RNA Pico Sensitivity Assay User Guide

For LabChip GX Touch/GXII Touch

CE

PN CLS140165, Rev. C

Contents

Specifications

Assay Specifications[1]

Table 1. Assay Specifications

Sensitivity	250 pg/µL Total RNA 500 pg/µL mRNA
Linear Concentration Range	500 - 5000 pg/µL Total RNA 625 - 5000 pg/µL mRNA
Quantitation Reproducibility	20% CV
Quantitation Accuracy	± 30% (for ladder as sample)
Size Range	100 - 6000 nucleotides
RNA Sample Volume	2 µL
Run Time	80 seconds per sample (about 2.5 hours for 96 samples)
Compatible Plate Types	384-well
Number of Samples per Chip Prep	Up to 96
Samples per Reagent Kit	Up to 480

Sample Conditions

Table 2. Sample Conditions

Additives	PerkinElmer 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	All 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 10 mM Tris. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.

1. All specifications pertaining to Total RNA and mRNA were determined using RNA diluted in water

Kit Contents

Storage: When not in use, store chips and reagents refrigerated at 4°C. Do not leave chips and reagents unrefrigerated overnight. Store the RNA Ladder at -70°C.

The kit contains enough reagents for 9 Low-throughput (LT) or 5 High-throughput (HT) chip preparations. Up to 48 samples can be tested with an LT chip prep. Up to 96 samples can be tested with an HT chip prep.

Table 3. Pico RNA Reagent Kit Contents, PN CLS960012

Reagent	Vial	Quantity
RNA Dye Concentrate	Blue	1 vial, 0.5 mL
Chip Storage Buffer	White	5 vials, 1.8 mL each
RNA Pico Gel Matrix	Red	5 vials, 0.510 mL each
RNA Pico Ladder (packaged separately, Part Number CLS760652)[a]	Yellow	2 vial, 0.04 mL
RNA Pico Marker	Green	1 vial, 0.8 mL
10X RNA Sample Buffer Concentrate	Purple	3 vials, 2.0 mL each

a. Additional RNA Pico Ladder can be ordered separately using Part Number CLS760652.

Table 4. Consumable Items

Item	Supplier and Catalog Number	Quantity
Spin Filters	Costar, Cat. # 8160	10
Ladder Tubes	Genemate, Cat. # C-3258-1	10, 0.2 mL
Detection Window Cleaning Cloth	VWR, Cat. # 21912-046	1
Swab	ITW Texwipe®, Cat. # TX758B	3
Buffer Tubes	E&K Scientific, Cat. # 697075-NC	10, 0.75 mL

Table 5. RNA LabChips

Item	Catalog Number
DNA 5K/RNA/CZE Chip for use with GX Touch/GXII Touch HT	Cat. # 760435
DNA 5K/RNA/CZE Chip for use with GX Touch/GXII Touch 24	Cat. # CLS138949

Safety Warnings and Precautions

WARNING!

For Research Use Only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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. As all chemicals should be considered 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 Concentrate contains DMSO. S24/25: Avoid contact with skin and eyes.

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
- Bio-Rad Hard-Shell® 384-well Skirted PCR Plates, Cat # HSP-38XX (recommended).
- PerkinElmer Hard-Shell thin-wall 96-well skirted PCR plate (blue), Cat # 6008870 (recommended).

Note: *Allow the chip and reagents to equilibrate to room temperature for 20-30 minutes before use.*

Preparing the Ladder Aliquots

Note: *The RNA Pico Ladder* (yellow cap 🟡) *should be kept on ice. Avoid multiple freeze-thaws.*

1. Spin down RNA Pico Ladder (yellow cap 🟡) and heat-denature at 70°C for 2 minutes. Afterward, immediately snap cool on ice for 5 minutes.
2. Prepare 4 µL aliquots in nuclease-free tubes. Store aliquots at -70°C.
3. When using frozen aliquots, do not heat-denature again. If needed, additional ladder can be ordered (Cat. # CLS760652).

Preparing the Gel-Dye Solution

Notes: *The Dye Solution contains DMSO and* ***must be thawed completely*** *before use.*

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

1. Vortex the thawed RNA Dye Concentrate (blue cap 🔵) for 10 - 15 seconds before use.
2. Add 90 µL of Pico RNA Dye Concentrate (blue cap 🔵) to one vial of Pico RNA Gel Matrix (red cap 🔴). Vortex until the solution is well mixed and transfer to a spin filter.

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

4 Discard filters, label and date the tubes.

5 Store in the dark at 4°C. Use within 3 weeks.

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

Preparing the Chip

1 Allow the chip to come to room temperature.

2 Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells (see Figure 1). For more details on how to set up a vacuum line see page 33.

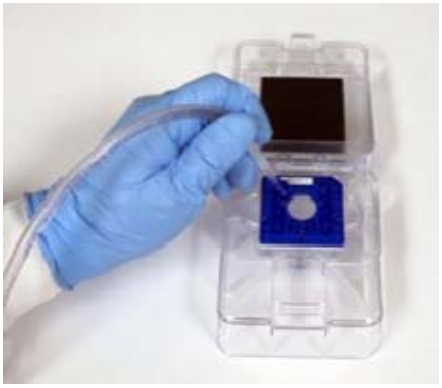

Figure 1. Using a vacuum to aspirate the chip wells is more effective than using a pipette.

3 Rinse and completely aspirate each active chip well (1, 3, 4, 7, 8 and 10) twice with nuclease-free water. Do not allow active wells to remain dry.

