placing the samples on ice for 5 minutes.

7 Spin down the plate at 3000 rpm for 5 minutes at room temperature to remove air bubbles.

8 Prepare the Small RNA Ladder (yellow cap):

a Thaw a **10 µL aliquot of Small RNA Ladder** (yellow cap) on ice.

b Add **114 µL of nuclease-free water or prepared 1X Sample Buffer** to the provided Ladder Tube.

c Add **6 µL of Small RNA Ladder** to the Ladder Tube.

d Mix thoroughly. Ensure there are no air bubbles in the Ladder Tube.

e Insert the Ladder Tube into the LabChip GX Touch/GXII Touch instrument.

Preparing the Buffer Tube

1 Add **750 µL nuclease-free water or prepared 1X Sample Buffer** 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 GX Touch/GXII Touch instrument.

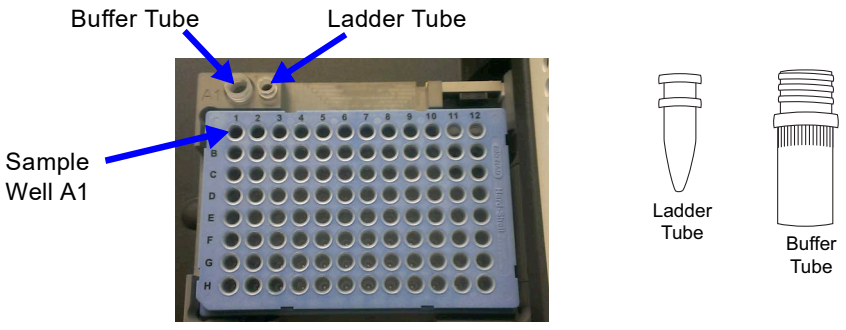

Figure 5. Locations of the Buffer Tube and Ladder Tube in the GX Touch/GXII Touch instrument

Inserting a Chip into the LabChip GX Touch/GXII Touch Instrument

1 Remove the PCR Cap strips and place the sample plate, Buffer Tube, and Ladder Tube into the instrument.

2 Remove the chip from the chip storage container and inspect the detection window. Clean BOTH sides of the detection 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 6).

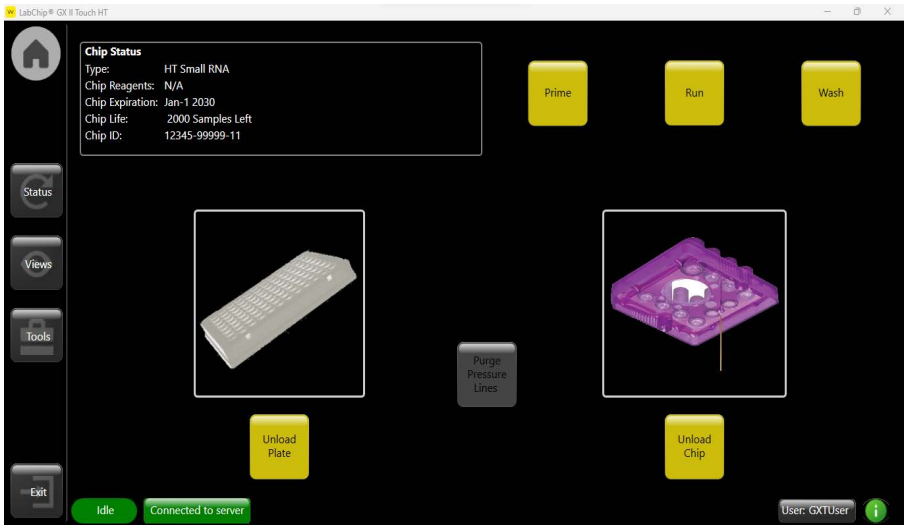

Figure 6. Home Screen

4 Insert the chip into the LabChip GX Touch/GXII Touch instrument (Figure 7) and close the chip door securely.

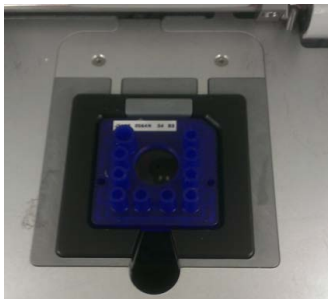

Figure 7. Chip in the LabChip GX Touch/GXII Touch instrument

5 Touch the **Load Plate** button on the Home screen (Figure 6) to retract the sample plate and move the sipper to the Buffer Tube. The Assay Choice screen opens (Figure 8).

