



Caliper LifeSciences

HT RNA LabChip[®] Kit, Version 2 LabChip GX/GXII User Guide

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Specifications

Linear Range	25 ng/μL – 250 ng/μL (Std Sens) 5 ng/μL – 50 ng/μL (High Sens)
Quantitation Reproducibility	20% CV
Size Range	100 – 6000 nucleotides (suitable for total RNA)
RNA Sample Volume	2 μL of user sample (Std Sens) 6 μL of user sample (High Sens)
Carry-Over	< 0.5% following 500 ng/μL sample
Run Time	80 seconds per sample (about 2.5 hours for 96-well plate)
Setup Time	Approximately 30 minutes to prepare chip and samples
Number of Samples per Chip Prep	200 samples
Chip Lifetime¹	2000 samples per chip
Samples per Reagent Kit	5 chip preps

Sample Conditions

Particulates	Sample should be spun down prior to analysis. All buffers should be filtered with a 0.22μm cellulose acetate filter.
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¹ Expected chip lifetime is based on use under normal laboratory conditions and adherence to Caliper preparation protocols, sample guidelines and storage conditions. Individual results may vary.

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HT RNA Reagent Kit (Part Number 760410)

Item	Vial	Quantity
RNA Dye Concentrate	Blue	1 vial of 0.5 mL
Chip Storage Buffer	White	2 vials, 1.8 mL each
RNA Gel Matrix	Red	2 vials, 1.6 mL each
RNA Ladder (packed separately)	Yellow	1 vial, 0.023 mL
RNA Marker	Green	1 vial, 0.8 mL
10X RNA Sample Buffer Concentrate	Purple	5 vials, 1.8 mL
Spin Filters	—	6 spin filters

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RNA Chip	—	1
Ladder Tubes	—	10, 0.2 mL PCR tubes
Centrifuge Tubes	—	5, 2.0 mL centrifuge tubes
Detection Window Cleaning Cloth	—	1 clean room cloth
Swab	—	3
Buffer Tubes	—	10, 0.75 mL tubes

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

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

Preparation Procedures

Additional Items Required

- MilliQ water: Molecular biology grade or better, 0.22-micron filtered
- 70%-isopropanol solution in DI water
- DEPC treated water (nuclease free)
- PCR cap strips

Note: Allow the chip and all reagents to equilibrate to room temperature before use (approximately 20 to 30 minutes).

Preparing the Gel-Dye Solution

Note: The dye contains DMSO and **must be thawed** completely before use. Gently vortex all kit reagents before use.

1. Gently vortex the thawed dye before use.
2. Transfer **75 μ L** of HT RNA Dye Concentrate (blue cap) to a 2.0 mL centrifuge tube provided with the reagent kit. Add **425 μ L** of HT RNA Gel Matrix (red cap) using a Reverse Pipetting Technique.
3. Vortex the solution until it is well mixed and spin down for a few seconds.
4. Transfer the mixture to a spin filter. Label and date the tube.
5. Centrifuge at **9200 rcf for 10 minutes at RT**. Store in the dark at 4 °C. Use within 5 days.
6. Use a centrifuge tube filled with 500 μ L of water to balance the centrifuge.

Preparing the RNA Samples and RNA Ladder

1. Prepare sample buffer by adding **620 μ L** RNA Sample Buffer Concentrate (purple cap) to **5580 μ L** DEPC treated water.

Note: This volume is enough for one full 96-well plate run (includes the samples, ladder, and buffer). You may adjust accordingly for partial plates. You may prepare a larger batch and store for future use.

2. Allow the HT RNA Ladder (yellow cap) to thaw on ice. (It is recommended that you aliquot the HT RNA Ladder to five 4- μ L aliquots for individual use.)
3. Pipette **4 μ L** of HT RNA Ladder into the provided 0.2 mL Ladder Tube and cover.
4. Pipette **2 μ L** of sample into individual wells of a microtiter plate or into RNase-free microcentrifuge tubes. (If running the High Sens. assay, pipette **6 μ L** of each sample.)
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.
5. Heat samples and ladder for **2 minutes at 70 °C**.
6. Snap cool the ladder and samples by immediately placing the tube/plate on ice for **5 minutes**.

