

Small RNA 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 Small RNA Ladder ● on ice. Avoid multiple freeze-thaws.

1. Thaw the Small RNA Ladder ● on ice. Prepare aliquots of ladder (10 μ L each) into DNase/RNase-free tubes.
2. Spin down the Small RNA Ladder ● and heat-denature at **70°C for 2 minutes**. Immediately snap cool **on ice for 5 minutes**.
3. Store aliquots at -70°C. When using frozen aliquots, do not heat-denature again.

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 **70 μ L** of RNA Dye Concentrate (blue cap ●) to **1 vial, 510 μ L** of the Small RNA 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 Gel-Dye solution to a spin filter and centrifuge at 9300 rcf for 10 min at room temperature.
6. Discard the filter. Label and date the tube. 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 Technique, 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 μ L (LT)** or **100 μ L (HT)** Small RNA 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.

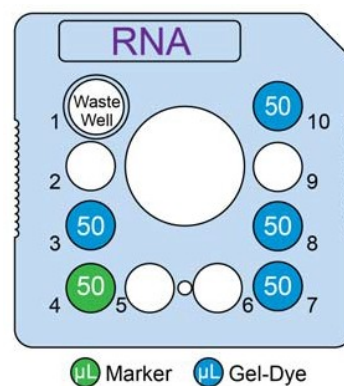


Figure 1. Low-Throughput Chip Preparation

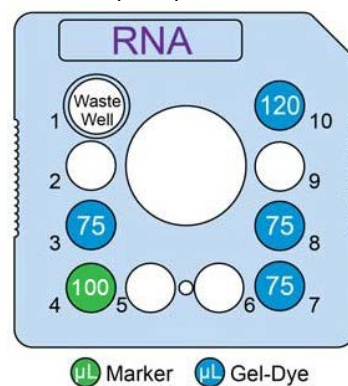


Figure 2. High-Throughput Chip Preparation

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RNA Sample, Ladder and Buffer Preparation

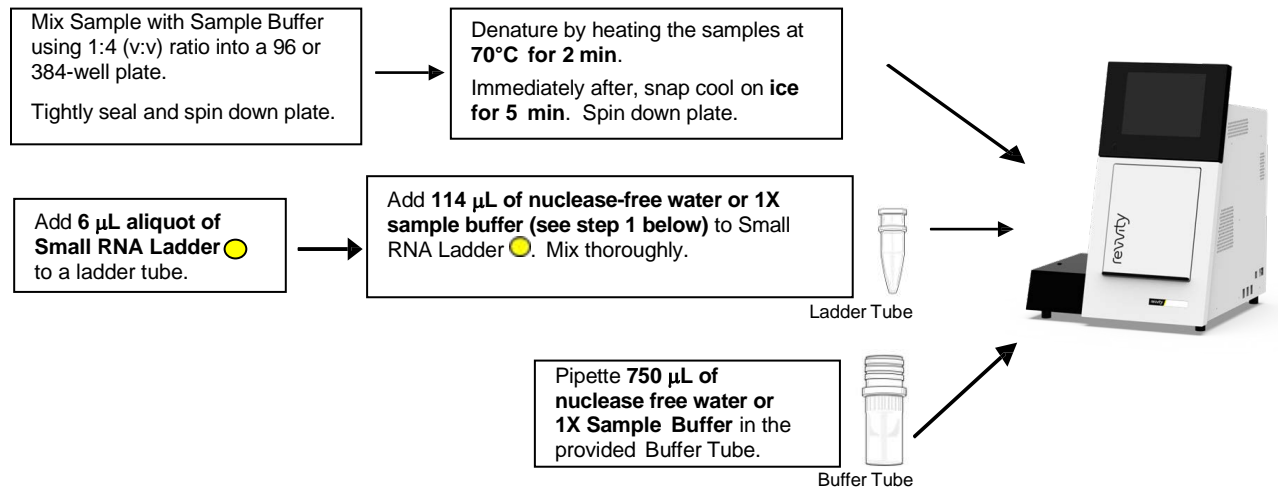


Figure 3. Sample, Ladder Tube and Buffer Tube Preparation

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. Nuclease-free water can be used in place of sample buffer for highest sensitivity. Samples can also be run undiluted.

Note: Due to sample evaporation, test only up to 48 samples 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 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 Sample Buffer or water** 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

The Small 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.

Small RNA Reagent Kit	CLS153530
Size Range	20-150 nt
Linear Concentration Range	100-10,000 pg/uL
Sensitivity¹	50 pg/μL
Sizing Reproducibility	CV < 5.0% or 4 nt
RNA Sample Volume	2 μL
Maximum Salt Concentration	10 mM Tris
Run Time	60 seconds per sample (~ 2.5 hours for 96 samples)
Compatible Plate Types	96-well or 384-well
Chip Lifetime	2000 samples (DNA 5K/RNA/CZE HT LabChip 760435) 750 samples (DNA 5K/RNA/CZE 24 LabChip CLS138949)
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 Maximum sensitivity is 50 pg/μL for samples (in TE buffer, 10 mM Tris, pH 8.0) run on-plate undiluted in sample buffer.

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LabChip Reagent CoA: <https://www.revvity.com/tools/COASearch>

For the complete *Small RNA Assay User Guide* (P/N CLS153470), go to: <http://www.revvity.com>.

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