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

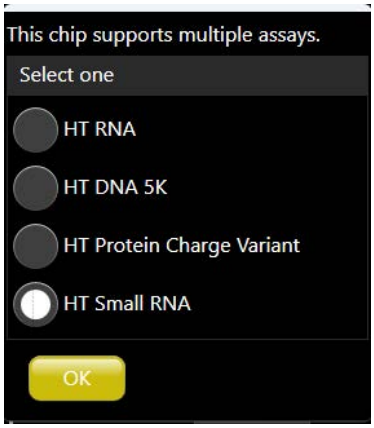

Figure 8. Assay Choice Screen

6 Touch the Small RNA assay and then touch **OK**.

Notes: *If performing multiple chip preps in one day, wash the chip in between chip preparations using the instrument and RNA Chip Storage buffer as described in* *"Washing and Repriming Chips" on page 19**.*

Be sure to periodically clean the O-rings on the top plate of the chip interface on the LabChip GX Touch/GXII Touch. Use the provided lint-free swab dampened with water to clean the O-rings using a circular motion. Allow the O-rings to dry before inserting a chip.

Operating Procedures

Running the Assay

Note: *The chip can be primed independently from running assays. Touch the Prime button on the Home screen (Figure 6 on page 13). Select the desired assay from the Assay drop-down list (see Figure 11 on page 16). Make sure the Buffer Tube is in the instrument. Touch the Prime button on the Chip Priming screen (Figure 9).*

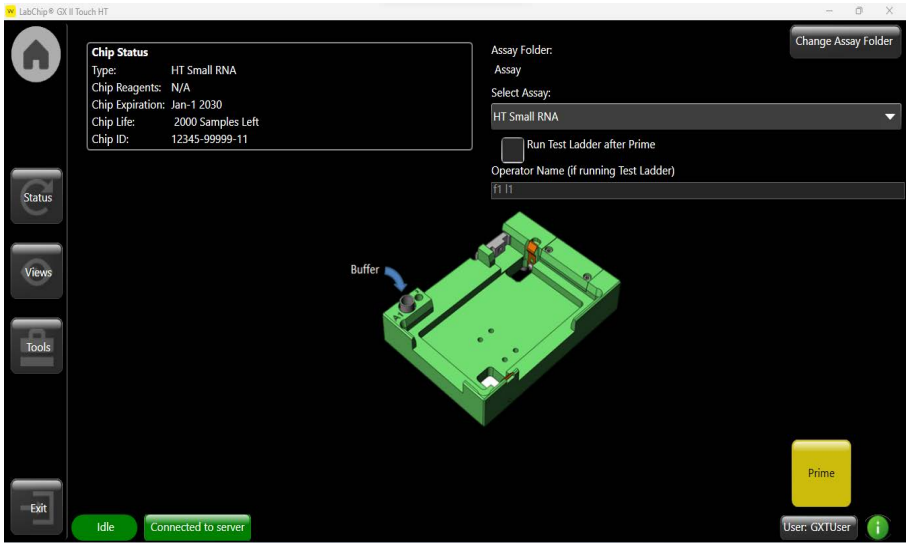

Figure 9. Chip Priming Screen

To run an assay:

1 Touch the **Run** button on the Home screen (see Figure 6 on page 13). The Select Wells tab opens (see Figure 10 on page 16).

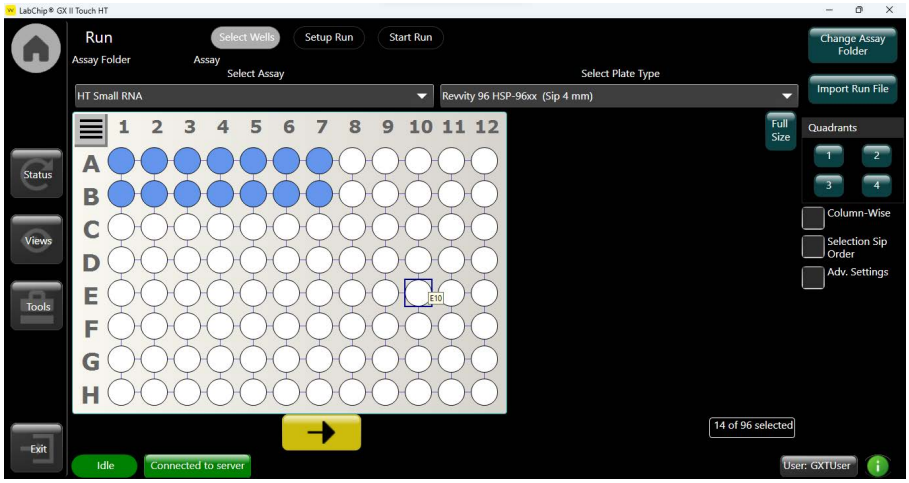

Figure 10. Select Wells Tab

2 Select the **HT Small RNA** assay in the Assay Type list (see Figure 11).

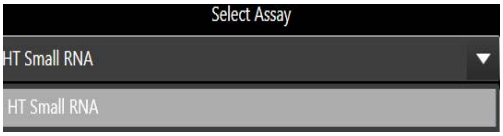

Figure 11. Assay Type Drop-Down List

3 Select the plate type, well pattern, and sip order (column or row). If desired, touch **Adv. Settings** to specify the number of times each well is sampled.

