



Caliper LifeSciences

HT RNA LabChip[®] Kit User Guide

Contents

Specifications	2
Sample Conditions	2
Kit Contents	3
Preparation Procedure	4
Additional Items Required	4
Preparing the Gel-Dye Solution	4
Preparing the RNA Samples and Ladder	4
Preparing the Buffer Strip Wells	5
Preparing the RNA Chip	5
Priming the Chip	6
Electrode Cleaning	7
Inserting a Chip onto the LC90	7
Running the HT RNA Assay	7
Storing the RNA Chip	8
Typical Results	10
Troubleshooting	12
LabChip Kit Essential Practices	14
General	14
Reagents	14
Chips	15
Samples	15
Electrode Cleaning	15
Chip Well Aspiration Using a Vacuum	15
Reordering Information	17
Customer Technical Support	17

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 max., then dye-gel mixture must be replaced.
Chip Lifetime¹	2000 samples per chip
Samples per Reagent Kit	5 chip preps

Sample Conditions

Particulates	Sample plates containing particulates or debris should be spun down prior to analysis. All buffers should be filtered with a 0.22μm cellulose acetate filter.
---------------------	---

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.

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

HT DNA 5K/RNA LabChip Kit (Part Number 760435)

RNA Chip	—	1
Ladder Strip Wells	—	4 strip wells
Centrifuge tubes	—	5, 2.0 mL centrifuge tubes
Detection Window Cleaning Cloth	—	1 clean room cloth
Swab	—	3
Buffer Strip Wells	—	4 strip wells

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
- PCR strip caps

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

1. Gently vortex the thawed dye before transferring.
2. Transfer **425µL** of HT RNA Gel Matrix (red cap) and **75µL** of HT RNA Dye Concentrate (blue cap) to a 2.0 mL centrifuge tube.
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 **RCF = 9200 for 10 minutes**. Store in the dark at 4 °C. Use within 5 days.
6. Use a centrifuge tube filled with 500 µL of water to balance the centrifuge.

Preparing the RNA Samples and RNA Ladder

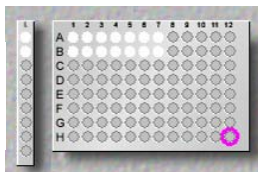
1. Prepare sample buffer by adding **620 µL** RNA Sample Buffer Concentrate (purple cap) to **5580 µL** DEPC treated water.

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-µL aliquots for individual use. Pipette **4 µL** of HT RNA Ladder into an RNase-free microcentrifuge tube).

NOTE: It is important to note that if not running an entire 96-well plate that the samples must be placed in a contiguous rectangle. Any blank wells must be filled with nuclease-free water as sample +sample buffer.

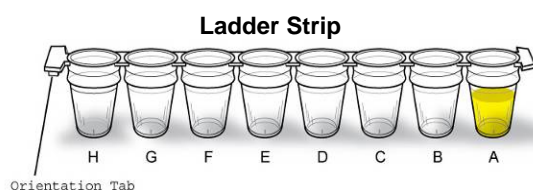
Example, 14
samples must be
split between 2 rows



3. Pipette **2 µL** of sample into individual wells of a microtiter plate or into RNase-free microcentrifuge tubes. (If running the High Sens. assay, pipette **6 µL** of each sample.)

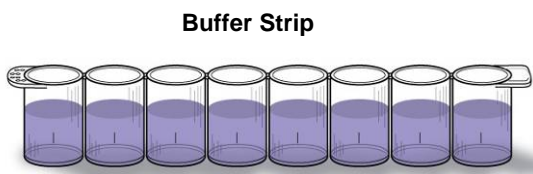
Samples in microtiter plates should be covered with PCR strip caps. Foil is not recommended as the adhesive may contaminate the samples. If diluting the samples use nuclease-free water.

4. Heat samples and ladder for **2 minutes at 70 °C**.
5. Snap cool the ladder and samples by immediately placing the tube/plate on ice for **5 minutes**.
6. Add **46 µL** of sample buffer (prepared in Step 1) to each sample. (If running the High Sens. assay, add **19 µL** of sample buffer to each sample.) Mix by pipetting up and down a couple of times. Cover with PCR strip caps and spin down the plate at 3000 rpm (1250 rcf) for 1 minute to remove air bubbles.
7. Add **96 µL** of sample buffer to the HT RNA Ladder and vortex the solution.
8. Transfer ladder to Well A of the ladder strip.
9. Insert the ladder strip into the groove marked “L” on the instrument’s plate holder with the well marked “A” nearest to the “A1” spot on the plate holder. An orientation tab on the opposite end of the ladder strip fits into a slot at the far end of the groove.



