RNA Assay Quick Guide
LabChip® GX Touch/GXII Touch

Notes:

- *Allow the chip and reagents to equilibrate to room temperature for at least 30 minutes before use.*
- *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.*

Preparation of Gel-Dye Solution

1. Vortex the thawed RNA Dye Concentrate for ***10 – 15 seconds before use.***
2. Transfer **75 µL** of RNA Dye Concentrate to a **2.0 mL** centrifuge tube provided with the reagent kit.
3. Add **425 µL** of RNA Gel Matrix using a Reverse Pipetting Technique.
4. Vortex the solution until it is well mixed and spin it down for a few seconds.
5. Transfer the solution to a spin filter. Use a centrifuge tube filled with **500 µL** of water (Milli-Q® or equivalent) to balance the centrifuge.
6. Centrifuge at **9300 rcf for 10 minutes at room temperature**.
7. Discard the filters, label, and date the tubes.
8. Store in the dark at **2-8°C**. Use within 5 days.

Low-Throughput (LT) Chip Preparation, up to 48 samples
High Throughput (HT) Chip Preparation, up to 192 samples

1. Rinse and aspirate each active well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q® or equivalent). Do not allow the active wells to remain dry.
2. 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 70% isopropanol to clean the chip detection window as needed.
3. Using a Reverse Pipetting Technique, add gel-dye solution to chip wells 3, 7, 8, and 10 as shown in **Figure 1 for LT** or as shown in **Figure 2 for HT.**
4. Add **50 µL (LT)** or **100 µl (HT)** RNA Marker to chip well 4 as shown in **Figure 1** and **Figure 2**, respectively.

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.*

5. Make sure the rims of the chip wells are clean and dry.
6. **IMPORTANT:** Ensure chip well 1 (waste well) is empty before placing the chip into the LabChip GX Touch/GXII Touch.

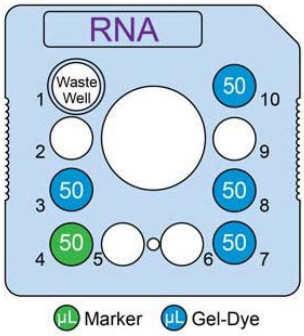

Figure 1. Low-throughput (LT) chip preparation

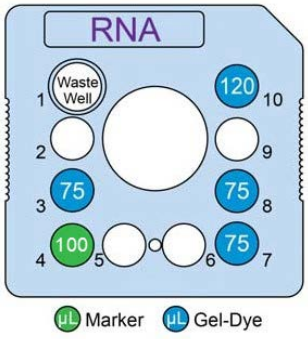

Figure 2. High-throughput (HT) chip preparation

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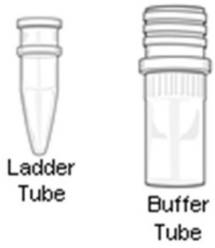
RNA Sample, Ladder, and Buffer Preparation

Note: *The RNA ladder should be kept on ice. Avoid multiple freeze/thaws.*
It is recommended that you aliquot the RNA ladder into five 4 µL lots for individual use.

1. Prepare Sample Buffer by adding **620 µL** RNA Sample Buffer Concentrate to **5580 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. Allow the RNA ladder to equilibrate to room temperature for at least 30 minutes.
3. Transfer **4 µL** RNA Ladder into RNase-free microcentrifuge tube or a well of the microtiter plate.
4. For each sample to be analyzed, pipette **2 µL** (RNA Std Sens) or **6 µL** (RNA High Sens) sample into individual microtiter plate wells (cover with PCR strip caps) or RNase-free microcentrifuge tubes.
5. Cover and heat the ladder and samples at **70°C for 2 minutes**.
6. Snap cool the samples and ladder by immediately placing the tubes and/or microtiter plate on **ice for 5 minutes**.
7. Add **46 µL** (RNA Std Sens) or **19 µL** (RNA High Sens) prepared sample buffer to each sample. Cover the samples with PCR strip caps and spin down the plate.
8. Add **96 µL** prepared sample buffer to the Ladder Tube.
9. Add **750 µL** prepared sample buffer to the provided Buffer Tube.



Chip Cleaning and Storage

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

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 a vacuum.
3. Each active well (1, 3, 4, 7, 8, and 10) should be rinse and aspirated twice with water (Milli-Q® or equivalent).
4. Add **120 µL** of RNA Chip Storage Buffer (white cap) to the active wells.
5. Place the chip back into the LabChip GX/GXII Touch.
6. Touch the **Wash** button.
7. Remove the chip from the instrument and place it into the storage container.
8. Add an additional **50 µL** of RNA Chip Storage Buffer to well 1.
9. Cover the wells with Parafilm® to prevent evaporation and store at 2-8°C until next use. If using the chip again within 24 hours it may be left at room temperature. Allowing the chip wells to dry may lead to changes in chip performance.

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Assay Specifications

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

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)
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	Up to 192 per HT chip prep Up to 48 per LT chip prep
Number of Chip Preps per Reagent Kit	5 (HT chip prep), 10 (LT chip prep)

For the complete RNA Assay User Guide, go to: http://www.perkinelmer.com