4 Touch the **Green Arrow** button. The Setup Run tab (Figure 12) opens.

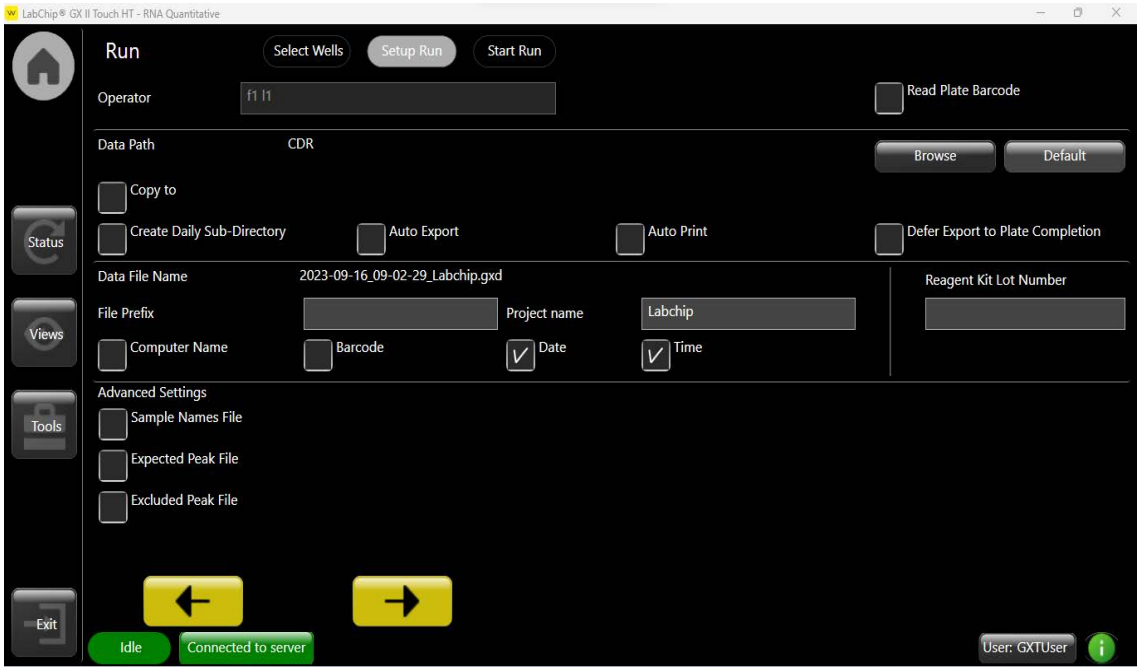

Figure 12. Setup Run Tab

5 Specify the operator name, the option to read the barcode, the destination of the file, the use of sample names, expected peaks, excluded peaks, the filename convention, and the auto export settings.

6 Touch the **Green Arrow** button. The Start Run tab (Figure 13) opens.

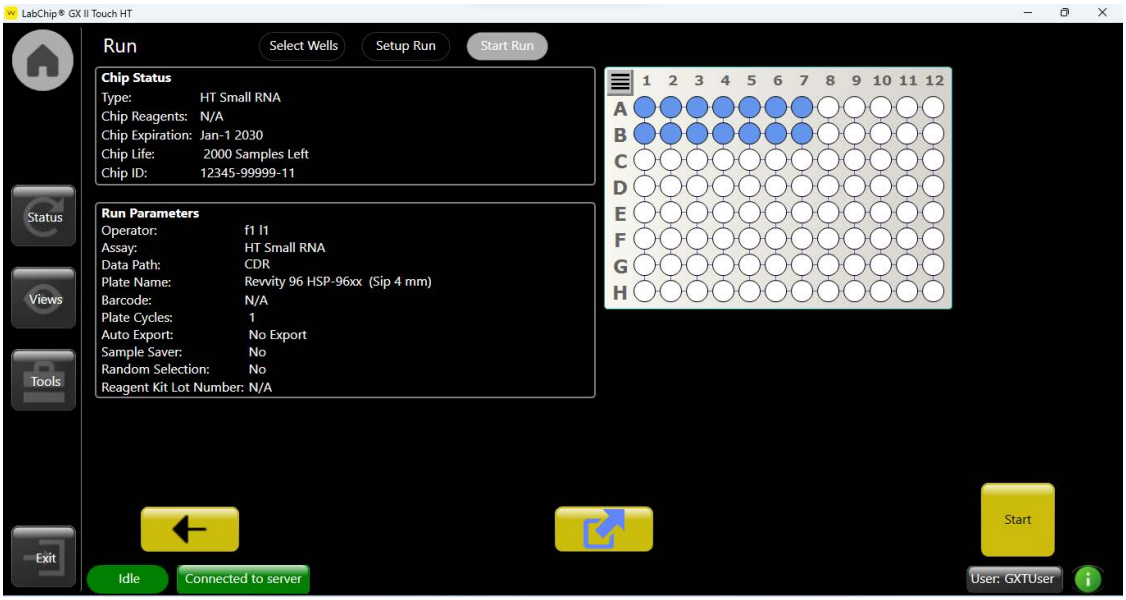

Figure 13. Start Run Tab

7 Touch **Start** to begin the run. If the chip has not been primed since the last time the chip door was opened, the chip is primed automatically at the start of the run.