Preparing the Buffer Strip Wells

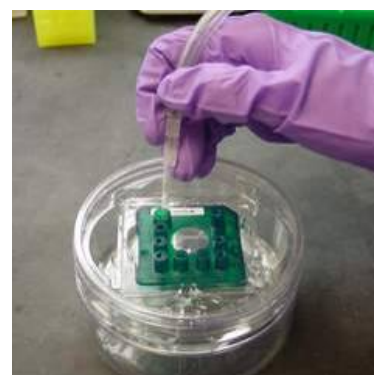
1. Add **200 µL** of sample buffer to each well of the buffer strip.



2. Insert the buffer strip into the groove marked “B” on the instrument’s plate holder.

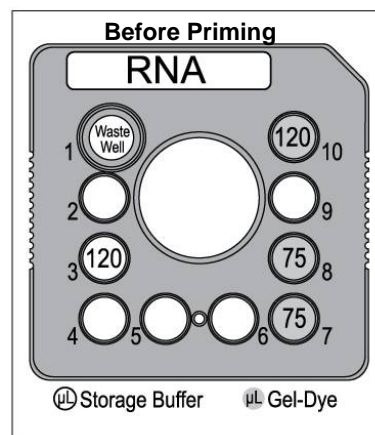
Preparing the RNA Chip

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 Milli Q water. Do not allow active wells to remain dry.



Using a vacuum to aspirate the chip wells is more effective than using a pipette. See page 15 for more details.

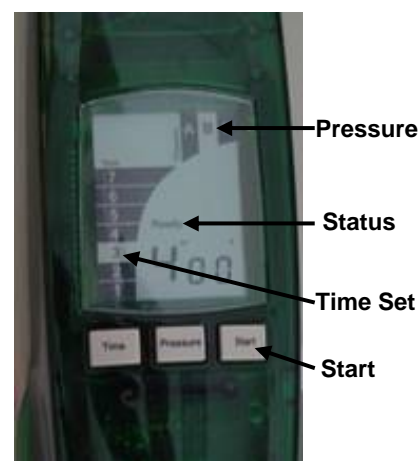
5. Add **75 µL** of gel-dye solution to Wells 7 and 8 and add **120 µL** of gel-dye to Well 10. Avoid introducing bubbles into the gel when pipetting. Use reverse pipetting technique described on page 14.
6. Add **120 µL** of HT RNA Storage Buffer (white cap) to Well 3.
7. Make sure the rims of the chip wells are free of adhesive residue.
8. Prime the chip as described in the table below:



Before priming, chip should have Buffer and Gel-Dye according to the image above.

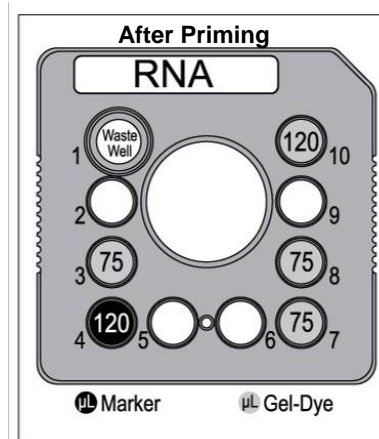
Priming the Chip

Set-up	Plug in the priming station and insure the <i>Ready</i> status appears on the display. Open the priming station by pressing the front lever.
Fill Reservoir	Fill the 0.2mL tube in the priming station with Milli Q water and replace it monthly.
Insert Chip	Place the chip in the priming station and close the cover. Make sure it snaps shut.
Set Pressure	Use the <i>Pressure</i> button set the pressure to [B].
Set time	Use the <i>Time</i> button to set the time to setting [3] (4 minutes).
Start	Press Start.
Remove Chip	Place one hand on the lid, and with the other hand press the lever to open the priming station.
Storage	Remove the chip and place it in the chip storage container.