4 If any water spills onto the top and bottom 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 dampened in water (Milli-Q® or equivalent) or alcohol to clean the chip 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).

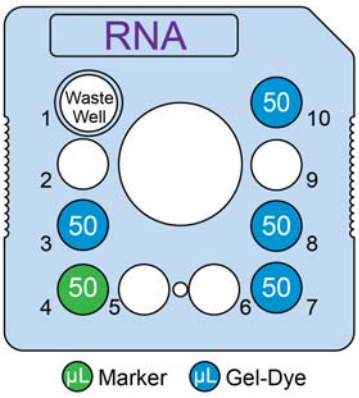

Figure 2. Low-throughput chip preparation.

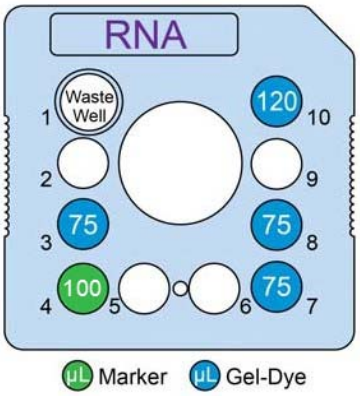

Figure 3. High-throughput chip preparation.

6 Add 50 µL (Low-throughput) or 100 µL (High-throughput) of RNA Pico 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.

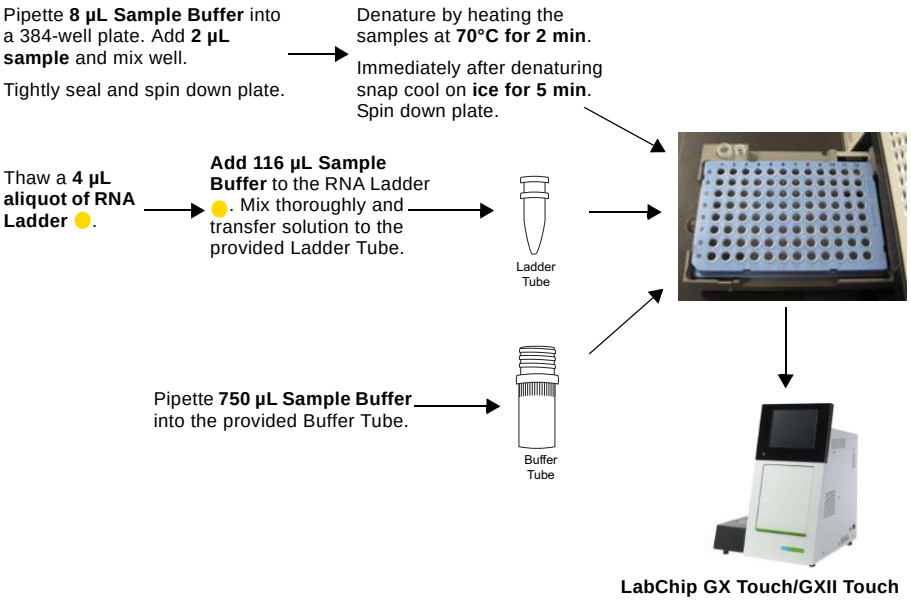

Figure 4. Sample, Ladder Tube and Buffer Tube preparation.

1 Prepare Sample Buffer by adding 200 µL of RNA Sample Buffer Concentrate (purple cap ●) to 1800 µL DEPC-treated or nuclease-free water.

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 Pipette 8 µL of Sample Buffer (maximum 48 samples) into individual wells of a microtiter plate or into RNase-free microcentrifuge tubes.

3 Add 2 µL of sample to each well and mix by pipetting up and down a few times. Avoid introducing bubbles. Samples in microtiter plates should be covered with PCR cap strips. Foil is not recommended as the adhesive may contaminate the samples. If diluting the samples, use nuclease-free water.

4 Denature samples by heating at 70°C for 2 minutes. Immediately after, snap cool on ice for 5 minutes. Spin down plate.

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.*

5 Prepare the RNA Ladder (yellow cap) by thawing a 4 µL aliquot. Add 116 µL of Sample Buffer to the RNA Ladder aliquot. Mix thoroughly and transfer solution to the provided Ladder Tube. Ensure there are no air bubbles in the Ladder Tube.

Preparing the Buffer Tube

1 Add 750 µL of RNA Sample Buffer solution 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.

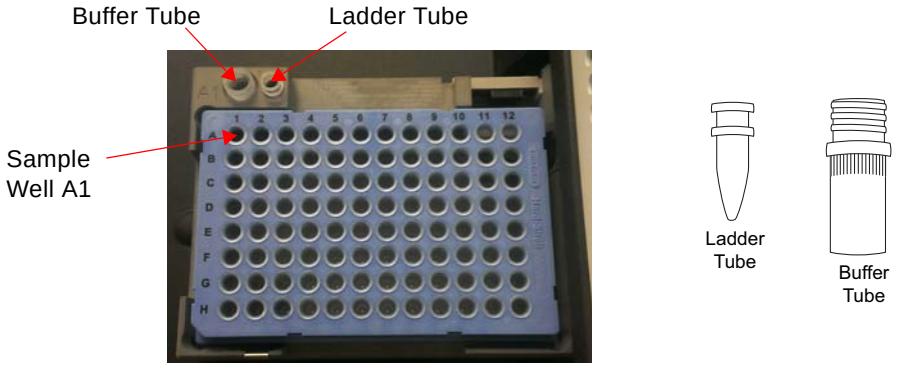

Figure 5. Locations of the Buffer Tube and Ladder Tube in the GX Touch/GXII Touch instrument.