Repriming Chips

If air bubbles or clogs in the chip channels are suspected, the chip can be reprimed to help remove air bubbles or clogs.

Note: *Place a Buffer tube with prepared 1X Sample Buffer solution or nuclease-free water into the instrument while priming chips.*

1 Touch the **Unload Chip** button on the Home screen to open the instrument door. The software automatically resets to require priming prior to running the chip again.

2 Place the chip and Buffer Tube into the instrument.

3 Close the chip door securely and choose the corresponding assay.

4 Touch the **Prime** button on the Home screen. The Prime screen opens.

5 Touch the **Prime** button on the Prime screen to reprime the chip.

Washing and Repriming Chips

Washing the chip clears all reagents from the chip channels. The chip can be immediately reprimed to help remove air bubbles, clogs, particulates, or residue.

1. Touch the **Unload Chip** button on the Home screen to open the instrument door.
2. Remove the chip from the instrument. Place the chip in the chip storage container, ensuring the sipper is submerged in fluid.
3. Thoroughly aspirate all fluid from the chip wells using a vacuum.
4. Rinse and completely aspirate each active well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
5. Add **120 µL** of **RNA Chip Storage Buffer** to each active well (1, 3, 4, 7, 8, and 10).
6. Place the chip in the LabChip GX Touch/GXII Touch instrument.
7. Place a Buffer Tube with **750 µL** of prepared **1X Sample Buffer** or nuclease-free water in the instrument.
8. Close the chip door securely.
9. Touch the **Wash** button on the Home screen. The Wash screen (Figure 14) opens.

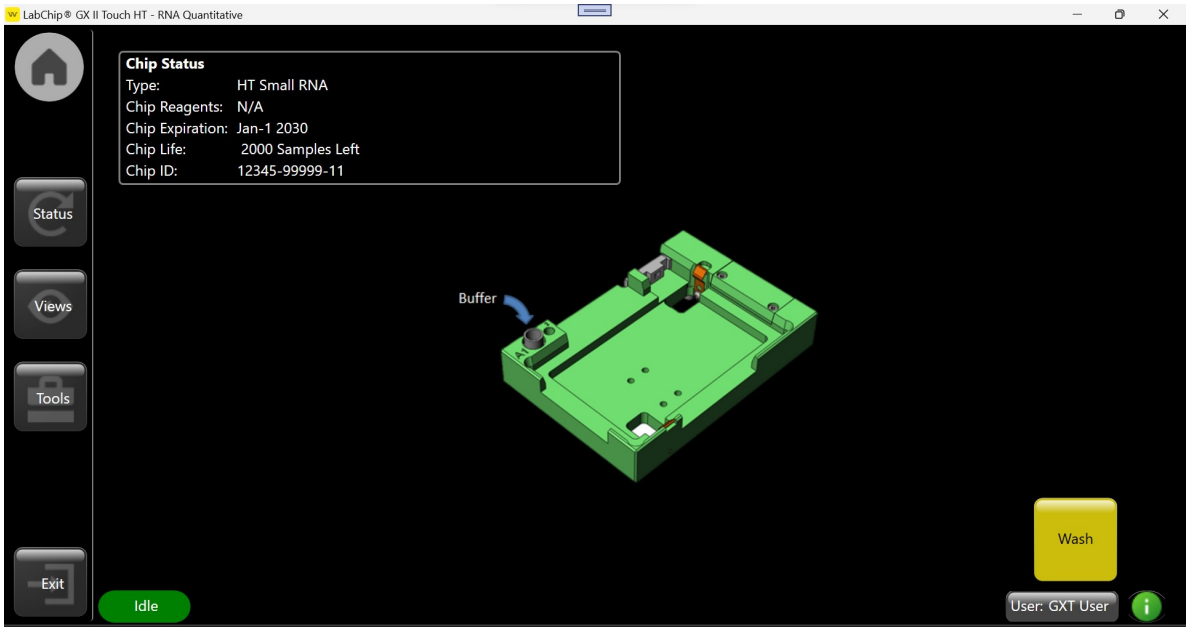

Figure 14. Wash Screen

10 Touch the **Wash** button on the Wash screen to start the chip wash.

11 After the completion of the wash cycle, touch the **Unload Chip** button on the Home screen to open the instrument door.

