

RNA Pico Sensitivity Assay Quick Guide

LabChip[®] GX Touch/GXII Touch

Note: Allow the chip and reagents to equilibrate to room temperature for about 20 minutes before use. *The Dye Concentrate must be completely thawed and vortexed before use.*

Preparation of Ladder Aliquots

1. Spin down RNA Pico Ladder ● and heat denature at **70°C for 2 minutes**. Afterward, immediately snap cool **on ice for 5 minutes**.
2. Prepare **4 µL aliquots** in nuclease-free tubes. Store aliquots at -70°C. Upon use of frozen aliquots, do not heat denature again. If needed additional ladder can be ordered (Part Number CLS760652).

Note: *The RNA Pico Ladder ● should be kept on ice. Avoid multiple freeze-thaws.*

Preparation of Gel-Dye Solution

1. Vortex the thawed Dye Solution for 20 seconds before use.
2. Transfer **90 µL** of Pico RNA Dye Solution (blue cap ●) to **1 vial** of the RNA Gel Matrix (red cap ●).
3. Vortex and invert the tube several times until the solution is well mixed and spin it down for a few seconds.
4. Transfer the solution into one spin filter and centrifuge at 9300 rcf for 5 min at RT.
5. Remove the filter in the tube and discard the filter.

Low-Throughput Chip Preparation - up to 48 samples

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

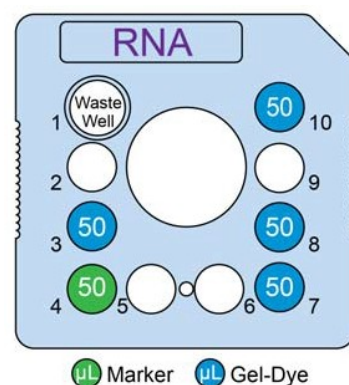


Figure 1. Low-Throughput Chip Preparation

High-Throughput Chip Preparation - up to 96 samples

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

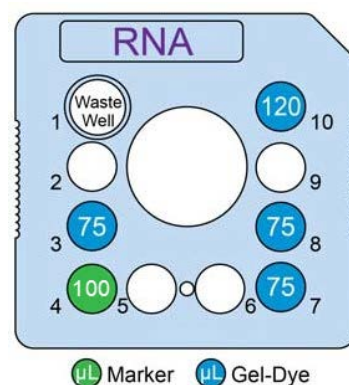


Figure 2. High-Throughput Chip Preparation

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

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RNA Sample, Ladder and Buffer Preparation

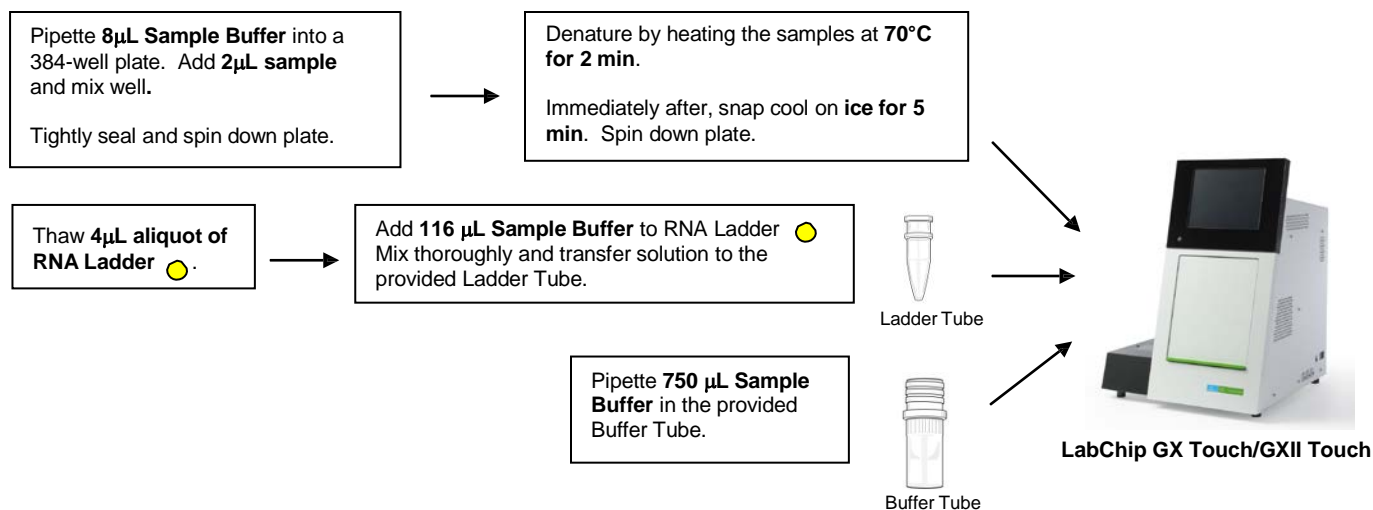


Figure 3. Sample, Ladder Tube and Buffer Tube Preparation

1. Prepare **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. The cleaning procedure can be conducted the following day, when running overnight.

1. Remove reagents from each well using a vacuum.
2. Rinse and thoroughly aspirate each active well (1, 3, 4, 7, 8 and 10) twice with molecular biology grade water.
3. Add **100 µL** of Storage Buffer to active wells.
4. Place the chip back on the LabChip GX Touch/GXII Touch and click the **Wash** button. Ensure a Buffer Tube with **750 µL** buffer or molecular biology grade water is in the buffer slot.
5. Remove the chip from the LabChip GX Touch/GXII Touch and place in chip container.
6. Add an additional **50 µL** of Storage Buffer to wells 1 and 4.
7. Make sure to cover all wells with Parafilm[®] and store at 4°C.

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Assay Specifications¹

Sensitivity	250 pg/μL Total RNA 500 pg/μL mRNA
Linear Concentration Range	500 – 5000 pg/μL Total RNA 625 – 5000 pg/μL 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
Number of Samples per Chip Prep	Up to 96
Samples per Reagent Kit	up to 480
For Research Use Only	

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

For complete RNA Pico Sensitivity Assay User Guide, go to:

<http://www.perkinelmer.com/labchipsystems>