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

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

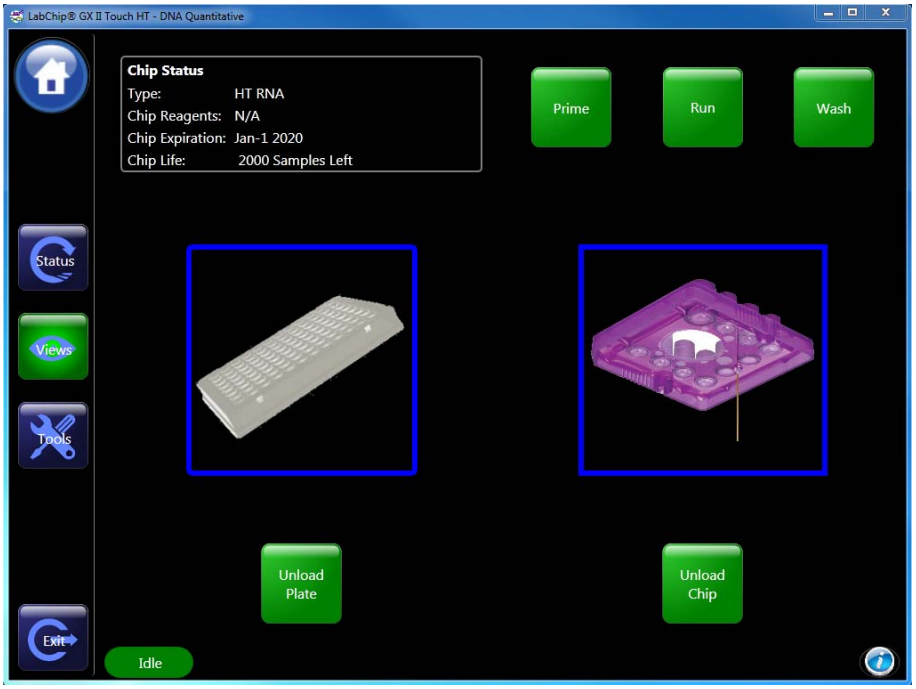

Figure 6. *Home* screen.

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

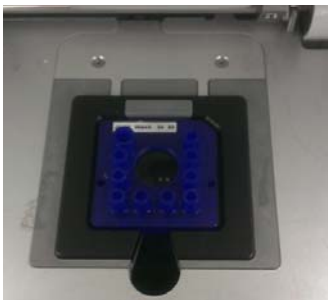

Figure 7. Chip in the LabChip GX Touch/GXII Touch instrument.

5 Touch the *Load Plate* button on the *Home* screen (Figure 6) to retract the sample plate and 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.*

6 The *Assay Choice* window will appear (Figure 8). Touch the desired assay and then touch *OK*.

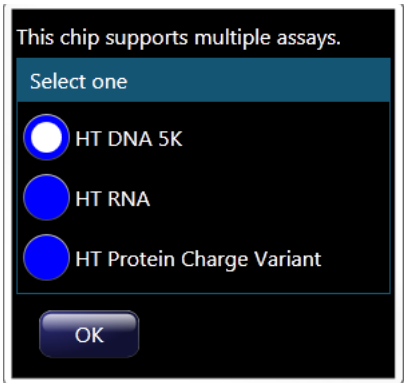

Figure 8. *Assay Choice* window.

Notes: *If performing multiple runs in a day, in between chip preparations the chip should be washed using the instrument and Chip Storage buffer as described in "Storing the Chip" on page 17.*

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.

Running the Assay

Note: *Chips can be primed independently from running assays. Touch the Prime button on the Home screen.* ***Make sure the Buffer Tube is placed on the instrument.***

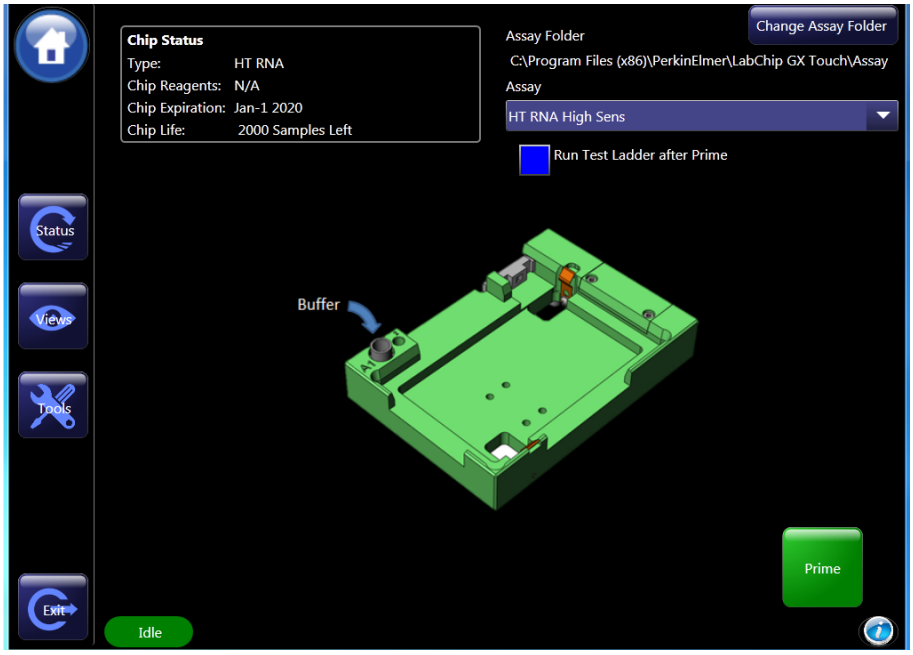

Figure 9. Chip priming screen.