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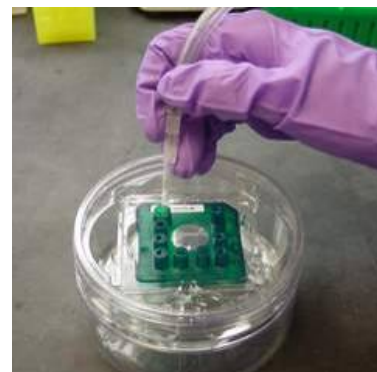
7. Add **46 μ L** of prepared sample buffer (prepared in Step 1) to each sample. (If running the High Sens assay, add **19 μ L** of sample buffer to each sample). Mix by pipetting up and down a couple of times. Cover with PCR cap strips and spin down the plate at **1250 rcf for 1 minute at RT** to remove air bubbles.
8. Add **96 μ L** of prepared sample buffer to the HT RNA Ladder.
9. Insert the Ladder Tube into the ladder slot on the LabChip GX instrument.

Preparing the Buffer Tube

1. Add **750 μ L** of sample buffer to the 0.75 mL Buffer Tube provided with the reagent kit.
2. Insert the Buffer Tube into the buffer slot on the LabChip GX instrument.

Preparing the RNA Chip

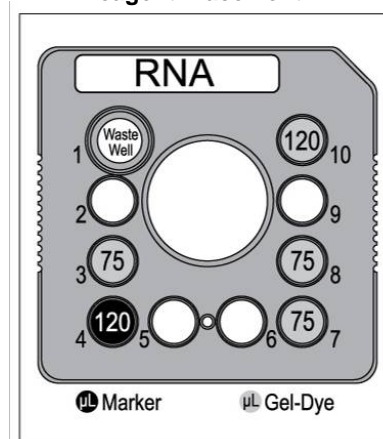
1. Allow the chip to come to room temperature and remove foil cover from chip wells.
2. Use a pipette tip attached to a vacuum line to dry the top and bottom chip surfaces and the top of the chip container. **DO NOT** run the tip over the central region of the detection window. Return the chip to the chip container when the bottom of the chip and the top of the chip container are dry.
3. Thoroughly aspirate all fluid from the chip wells using vacuum line with chip placed in the container and sipper immersed in fluid.
4. Each active well (1, 3, 4, 7, 8 and 10) should be rinsed and completely aspirated twice, with nuclease free water. Do not allow active wells to remain dry.
5. Add **75 μ L** of Gel-Dye solution to chip wells 3, 7 and 8 and add **120 μ L** of Gel-Dye to chip well 10 using a Reverse Pipetting Technique.
6. Add **120 μ L** HT RNA Marker (green cap) to chip well 4.
7. Make sure the rims of the chip wells are free of adhesive residue.
8. Place the chip in the LabChip GX instrument to begin the assay.



Using a vacuum to aspirate the chip wells is more effective than using a pipette. See page 14 for more details.

NOTE: Be sure to periodically clean the O-rings on the top plate of the chip interface on the LabChip GX instrument. Use the provided lint free swab dampened with DI water or 70%-isopropanol solution in DI water to clean the O-rings, using a circular motion. Allow the O-rings to dry before inserting a chip.

Reagent Placement



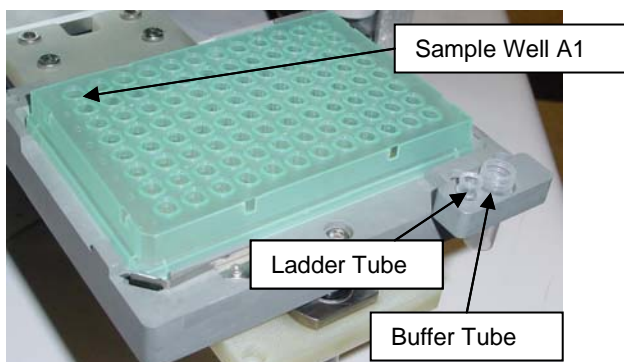
Add Marker and Gel-Dye according to the image above.