12 Return the chip to the chip storage container. Verify the sipper is submerged in fluid.

13 Thoroughly aspirate all fluid from the chip wells using a vacuum.

14 Prepare the chip as described in “Preparing the Chip” on page 9.

15 Place the chip into the LabChip GX Touch/GXII Touch instrument.

16 Close the chip door securely.

17 Touch the **Run** or **Prime** button on the Home screen.

18 If air bubbles are not dislodged after a reprime: Fill all active wells with 100 µL of RNA Chip Storage Buffer, then suction the sipper with a vacuum line as shown in Figure 15 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.

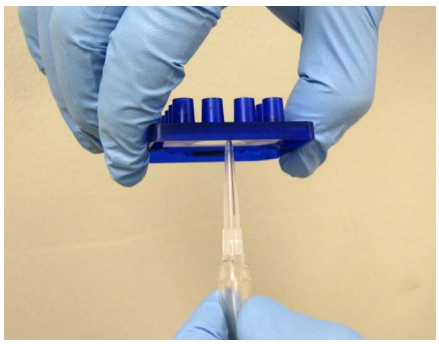

Figure 15. Removing an air bubble or clog by suctioning the sipper with a vacuum

Cleaning and Storing the Chip

After use, the chip must be cleaned and stored in the chip container. The chip can be washed the following morning when running overnight.

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, 3, 4, 7, 8, and 10) twice with water (Milli-Q® or equivalent).

4 Add **120 µL RNA Chip Storage Buffer** (white cap ◯) to the active wells.

5 Place the chip in the LabChip GX Touch/GXII Touch instrument. Ensure a buffer tube with **750 µL RNA Sample Buffer** or water is in the Buffer slot.

6 Touch the **Wash** button on the Home screen. The Wash screen (Figure 16) opens.

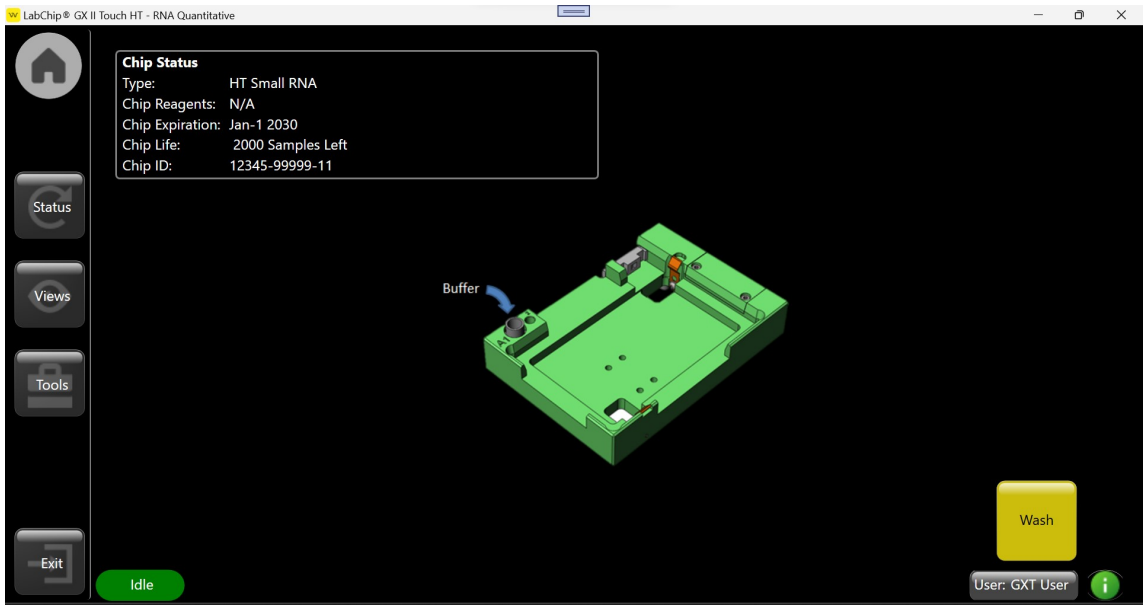

Figure 16. Wash Screen

7 Touch the **Wash** button on the Wash screen to start the chip wash.

8 When the chip wash is complete, touch the **Unload Chip** button, remove the chip from the instrument and place the chip in the chip storage container.

9 Add an additional **50 µL RNA Chip Storage Buffer** to well 1.

10 Cover the wells with Parafilm® to prevent evaporation and store at 2-8°C. Storing a chip with dry wells may clog the chip. If using the chip again within 24 hours, the chip can be stored at room temperature.

Chip Cartridge Cleaning

Daily

1 Inspect the inside of the chip cartridge and O-rings for debris.

2 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.

3 Clean the electrodes with the provided lint-free swab dampened with water (Milli-Q® or equivalent).

Monthly

1 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 GX Touch/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.

2 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).