1 Touch the *Run* button (see Figure 9).

2 Select the appropriate assay type (see Figure 10). For RNA Pico Sensitivity the available assay types are:

- **RNA Pico Sensitivity:** For analysis of Eukaryotic Total RNA.
- **RNA Pico mRNA:** For analysis of mRNA.

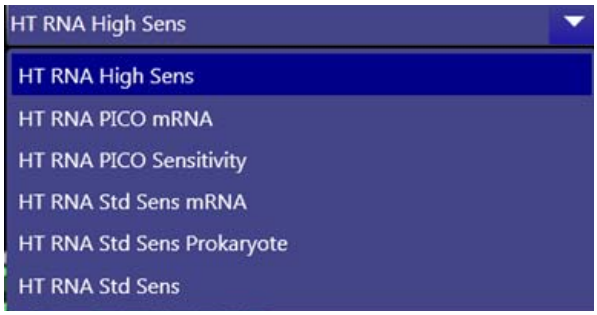

Figure 10. *Assay Choice* window.

3 Select the 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 11). Touch the *green arrow* button.

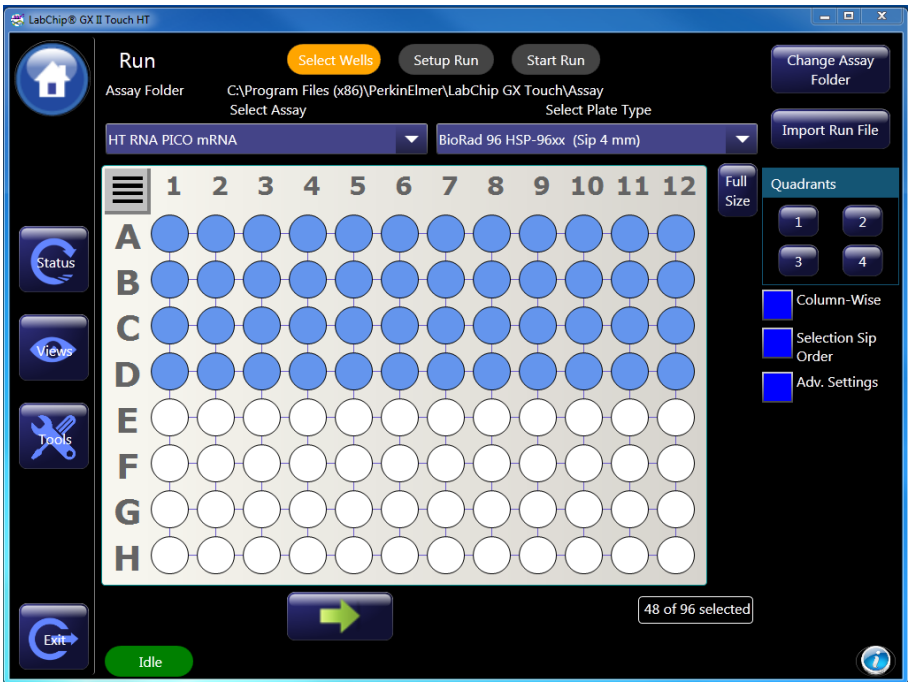

Figure 11. Selecting wells.

4 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 12). Touch the *green arrow* button.

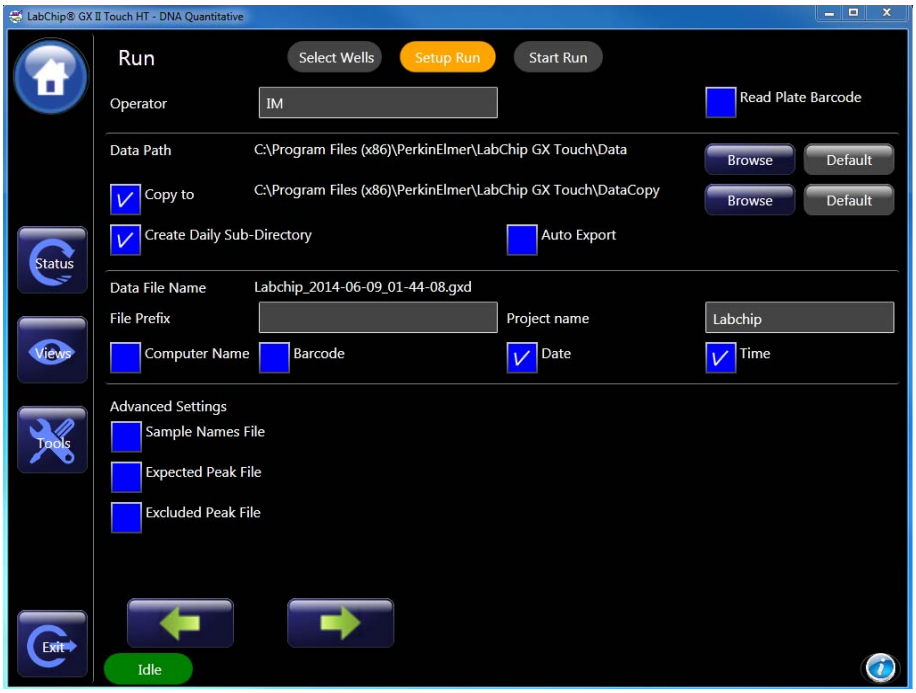

Figure 12. Run setup screen.