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Inserting a Chip into the LabChip GX Instrument

1. Check that the sample plate, Buffer Tube, and Ladder Tube are placed appropriately on the instrument.
2. Remove the chip from the chip storage container and inspect the chip window. Clean BOTH sides of the chip window with the Caliper-supplied cleanroom cloth dampened with a 70%-isopropanol solution in DI water.
3. Eject the chip cartridge by pressing the **CHIP** button on the instrument front panel.
4. Release the cartridge latch, insert the chip into the LabChip GX instrument, refasten the latch and push the cartridge into the instrument.
5. Press the **EJECT** button on the instrument front panel to retract the sample plate and send the sipper to Buffer Tube.



Running the HT RNA Assay

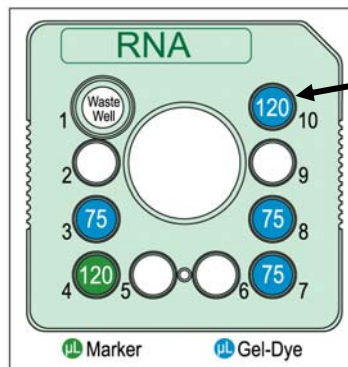
1. Start the LabChip GX software.
2. On the main screen, click on the *Run* button in the upper left corner of the LabChip GX Software.
3. The *Start Run* dialog box will pop up with tabs listed as *Output*, *Run* and *Advanced*.
4. In the *Run Tab*, select the appropriate assay type, operator name, plate name, well pattern and barcode option.
 - For HT RNA assays appropriate assay types are:
 - *HT RNA Std Sens*: For detection of RNA in 25 ng/ μ L to 250 ng/ μ L range.
 - *HT RNA High Sens*: For detection of RNA in 5 ng/ μ L to 50 ng/ μ L range. (Requires a higher sample volume).
5. In the *Output Tab* select the destination of the file, the filename convention and any additional data to autoexport.
6. In the *Advanced Tab* select the number of times each well is sampled, the inclusion of any sample names and any expected peaks.
7. Click *Start* to begin the run.

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Refresh the gel+dye solution in Well 10 after 100 samples or 3 hours.

After one full 96-well plate has been run or if gel+dye solution was placed in the chip more than 3 hours ago, we recommend the user should refresh the gel+dye solution in well 10. To do this, press “Chip” button on the instrument to eject the chip cartridge. Remove the residual gel+dye solution in well 10 (this can be done either by pipette or by vacuum aspirator...the chip does not need to be removed from the instrument nor does the well need to be washed or rinsed). After the previous gel+dye solution has been removed, add 120ul of fresh, unused gel+dye into well 10. (This could be the leftover gel+dye mixture from an earlier filtered gel+dye prep). Only well 10 needs to be refreshed, you may leave the other remaining reagents in the chip. Once well 10 has been refreshed, you may close the chip cartridge, press “Chip” button to insert the chip back into instrument and then start your next RNA run.



Refresh the gel+dye solution in Well 10 after 100 samples or 3 hours.

Storing the RNA Chip

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

1. Remove the reagents from each well of the chip, using vacuum.
2. Each active well (1, 3, 4, 7, 8 and 10) should be rinsed and aspirated twice, using nuclease free water.
3. Add **80 µL** of HT RNA Storage Buffer (white cap) to the active wells.
4. Place the chip in the LabChip GX instrument and click the *Wash* button in the left corner of the LabChip GX Software.
5. Remove the chip from the instrument and place it in the plastic storage container. Add an additional amount of Storage Buffer to well 1. Cover the wells with parafilm to prevent buffer 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 DI water or 70%-isopropanol solution in DI water 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.