3 Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

Results

Small RNA Ladder Result

The electropherogram of a typical Small RNA ladder using the Standard Sample Workflow is shown in Figure 17. Following the lower marker, ladder fragments in order of increasing migration time correspond to 4, 25, 45, 65 and 100 nt.

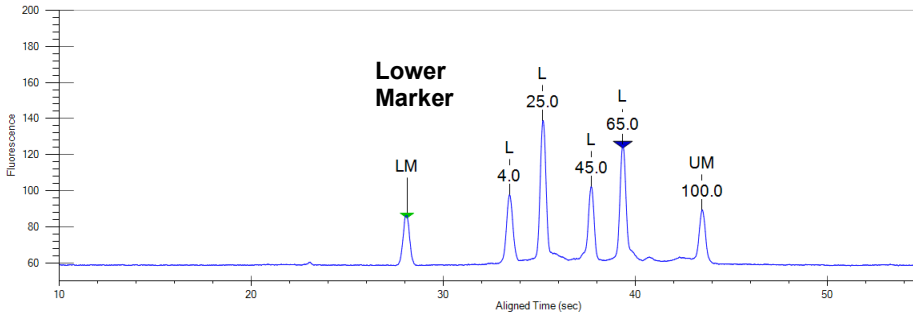

Figure 17. Typical Small RNA Ladder

Small RNA Sample Result

Figure 18 shows the electropherogram for a small RNA fragment (20 nt, IDT laboratories) measured using the Small RNA Assay. The main peak (measured 21.4 nt) can be quantified using the LabChip GX Reviewer software.

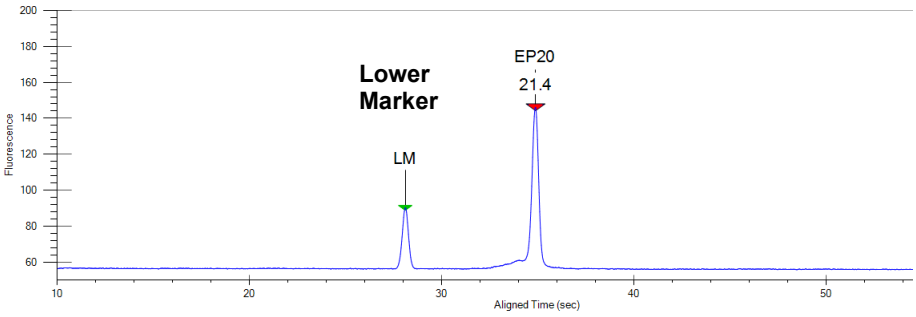

Figure 18. Electropherogram for a typical Small RNA sample

Concentration Values

The value for concentration is calculated based on the **Dilution Correction** value specified in the Analysis tab of the Analysis Settings window. By default, the Dilution Correction value is set to 1, giving the on-plate sample concentration. To give the undiluted sample concentration, the Dilution Correction value must be edited for the dilution used. For example, if the sample is diluted 1:1, set the Dilution Correction value to 2.0.

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:

- Air bubble in sipper introduced during chip priming.

What to do:

- Reprime the chip. See “Repriming Chips” on page 18 for instructions on how to reprime the chip.

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 18 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 “Repriming Chips” on page 18 for instructions on how to reprime the chip.

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 µL (minimum volume is 100 µL).

Symptom: No marker peaks but sample peaks are present.

Possible causes:

1 No Small RNA Marker added to chip well 4. The Small RNA marker may not have been put into the marker well during chip prep or the chip may have remained idle in the instrument for an extended period of time.

2 If there is Small RNA Marker in chip well 4, the marker channel may be clogged.

What to do:

1 Add or replenish the Small RNA Marker in the chip:

   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 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® or equivalent).

e Add Small RNA Marker (green cap) to chip well 4.

f Insert the chip back into the instrument.

g Restart the run.

2 Reprime the chip to unclog the marker channel. See “Repriming Chips” on page 18 for instructions on how to reprime the chip.

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

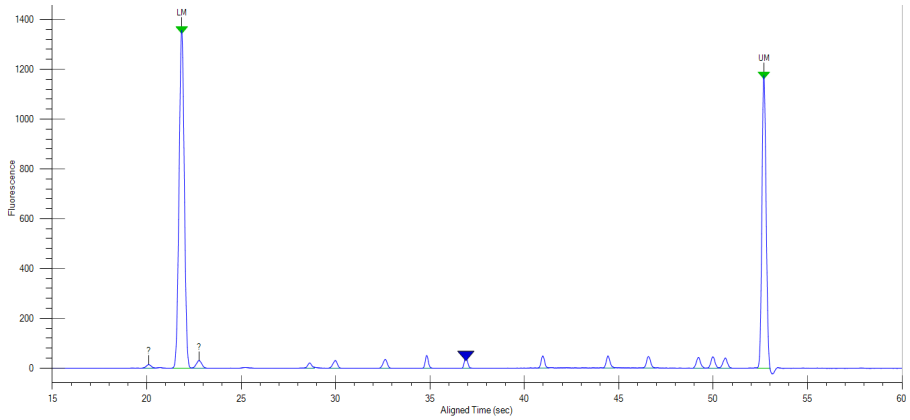

Figure 19. Small ladder peaks in sample well caused by delayed sip

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 18 for instructions on how to reprime the chip.