5 Touch *Start* to begin the run (Figure 13).

Figure 13. Starting a run.

Storing the Chip

After use, the chip must be cleaned and stored in the chip container. The procedure below can be conducted the following day when running overnight.

1. Place the chip into the plastic storage container. The sipper should be submerged in the fluid reservoir.
2. Remove the reagents from each well of the chip using vacuum.
3. Each active well (1, 3, 4, 7, 8 and 10) should be rinsed and aspirated twice with water (Milli-Q® or equivalent).
4. Add 120 µL of Storage Buffer (white cap ◯) to the active wells.
5. Place the chip in the LabChip GX Touch/GXII Touch instrument and touch the *Wash* button in the upper right corner in the *Home* Screen.

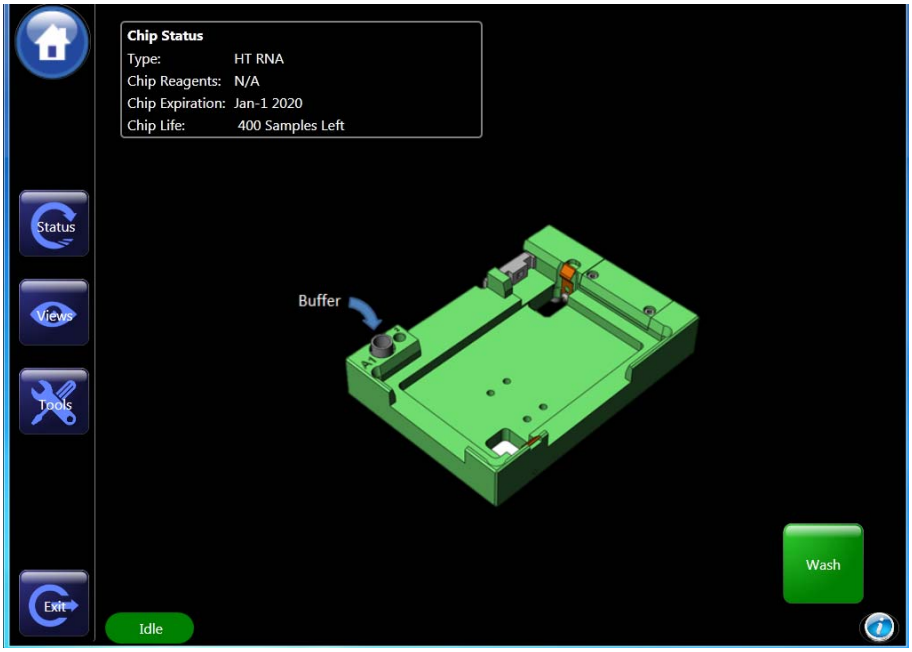

Figure 14. *Wash* screen.

6. Remove the chip from the instrument and place it in the plastic storage container. Add an additional amount of Storage Buffer to well 1.
7. Cover the wells with Parafilm® to prevent evaporation and store at 4°C. Storage of a chip with dry wells may cause it to become clogged.

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 GX Touch/GXII 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

RNA Pico Sensitivity Ladder Result

The electropherogram of a typical RNA Pico Sensitivity ladder using the Standard Sample Workflow is shown in Figure 15. Following the lower marker, ladder fragments in order of increasing migration time correspond to 200, 500, 1000, 1500, 2000, 3000, 4000 and 6000 nt.

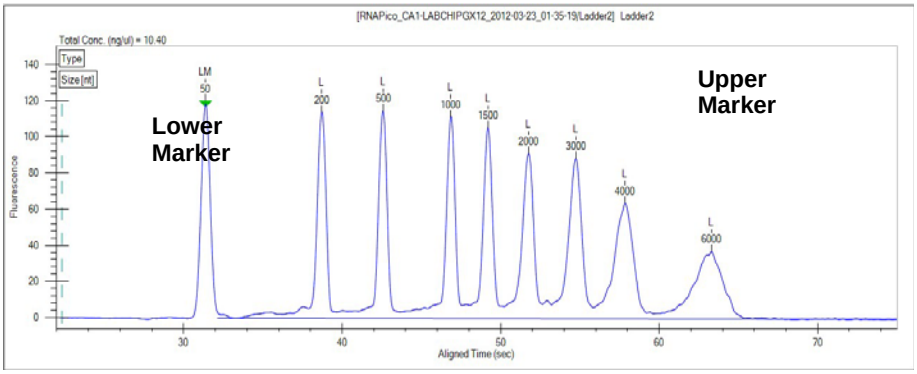

Figure 15. Typical RNA Pico Sensitivity ladder.

RNA Pico Eukaryote Sample Result

The electropherogram for a typical total RNA sample is shown in Figure 16. Your results may vary depending on the type of total RNA.