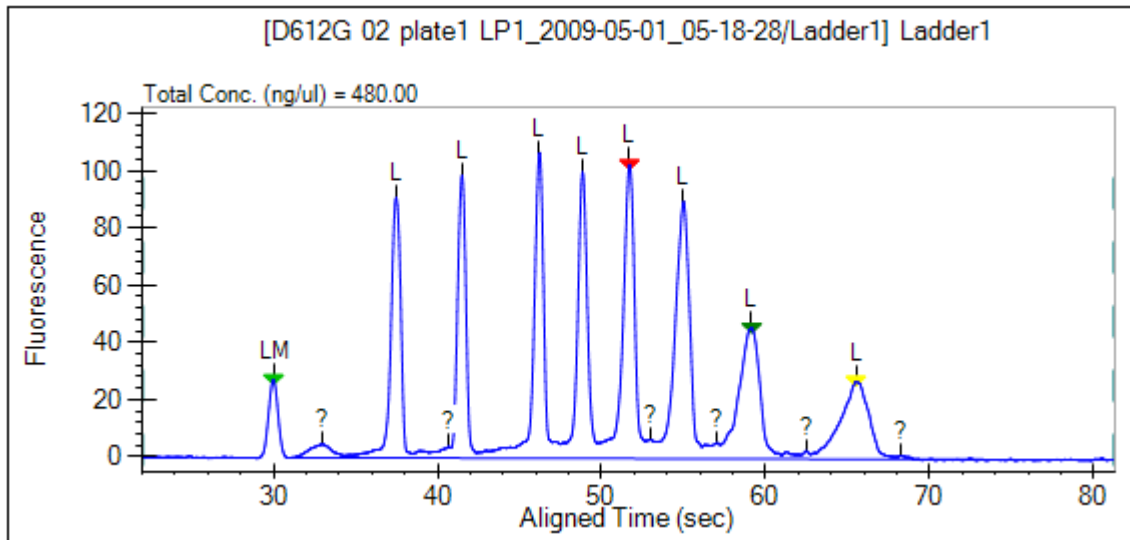
2. Monthly

- 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 instrument. Soak O-rings in DI water for a few minutes. Clean the O-ring faces by rubbing between two fingers.
- 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 DI water.
- C) Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

Results

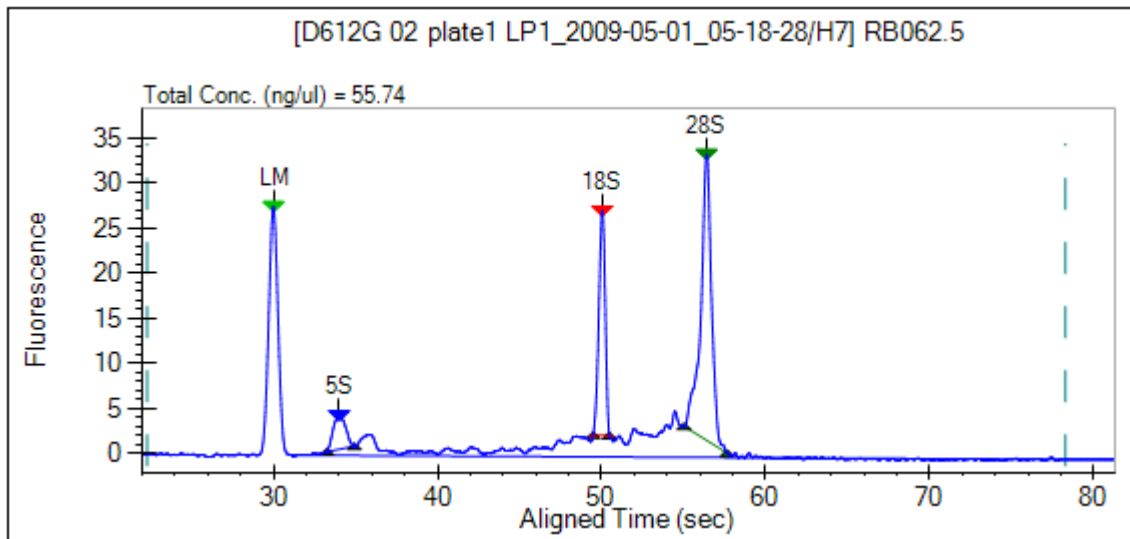
HT RNA Ladder Result

- Electropherogram of a typical RNA ladder is shown below:



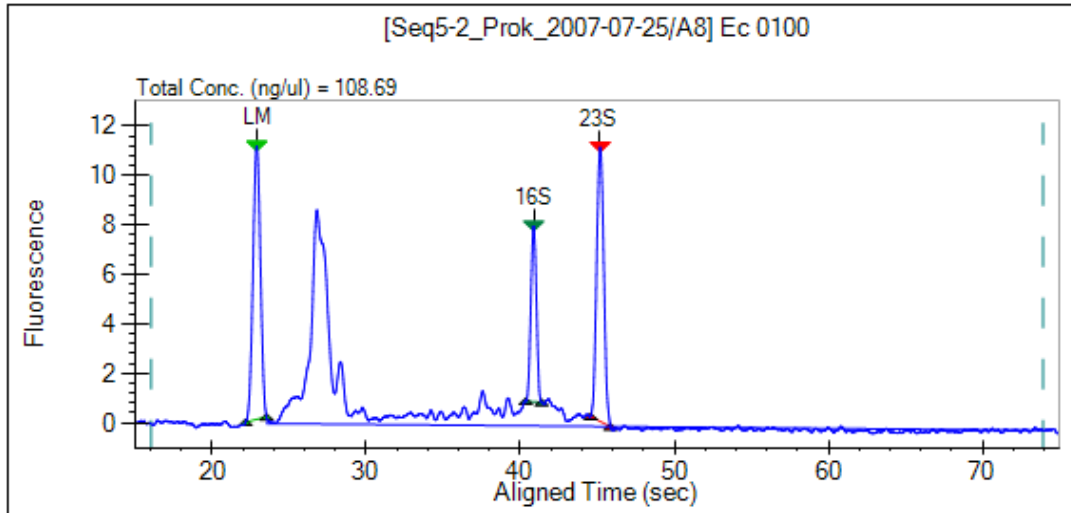
HT RNA Eukaryote Sample Result

- The electropherogram for typical total RNA samples is shown below. Your results may vary depending on the type of total RNA.



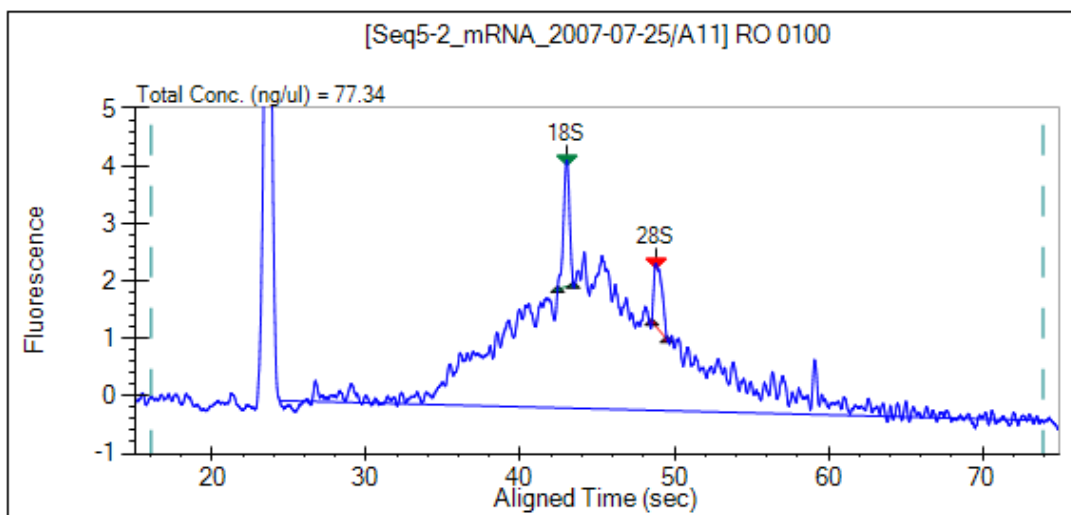
HT RNA Prokaryote Sample Result

- The electropherogram for typical Prokaryote Total RNA samples is shown below. Your results may vary depending on the type of total RNA.