Symptom: Unexpected sharp peaks.

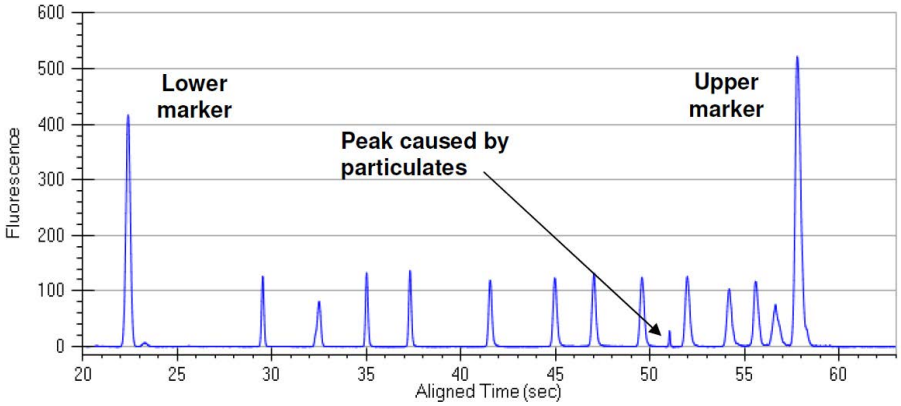

Figure 20. Unexpected sharp peak

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-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.

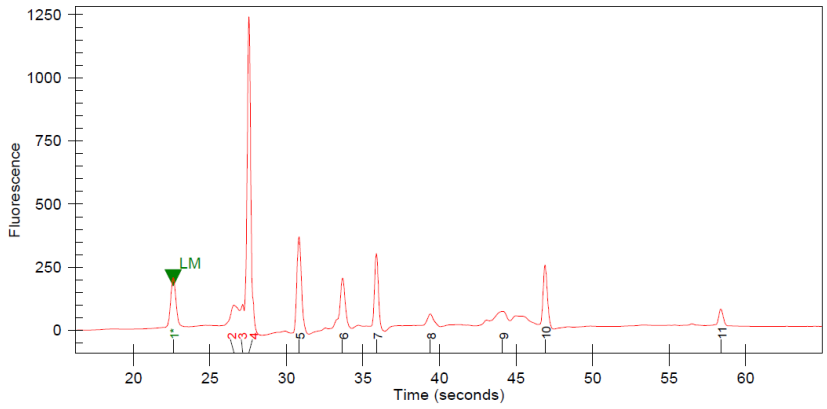

Figure 21. Humps in several electropherograms

Possible causes:

- Electrode 7 is dirty and has contaminated the Gel-Dye solution in well 7.

What to do:

- 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.

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 marker is:

- *Small RNA Assay Lower Marker: 25.5 - 30 seconds*

Possible causes:

1. Incorrect Gel-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 in the separation channel will slow down migration, and less dye in the separation channel will speed up migration.*

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.
4. A pressure leak or current leak can slow peak migration.

What to do:

1. Prepare fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye solution. See “Washing and Repriming Chips” on page 19 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 for 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 spinning the sample plate (e.g. 3000 rpm for 5 minutes) before starting a new run. The debris can be flushed out of the chip by washing and re-priming the chip. See “Washing and Repriming Chips” on page 19 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.

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 (see page 35).

General

- Allow the chip, sample plate, and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use. Thaw the Small RNA Ladder on ice. (It is recommended to denature and aliquot the Small RNA Ladder into five 10 µL aliquots for individual use after thawing the vial for the first time.)
- Use only consumables that are within their expiration date.
- 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 (see page 32) will help avoid introducing bubbles into the chip when pipetting the gel.

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 90 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