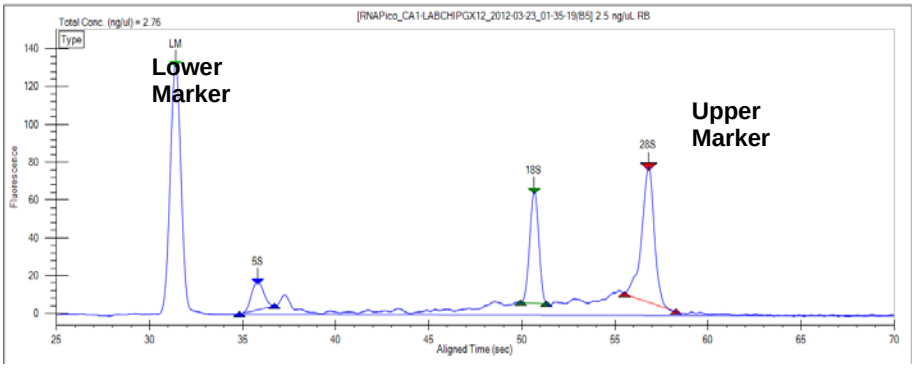

Figure 16. Electropherogram for a typical total RNA sample.

RNA Pico mRNA Sample Result

The electropherogram for a typical mRNA sample is shown in Figure 17. Your results may vary depending on the type and concentration of mRNA.

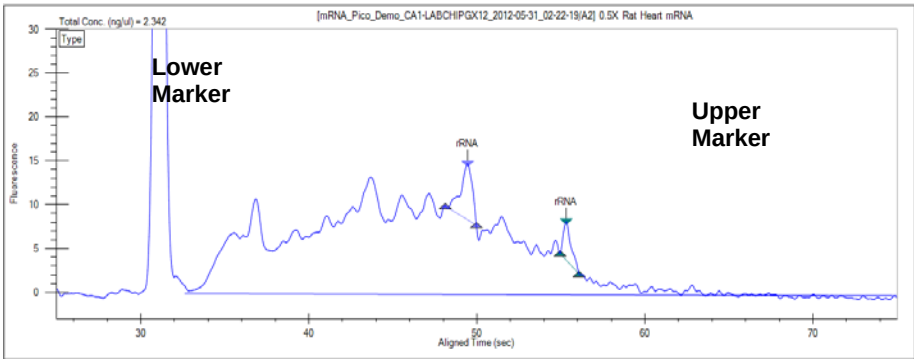

Figure 17. Electropherogram for a typical mRNA sample.

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 27 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 27 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 you suspect there may be debris in your samples, spin the sample plate down in a centrifuge (e.g. 3000 rcf for 5 minutes). Unclog the sipper by repriming the chip. See "LabChip Kit Essential Practices" on page 27 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 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.
- Ensure that chip well 4 is rinsed and completely aspirated twice with water (Milli-Q® or equivalent).
- Add 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 “LabChip Kit Essential Practices” on page 27 for instructions on how to reprime the chip.

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

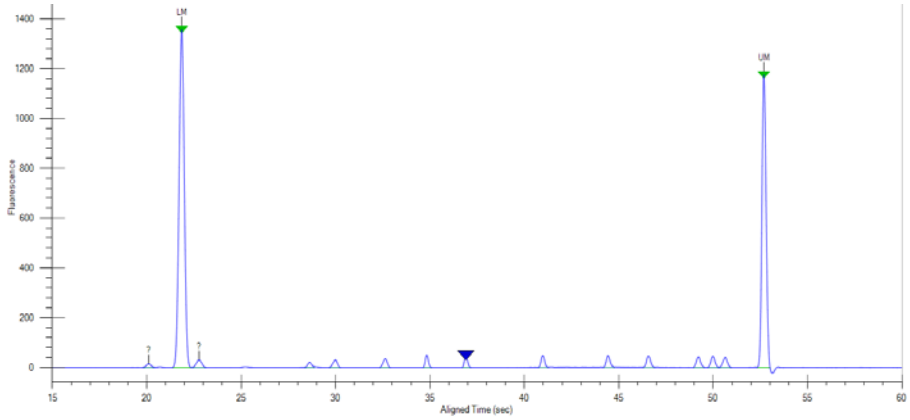

Figure 18. Small ladder peaks in sample well caused by delayed sip.

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 “LabChip Kit Essential Practices” on page 27 for instructions on how to reprime the chip.

Symptom: Unexpected sharp peaks.

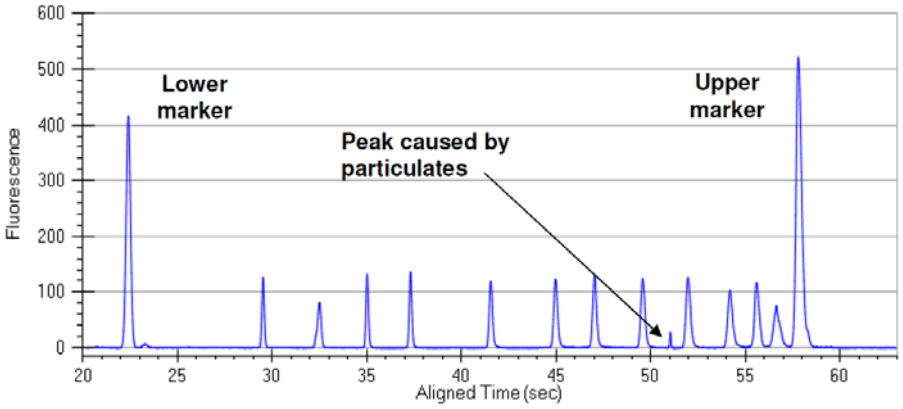

Figure 19. Unexpected sharp peak.