NOTE: Be sure to periodically clean the O-rings on the top plate of the priming station. 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 priming a chip.

9. Aspirate the contents of Wells 3 and 4 using vacuum.
10. Add **75 µL** of gel-dye solution to Well 3. Avoid introducing bubbles into the gel when pipetting. Use reverse pipetting technique described on page 14.
11. Add **120 µL** HT RNA Marker (green cap) to Well 4.



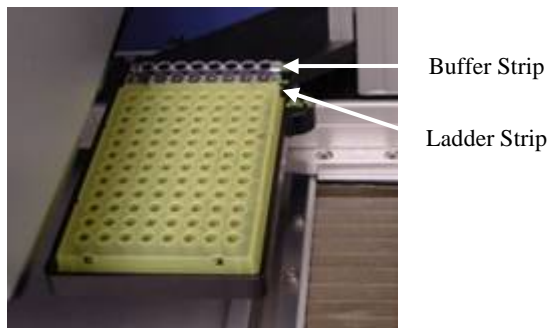
After Priming, add Marker and Gel-Dye according to the image above.

Electrode Cleaning

1. Fill the cleaning chip with 1.2 mL of DEPC treated water.
2. Insert the cleaning chip into the instrument and close the chip holder in order to rinse the electrodes.
3. Leave the chip in the instrument for 2 minutes.
4. Remove the cleaning chip and allow the electrodes to dry for approximately 1 minute.

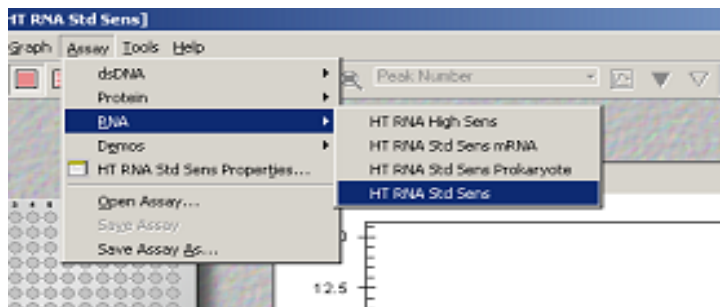
Inserting a Chip onto the LC90

1. Check that the sample plate, buffer strip, and ladder strip are placed on the instrument accordingly.
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 Milli Q water.
3. Insert the chip into the LC90 instrument.
4. Click the Load Plate icon on the LabChip HT software toolbar to position the plate holder so the sipper is immersed in the Buffer Trough. Avoid exposing the sipper tip to the air for extended periods of time. Keep the sipper tip immersed in the Buffer Trough whenever possible.



Running the HT RNA Assay

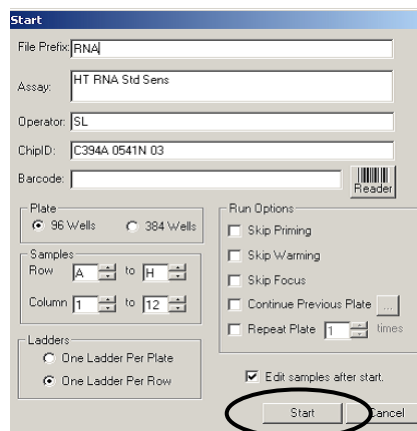
1. Start the LabChip HT software.
2. On the menu bar, select Assay and click on *RNA*. A list of assay choices will appear. Select the appropriate assay script:
 - *HT RNA Std Sens.*: For detection of RNA in 25 ng/μL to 250 ng/μL range.
 - *HT RNA High Sens.*: For detection of RNA in 5 ng/μL to 50 ng/μL range. (Requires a higher sample volume).



3. Click *Start...* on the upper left corner of the LabChip HT window.

4. The Start menu will appear.

- Enter the *File Prefix* (the name you want your file to be called), *Operator* (your initials), and *ChipID* (the unique ID number on each chip and chip box – e.g., C123A 0377N 04).
- Select plate type: *96 Wells* or *384 Wells*. *Make sure that the proper sipper alignment file is selected prior to plate type setting. An Incorrect selection of alignment file can cause the sipper to brake.*
- If you are running fewer than 96 samples, select the rows and columns used in your experiment. Must be one contiguous rectangular region.
- Click *Start*.

