RNA Assay Quick Guide
LabChip® GX Touch/GXII Touch

Notes:

- *Allow the chip and refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use. Thaw the RNA Ladder on ice.*
- *RNA Dye contains DMSO and must be thawed completely before use.*
- *The dye is light sensitive. Do not expose the Dye or Gel-Dye solution to light for any length of time.*
- *Keep the prepared Gel-Dye solution in the dark.*

Preparation of Gel-Dye Solution

1. Vortex the thawed RNA Dye Concentrate for ***10 to 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 Gel-Dye solution until it is well mixed and spin down for a few seconds.
5. Transfer the Gel-Dye solution to a spin filter. Use a centrifuge tube filled with **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 filter.
8. Label and date the tube.
9. 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 completely 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 or bottom chip surfaces during rinsing, aspirate using the vacuum line. DO NOT run the tip over the detection window. Use the provided Detection Window Cleaning Cloth dampened in water (Milli-Q® or equivalent) or 70% isopropanol to clean the 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 (LT)** or **Figure 2 (HT).**
4. Add **50 µL (LT)** or **100 µl (HT)** RNA Marker to chip well 4 as shown in **Figure 1 (LT)** or **Figure 2 (HT)**.

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.

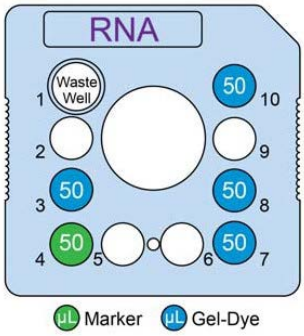

Figure 1. Low-throughput (LT) chip preparation

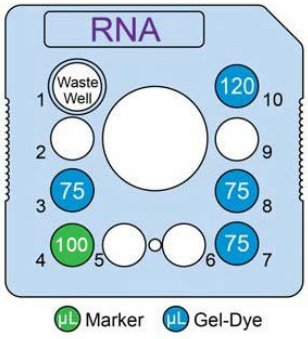

Figure 2. High-throughput (HT) chip preparation

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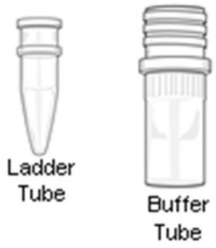
RNA Sample, Ladder, and Buffer Preparation

1. Prepare 1X 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 thaw on ice. (Avoid multiple freeze/thaws. It is recommended to aliquot the RNA ladder into five 4 µL lots for individual use after thawing the vial for the first time.)
3. Transfer **4 µL** RNA Ladder into the provided 0.2 mL Ladder Tube and cover.
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 cap strips.
5. 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 1X Sample Buffer to each sample. Mix by pipetting up and down a few times. Avoid creating air bubbles. Cover the samples with PCR strip caps and spin down the plate at 3000 rpm for 5 minutes.
8. Add **96 µL** prepared 1X Sample Buffer to the Ladder Tube.
9. Add **750 µL** prepared 1X 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 chip storage container. Verify the sipper is submerged in the fluid reservoir.
2. Remove the reagents from each well of the chip using a vacuum.
3. Rinse and completely aspirate each active well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q® or equivalent).
4. Add **120 µL** of RNA Chip Storage Buffer (white cap) to the active wells.
5. Place the chip back into the LabChip GX Touch/GXII Touch.
6. Touch the **Wash** button on the Home screen.
7. Touch the **Wash** button on the Wash screen.
8. When the chip wash is complete, remove the chip from the instrument and place the chip into the chip storage container.
9. Add an additional **50 µL** RNA Chip Storage Buffer to well 1.
10. Cover the wells with Parafilm® to prevent evaporation and store at 2-8°C. Storing a chip with dry wells may clog the chip. If using the chip again within 24 hours, the chip can be stored at room temperature.

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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 samples)
Setup Time	Approximately 30 minutes to prepare chip and samples
Samples per Chip Prep	Up to 192 samples per HT chip prep Up to 48 samples per LT chip prep
Chip Preps per Reagent Kit	5 HT chip preps or 10 LT chip preps
Chip Lifetime	HT: 2000 samples 24: 750 samples

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

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