RNA Pico Sensitivity Assay Quick Guide
LabChip® GX Touch/GXII Touch

Notes: Allow the chip and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use.
The RNA Dye Concentrate must be thawed completely and vortexed before use.

Preparation of Ladder Aliquots

Note: Thaw the RNA Pico Ladder on ice. Avoid multiple freeze-thaws.

1. Thaw the RNA Pico Ladder on ice.
2. Spin down the RNA Pico Ladder and heat-denature at **70°C for 2 minutes**. Immediately snap cool **on ice for 5 minutes**.
3. Prepare five **4 µL aliquots** in nuclease-free tubes. Store aliquots at -70°C. When using frozen aliquots, do not heat-denature again. If needed, additional RNA Pico Ladder can be ordered (P/N CLS760652).

Preparation of Gel-Dye Solution

NOTE: The prepared volume of Gel-Dye solution is enough for one HT (High-Throughput) or two LT (Low-Throughput) chip preps.

Warning: 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.

1. Allow the chip and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use.
2. Vortex the thawed RNA Dye Concentrate for 10 - 15 seconds before use.
3. Transfer **90 µL** of RNA Dye Concentrate (blue cap) to **1 vial** of the RNA Pico Gel Matrix (red cap).
4. Vortex and invert the tube several times until the solution is well mixed and spin it down for a few seconds.
5. Transfer the solution into a spin filter and centrifuge at 9300 rcf for 10 min at RT.
6. Discard the filter, label and date the tube, and store in the dark at 2-8°C. Use within 5 days.

Low-Throughput (LT) Chip Preparation - up to 48 samples and
High-Throughput (HT) Chip Preparation - up to 96 samples

1. Rinse and completely aspirate each active well (1, 3, 4, 7, 8, and 10) twice with nuclease-free water.
2. Using a Reverse Pipetting Techniques, add Gel-Dye solution to chip wells 3, 7, 8 and 10 as shown in **Figure 1 (LT)** or **Figure 2 (HT)**.
3. Add **50 µL (LT)** or **100 µL (HT)** RNA Pico Marker to chip well 4 as shown in **Figure 1 (LT)** or **Figure 2 (HT)**.
4. Clean both sides of the chip window with the supplied clean room cloth dampened with 70% isopropanol.
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.

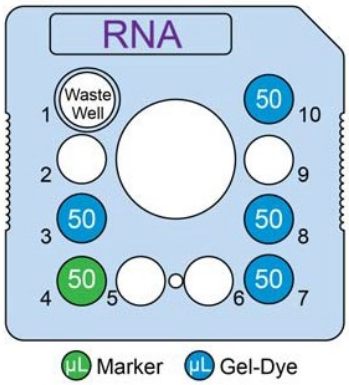

Figure 1. Low-Throughput Chip Preparation

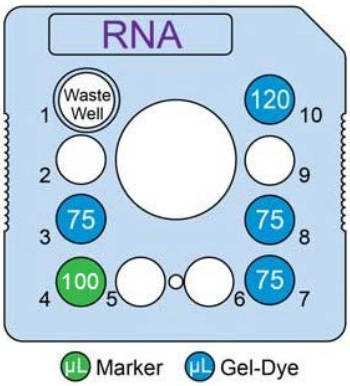

Figure 2. High-Throughput Chip Preparation

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RNA Sample, Ladder and Buffer Preparation

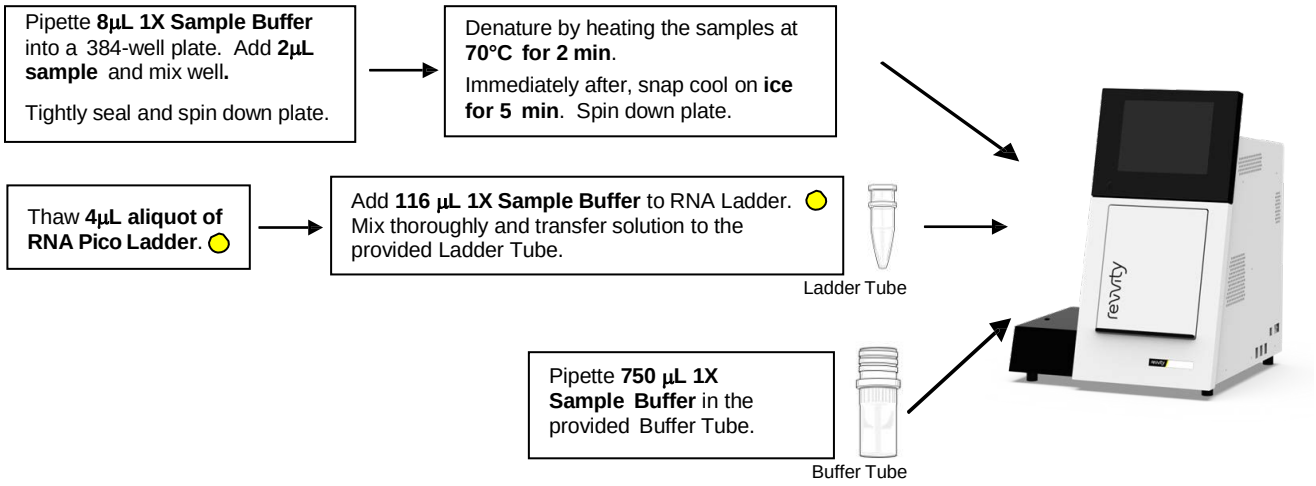

Figure 3. Sample, Ladder Tube and Buffer Tube Preparation

1. Prepare 1X **Sample Buffer** by adding **200 µL** RNA Sample Buffer Concentrate 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. Prepare sample, Ladder Tube and Buffer Tube according to **Figure 3**. For sample heat denature, if a 384-well thermocycler or heat block is not available, sample plate can be heated by placing plate on top of one heat block, and then placing another heat block on top of the plate.

Note: *Due to sample evaporation, test up to 48 samples only per run. For example, if analyzing 96 samples, test samples in a total of 2 runs.*

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 reagents from each well 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 to the active wells.
5. Place the chip back into the LabChip GX Touch/GXII Touch. Ensure a Buffer Tube with **750 µL RNA Chip Storage Buffer** is in the buffer slot.
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[1]

The RNA Pico Sensitivity 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.

Sensitivity	250 pg/µL Total RNA 500 pg/uL mRNA
Linear Concentration Range	500 – 5000 pg/µL Total RNA 625 – 5000 pg/uL mRNA
Quantitation Accuracy	± 30% (for ladder as sample)
Quantitation Reproducibility	20% CV
Size Range	100 – 6000 nucleotides
RNA Sample Volume	2 µL
Maximum Salt	10 mM Tris
Run Time	80 seconds per sample (about 2.5 hours for 96 samples)
Compatible Plate Types	384-well
Chip Lifetime	HT: 2000 samples 24: 750 samples
Samples per Chip Prep	Up to 96 samples per HT chip prep (divided into 2 runs of 48 samples) Up to 48 samples per LT chip prep
Chip Preps per Reagent Kit	5 HT chip preps or 10 LT chip preps
For Research Use Only	

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

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

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