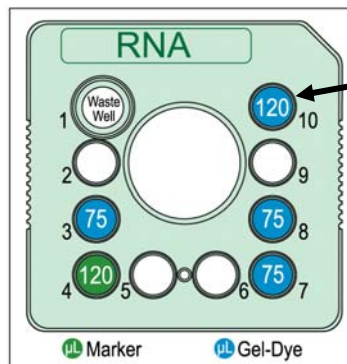


5. The Edit Samples menu will open.

- Enter *Sample Name*, *Sample Comment*.
- Click *OK*.

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, open 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, 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 Milli Q water.
3. Add **80 µL** of HT RNA Storage Buffer (white cap) to the active wells.
4. Place the chip in the priming station and follow the same procedure outlined in 'Priming the Chip'.

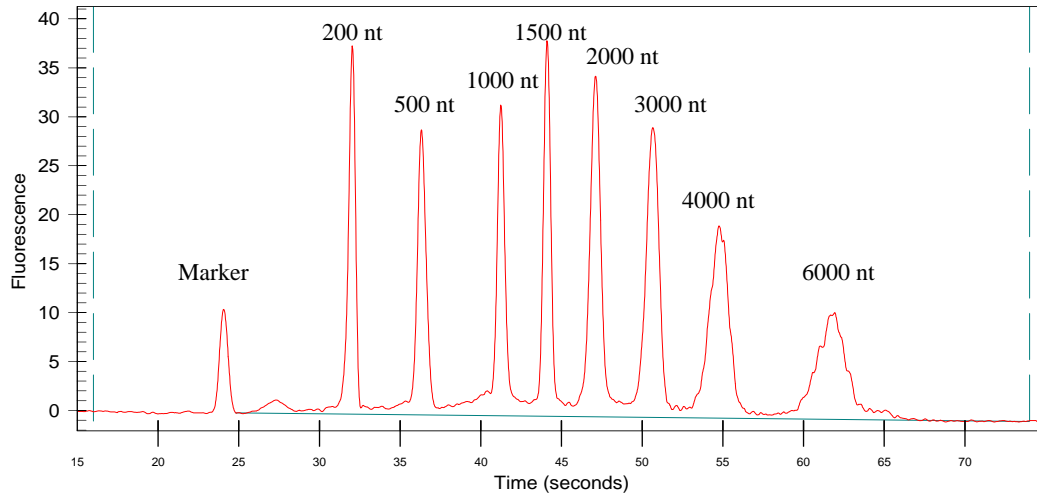
5. Remove the chip from the priming station and place it in the plastic storage container. Add an additional amount of Storage Buffer to well 1. Cover the wells with parafilm to prevent buffer evaporation and store in the fridge at 4 °C. Storage of a chip with dry wells may cause it to become clogged.

Results

To view your results, click on the sample in the plate view or electropherogram.

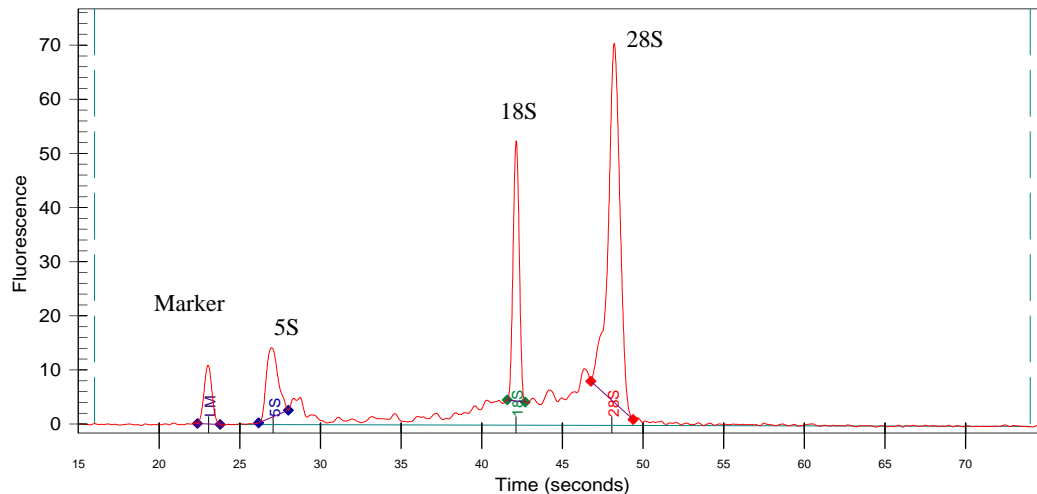
HT RNA Ladder Result

- Electropherogram of a typical RNA ladder is shown below:



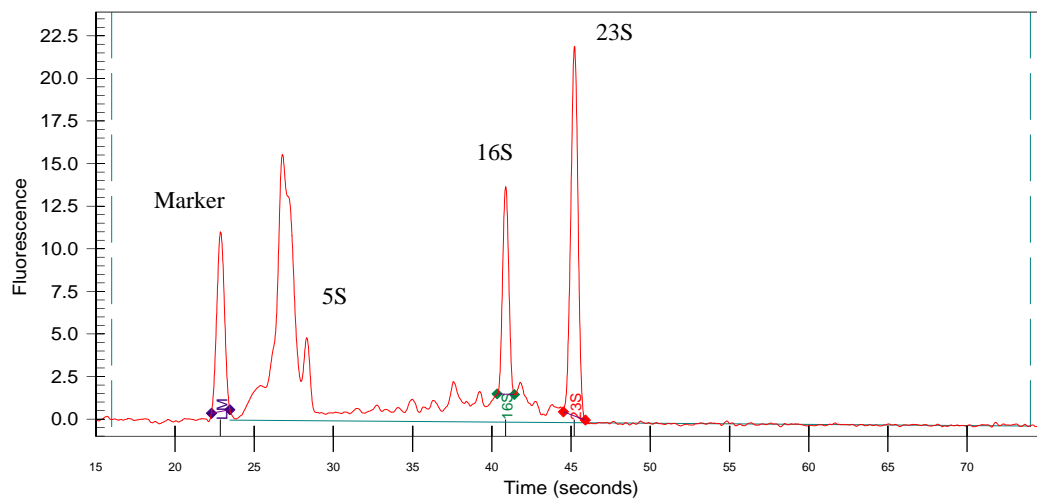
HT RNA Eukaryote Sample Result

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



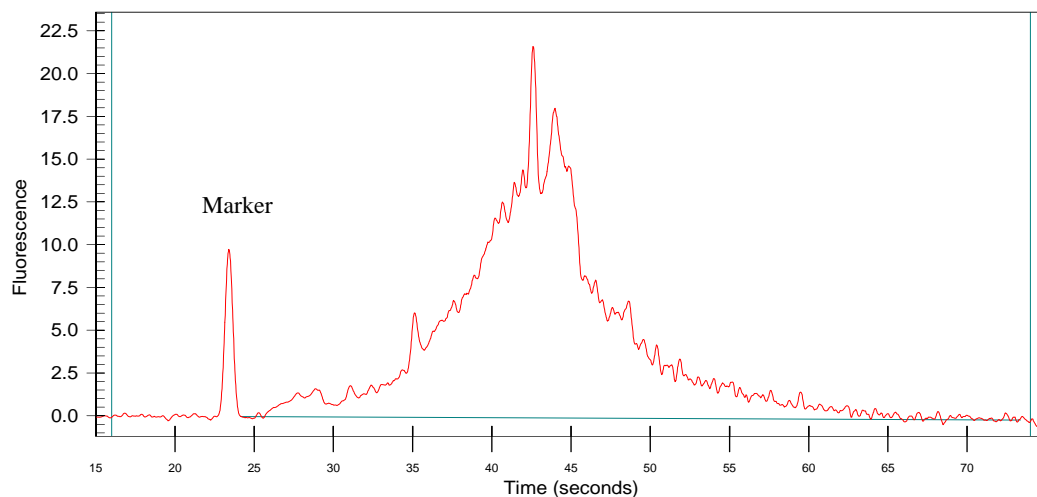
HT RNA Prokaryote Sample Result

- 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.