HT RNA mRNA Sample Result

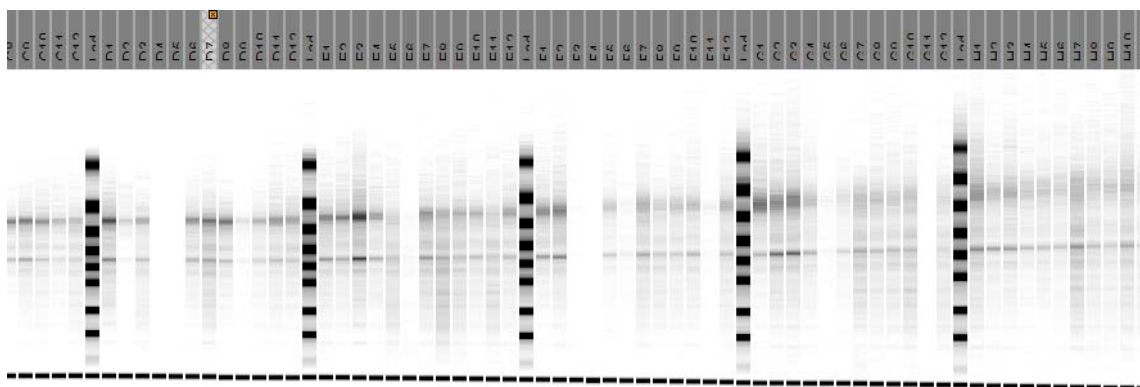
- The electropherogram for a typical mRNA sample is shown below. Your results may vary depending on the type and concentration of mRNA.



Troubleshooting

Symptom: Migration Time Shift

- The sample peaks migrate past the detector slower than expected or migrate at different times from one sample to another.



Possible causes:

- There is debris inside the chip channels causing a change in the electric field distribution.
- The chip has reached the end of its life and should be replaced.
- The Gel-Dye mixture is not formulated correctly or is past the expiration date.

What to do:

- If you suspect there may be debris in your samples, spin the sample plate down in a centrifuge. Unclog the chip by washing and repriming the chip. See the section entitled “LabChip Kit Essential Practices – Chips” for instructions on how to wash and reprime the chip.
- Retire this chip.
- Check the expiration date of the reagents and re-make the Gel-Dye formulation.

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.

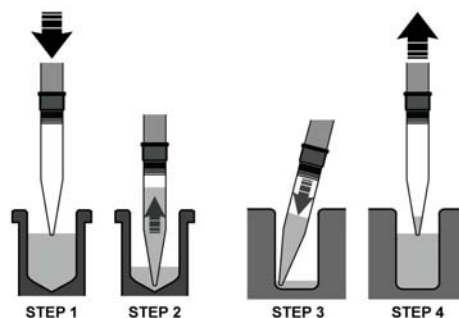
For assay and instrument troubleshooting, refer to the LabChip HT Software Help file or call Caliper Technical Support at 1-877-LABCHIP.

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 Caliper-supplied clean room cloth can be used on the chip to clean the detection window.
- Water used for chip preparation procedures must be 0.22-micron filtered deionized, molecular biology grade and nuclease free.
- Use of the “Reverse Pipetting Technique” (described below) will help avoid introducing bubbles into the chip when pipetting the gel.

Reverse Pipetting Technique

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



Reagents

- Store RNA ladder at -70 °C and all other 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.

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- Protect the dye and Gel-Dye mixture from light. Store in dark at 4 °C when not in use.
- The Gel-Dye mixture expires 5 days after preparation.