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) water 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.

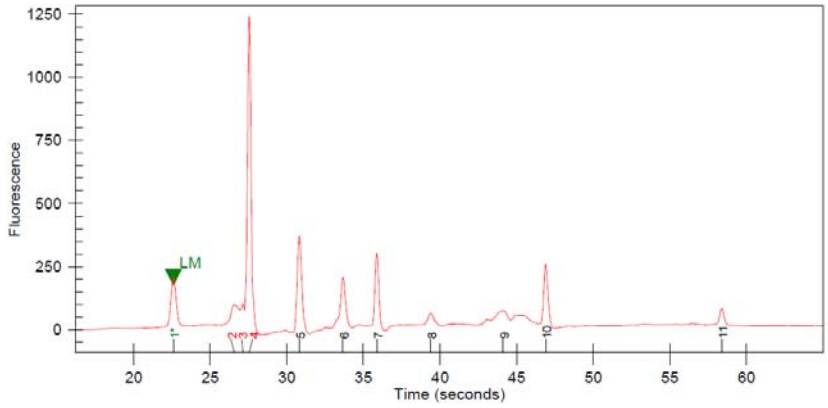

Figure 20. Humps in several electropherograms.

Possible causes:

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

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.

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 PerkinElmer prior to shipment.*

Normal migration time windows for the markers are:

- *RNA Pico Sensitivity Assay Lower Marker: 28 - 33 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 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 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 mixture. See “LabChip Kit Essential Practices” on page 27 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 PerkinElmer. 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 rcf for 5 minutes) before starting a new run. The debris may be flushed out of the chip by washing and re-priming the chip. See "LabChip Kit Essential Practices" on page 27 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 PerkinElmer Technical Support at 1-800-762-4000.

General

- Allow the chip, sample plate and all reagents to equilibrate to room temperature before use (approximately 20 to 30 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 PerkinElmer-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. PerkinElmer, 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

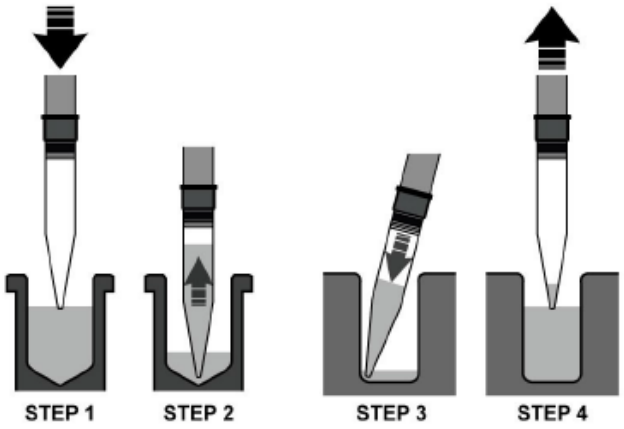

Figure 21. Reverse pipetting.

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 4°C when not in use.
- The LabChip dye contains DMSO and should 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 mixture from light. Store in the dark at 4°C when not in use.
- The Gel-Dye mixture expires 3 weeks after preparation.

Chips

Repriming Chips

Note: *Buffer tubes filled with 1X sample buffer or water should be placed into the instrument while priming or washing chips.*

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

Washing and Repriming Chips

- Touch the *Unload Chip* button on the *Home* screen to open the instrument door.
- Return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from the chip wells using a vacuum line.
- Ensure that each active well (1, 3, 4, 7, 8 and 10) is rinsed and completely aspirated twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
- Add 120 µL of Chip Storage Buffer to each active well (1, 3, 4, 7, 8 and 10).
- Place the chip in the LabChip GX Touch/GXII Touch instrument.
- Close the chip door securely.
- Touch the *Wash* button on the *Home* screen (Figure 22).

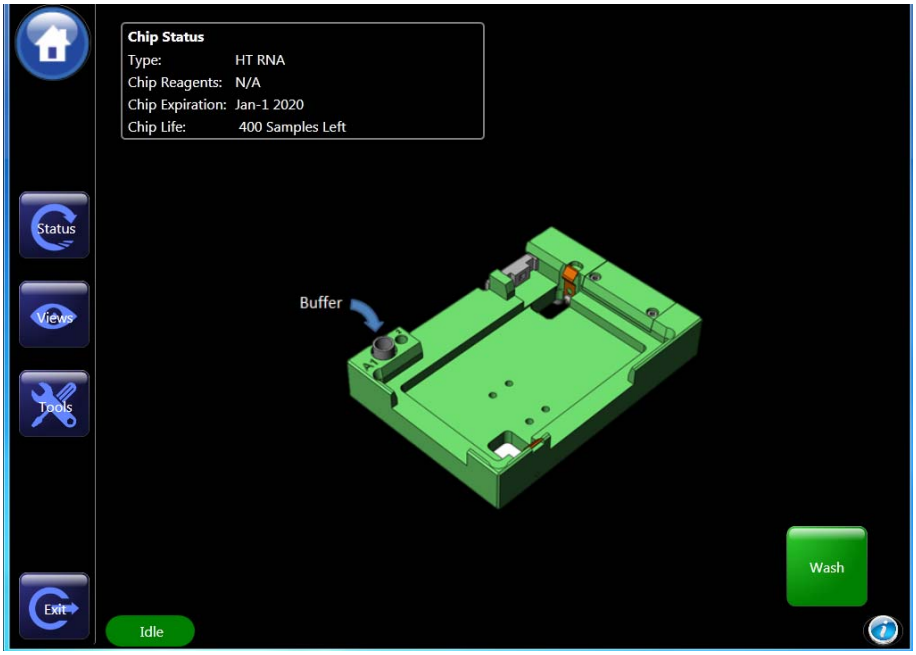

Figure 22. *Wash* screen.