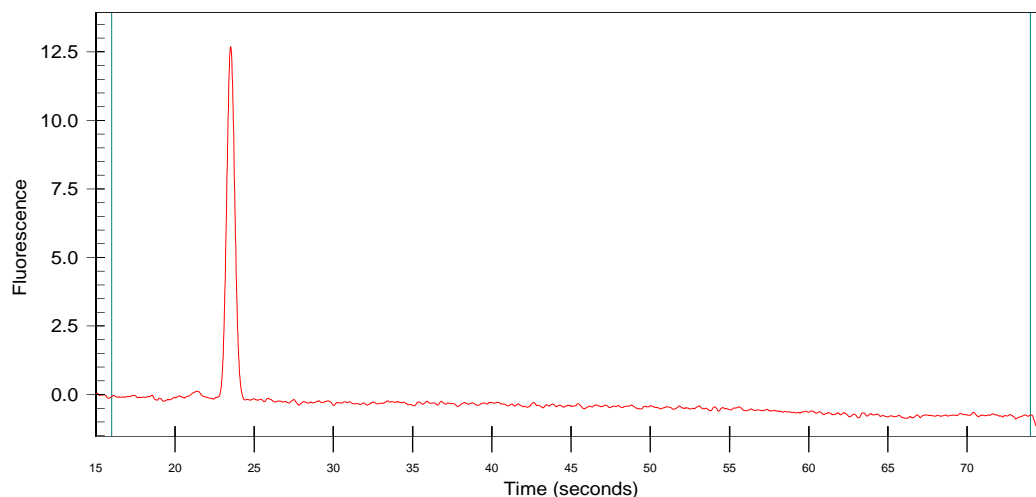
Troubleshooting

Symptom: No Ladder or Sample Peaks Appear

- Marker peak is apparent, but no ladder or sample peaks are visible.
- Marker peak is about twice its normal height.

Possible causes:

- A) Sipper clog due to air bubbles.
- B) The sipper is not immersed in the sample due to low sample volume on the plate.
- C) The sipper is not immersed in the sample due to an incorrect sipper height setting.



Example electropherogram result when sipper is clogged.

What to do:

A) Unclog the sipper by applying pressure to the Marker Well, (well 4) using a 10 ml syringe.

- Open the chip holder.
- Pull the syringe plunger to the 10 mL mark and place it over the Marker Well, as shown in the photo.
- Depress the syringe to about the 6mL mark and hold for 20 seconds.
- Release the syringe and allow it to return to the 10mL mark before removing the syringe from the chip well.
- Close the chip holder and re-start the run.



B) Look for bubbles in the samples

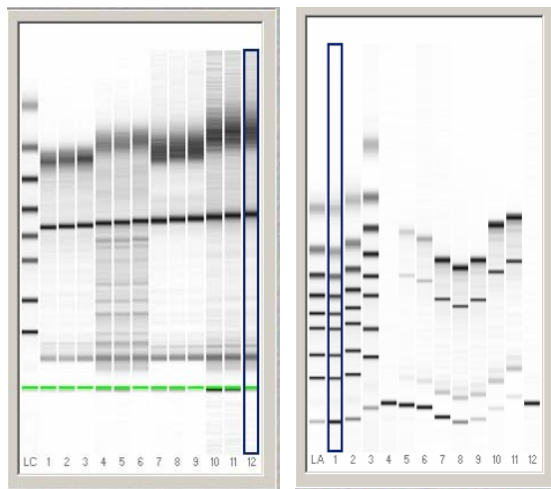
- Manually insert a larger volume pipette tip into sample well to dislodge the bubble.

C) Be sure that the correct sipper height is being used.

- Use 0.5mm for samples on a 96-well plate. Use 1mm for samples on a 384-well plate.

Symptom: Migration Time Shift

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



Examples of inconsistent peak migration.

Possible causes:

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

What to do:

- A) Clear the chip channels
 - Remove the Gel-Dye and marker from the chip.
 - Clean the wells 2 times with DI water and fill with storage buffer.
 - Prime chip for 4 min.
 - Rinse wells 2 times with Milli Q water and refill with Gel-Dye mixture.
- B) Retire this chip.
- C) 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.

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.