Chips

- Repriming Chips:
 - Press the **CHIP** button on the front instrument panel to eject the chip cartridge.
 - Reinsert the cartridge by pushing the cartridge back into the instrument.
 - Press the *Run* button on the main screen of the LabChip GX software.
- Washing and Repriming Chips:
 - Press the **CHIP** button on the front instrument panel to eject the chip cartridge.
 - Open the chip cartridge and 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 nuclease free water. Do not allow active wells to remain dry.
 - Add **80 µL** of Chip Storage Buffer to each active well (1, 3, 4, 7, 8 and 10).
 - Place the chip in the LabChip GX instrument.
 - Reinsert the cartridge by engaging the latch and pushing the cartridge back into the instrument.
 - Press the *Wash* button on the main screen of the LabChip GX software.
 - After completion of the wash cycle, Press the **CHIP** button on the front instrument panel to eject the chip cartridge.
 - Open the chip cartridge and 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 nuclease free water. Do not allow active wells to remain dry.
 - Add **75 µL** of Gel-Dye solution to Wells 3, 7, 8 and add **120 µL** of Gel-Dye to chip well 10 using a Reverse Pipetting Technique.
 - Add **120 µL** HT RNA Marker (green cap) to chip well 4.
 - Place the chip in the LabChip GX instrument.
 - Reinsert the cartridge by engaging the latch and pushing the cartridge back into the instrument.
 - Press the *Run* button on the main screen of the LabChip GX software.

¹ Caliper Life Sciences 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.

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- Other Considerations:
 - RNA chips should be stored refrigerated prior to first use.
 - Cover the active wells on the chip with adhesive foil 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-prepare the chip as long as the maximum number of samples per chip prep is not exceeded.

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 prior to analysis. For troublesome samples a suggested spin of **3000 rcf for 5 mins at RT** should be sufficient.
- Up to two 96-well plates or half of a 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, Caliper recommends vacuuming the wells instead. This can be accomplished by attaching a permanent pipette tip to a house vacuum line with trap (Figures 1a and 1b). To avoid contamination, use a new pipette tip over the permanent tip for each chip aspirated (Figure 2).

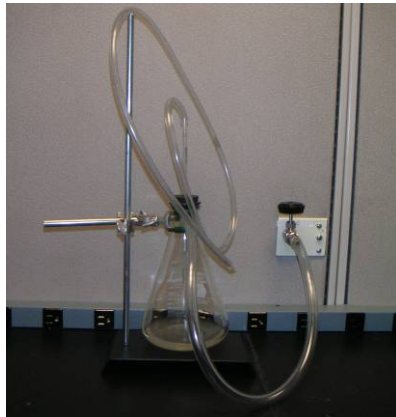


Figure 1a



Figure 1b

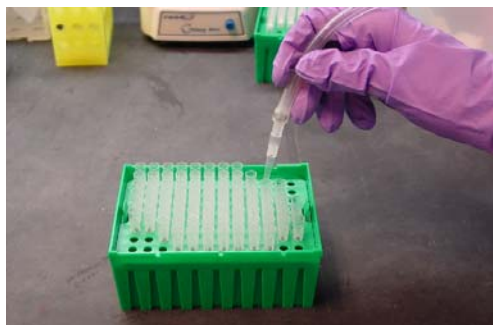


Figure 2

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Reordering Information

<u>Product</u>	<u>Part Number</u>
HT DNA 5K/RNA LabChip, Ver 2	760435
HT RNA Reagent Kit	760410
Buffer Tube	E&K Scientific 697075-MC
Ladder Tube	Genemate C-3258-1
2.0 ml Centrifuge Tubes	Eppendorf 022363352

Customer Technical Support

Caliper Life Sciences
68 Elm Street
Hopkinton, MA 01748-1668
Phone: 1-877-LABCHIP (522-2447)
Fax: 1-508-435-3439

For additional assay and instrument troubleshooting, refer to the LabChip HT Software Help file. Call Caliper Technical Support at 1-877-LABCHIP.

The chip and reagents supplied with this kit are sold with limited rights of use. The chip may only be used with the specific quantity of reagents supplied with this kit. The purchaser has no right or license to refurbish, reuse, remanufacture, or otherwise use the chip with any other reagents than those specifically supplied in this kit. For more information on the terms and conditions of use of these chips and reagents, please read your LabChip GX User Guide. Caliper, the Caliper logo, LabChip, and the LabChip logo are registered trademarks of Caliper Life Sciences.

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