- After the completion of the wash cycle return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from the chip wells using a vacuum line.
- Ensure that each active well (1, 3, 4, 7, 8 and 10) is rinsed and completely aspirated twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
- Add Gel-Dye solution to chip wells 3, 7, 8 and 10 using a Reverse Pipetting Technique as shown in Figure 21.
- Add 50 µL (Low-throughput) or 75 µL (High-throughput) Marker (green cap ●) to chip well 4. Please note that the marker well may need to be replenished if the chip is in idle mode on the instrument for an extended period of time.
- Place the chip into the LabChip GX Touch/GXII Touch instrument.
- Close the chip door securely.
- Touch the *Run* or *Prime* button on the *Home* screen.

- 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 of Chip Storage Buffer. Then suction the sipper with a vacuum line as shown in Figure 23 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.

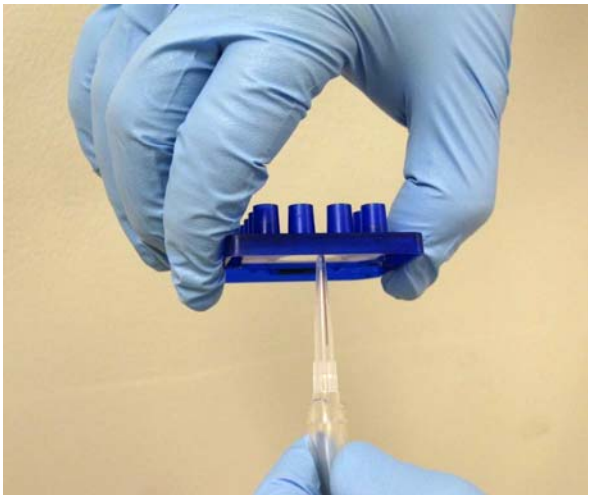

Figure 23. Removing an air bubble or clog by suctioning the sipper with a vacuum line.

Other Considerations:

- Chips should be stored refrigerated prior to first use.
- Cover the active wells on the chip with Parafilm® and store at 4°C. If using the chip again within 24 hours it may be left at room temperature.
- 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 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.
- PerkinElmer 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.
- Sample plates containing gas bubbles and/or particulate debris should be spun down at 3000 rpm (1250 rcf) prior to analysis.
- Up to 96 samples in a 96-well or 384-well plate can be run with a single chip preparation.

Chip Well Aspiration Using a Vacuum

Aspirating with a pipette can leave used reagents in the chip wells. For this reason, PerkinElmer recommends vacuuming the wells instead. This can be accomplished by attaching a permanent pipette tip to a house vacuum line with trap (Figure 24). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 25).

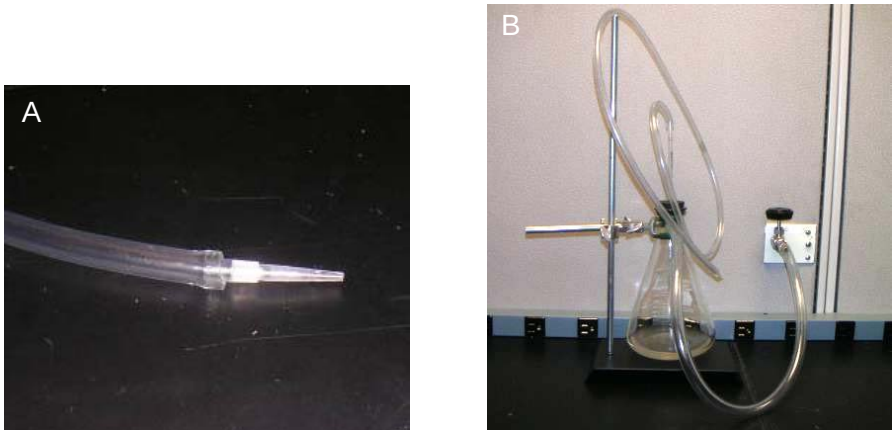

Figure 24. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap.

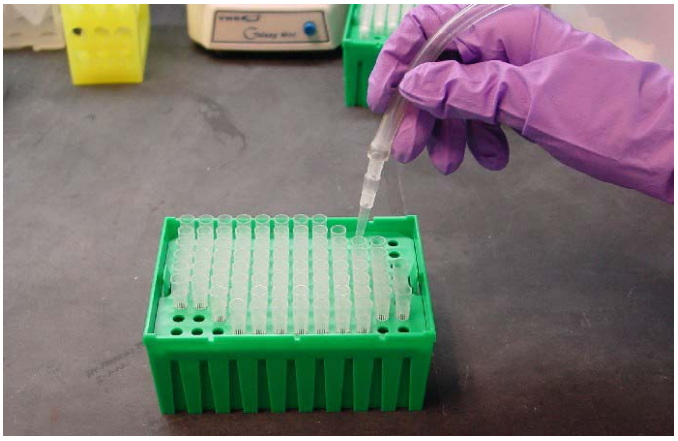

Figure 25. Replacing the disposable pipette tip.

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

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