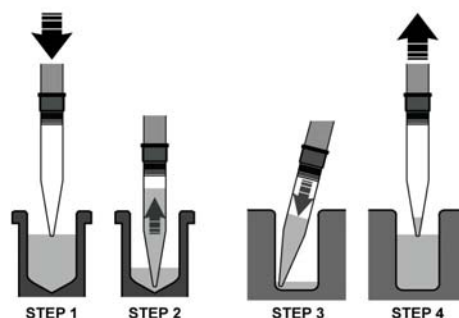
For assay and instrument troubleshooting, refer to the LabChip HT Software Help file or call Caliper Technical Support at 1-877-LABCHIP

General

- Allow the chip, sample plate and all reagents to equilibrate to room temperature before use (approximately 20 to 30 minutes).
- Clean the chip priming station weekly and the LC90 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. Use of other, non-approved wipes may leave fluorescent debris, which can cause erratic focusing.
- Do not use transfer pipettes or other liquid handling tools that can contaminate liquids with fibers or other debris.
- Use only filter pipette tips.
- Water used for chip preparation procedures must be 0.22-micron filtered deionized, molecular biology grade.
- 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 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.

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.

- 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 dark at 4 °C when not in use.
- The gel-dye mixture expires 5 days after preparation.

Chips

- 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 at 3000 rpm (1250 rcf) prior to analysis.
- Up to two 96-well plates or half of a 384-well plate can be run with a single chip preparation.

Electrode Cleaning

Prior to inserting a prepared RNA chip into the instrument, Caliper recommends cleaning the instrument electrodes daily. If there is RNase contamination, electrodes should be cleaned between each run.

- Fill the cleaning chip with 1.2 mL of DEPC treated water. The cleaning chip can be filled from any well. All wells are connected together by a central U-shaped channel. (The cleaning chip is shipped with the instrument accessory kit).
- Insert the cleaning chip into the instrument and close the chip holder. This immerses the electrodes in water.
- Allow for a minimum of 2 minutes of incubation before removing the chip from the instrument.

- Remove the cleaning chip and allow the electrodes to air dry for approximately five minutes.

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 b). To avoid contamination, use a new pipette tip over the permanent tip for each chip aspirated (Figure 2).

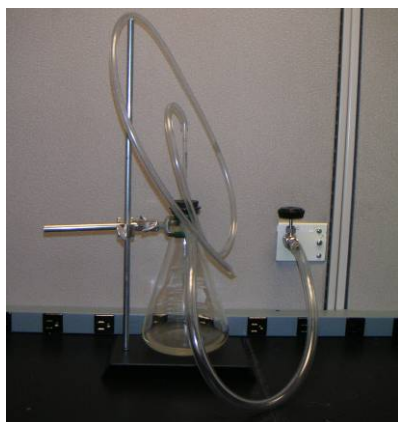


Figure 1a



Figure 1b

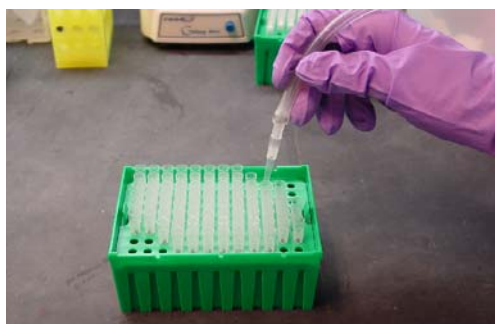


Figure 2

Reordering Information

<u>Product</u>	<u>Part Number</u>
HT DNA 5K/RNA LabChip, Ver 2	760435
HT RNA Reagent Kit	760410
Buffer Strip Wells	Corning 2593
Ladder Strip Wells	Nunc 248909
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 AMS90SE™ User Guide or LabChip 90 User Guide. Caliper, the Caliper logo, LabChip, and the LabChip logo are registered trademarks of Caliper Life Sciences.

© Copyright Caliper Life Sciences 2010
<http://www.caliperLS.com>

Doc 450594

This product is provided under an agreement between Molecular Probes, Inc. (a wholly owned subsidiary of Invitrogen Corporation) and Caliper Life Sciences Inc., and the manufacture, use, sale or import of this product is subject to one or more of U.S. Patents and corresponding international equivalents, owned by Molecular Probes, Inc. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer in conjunction with Caliper Life Sciences Inc.'s automated electrophoresis system, where such research does not include testing, analysis or screening services for any third party in return for compensation on a per test basis. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, USA. Tel: (541) 465-8300, Fax: (541) 335-0354.