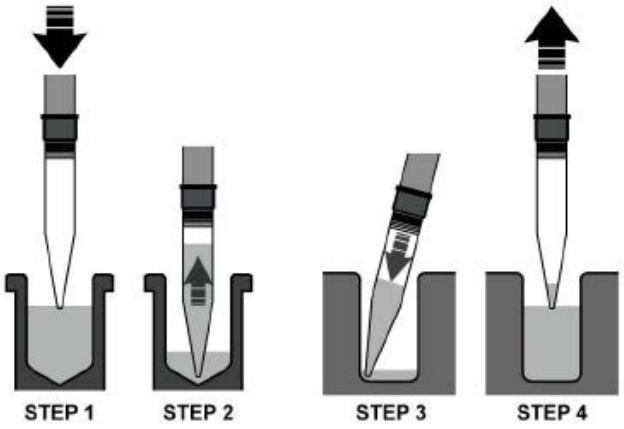

Figure 22. Reverse pipetting

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 reagents at 2-8°C when not in use. Store Small RNA Ladder at -70°C when not in use.
- All refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.
- Allow the Small RNA Ladder to thaw on ice. Aliquot the Small RNA Ladder to five 10 µL aliquots for individual use after thawing for the first time.
- The RNA Dye contains DMSO and must be thawed completely before use. It is recommended that you prepare aliquots to reduce the time required for thawing.
- Gently vortex all kit reagents before use.
- Dispense reagents into chip wells slowly without introducing air bubbles. Insert the pipette tip vertically and to the bottom of the chip well.
- Protect the Dye and Gel-Dye solution from light. Store in the dark at 2-8°C when not in use.
- The Gel-Dye solution expires 5 days after preparation.

Chips

- Store chips at 2-8°C prior to first use.
- After use, cover the active chip wells with Parafilm® and store at 2-8°C. If using the chip again within 24 hours, store the chip at room temperature (20 - 25°C).
- 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.
- Keep the sipper submerged in fluid at all times and do not expose 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 chip to dust by keeping the chip 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 idle on the instrument for up to 8 hours. This workflow allows analysis of samples as needed throughout the day without having to re-prep the chip, as long as the maximum number of samples per chip prep is not exceeded.
- Revvity recommends the chip be re-prepared after it has been idle for 8 hours.

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 48 samples in a 384-well plate can be processed in a single run. Up to 96 total samples can be processed with an HT chip prep. Up to 48 total samples can be processed with an LT chip prep.

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 23). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 24).

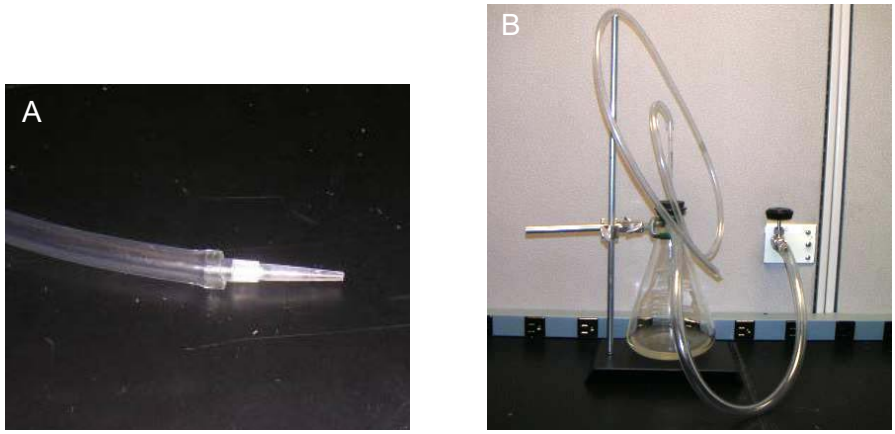

Figure 23. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap

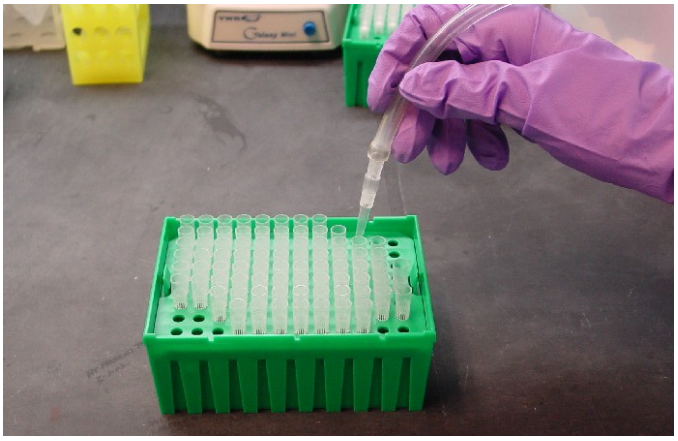

Figure 24. Replacing the disposable pipette tip

Reordering Information

Table 6. Reordering Information

Product	Part Number
Small RNA Assay Reagent Kit	CLS153530
Small RNA Ladder	CLS153594
DNA 5K/RNA/CZE HT Chip (GX Touch/GXII Touch HT)	760435
DNA 5K/RNA/CZE 24 Chip (GX Touch/GXII Touch HT or 24)	CLS138949
Detection Window Cleaning Cloth	VWR®, Cat. # 21912-046
Swab	ITW Texwipe®, Cat. # TX758B
Revvity 96-well PCR Plate	6008870
Revvity 384-well PCR Plate	6008910

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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 additional assay and instrument troubleshooting, refer to the LabChip GX Touch Software Help file or the *LabChip GX Touch User Manual*.

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