FFA120 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 FFA120 Assay requires one of the following chip types:
  - DNA 5K/RNA/CZE HT Chip, P/N 760435, or
  - DNA 5K/RNA/CZE 24 Chip, P/N CLS138949

Preparing the Ladder Aliquots

NOTE: Avoid multiple freeze-thaws of the FFA120 Ladder.

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. Thaw the **FFA120 Ladder** on ice or at 4ºC.
2. Vortex the thawed FFA120 Ladder for 10 - 15 seconds to mix and then spin down for a few seconds before each use.
3. Aliquot 65 µL into two nuclease-free tubes, up to 5 cycles of freeze-thaw.
4. Store the FFA120 Ladder aliquots at -20ºC until the expiration date.

Preparing the Gel Solution

NOTE: The prepared 510 µL of Gel solution is enough for one HT (High-Throughput) or two LT (Low-Throughput) chip preps

1. Allow the chip and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use.
2. Transfer 1 vial, 510 µL of **FFA120 Gel Matrix** to a spin filter. Use a centrifuge tube filled with 510 µL of water to balance the centrifuge.
3. Centrifuge at 9300rcf for 10 minutes at room temperature.
4. Discard the filter.
5. Label and date the tube. Store at 2-8°C.
6. The filtered Gel Solution (stored at 2-8°C) is stable until it reaches the Gel Solution expiration date.

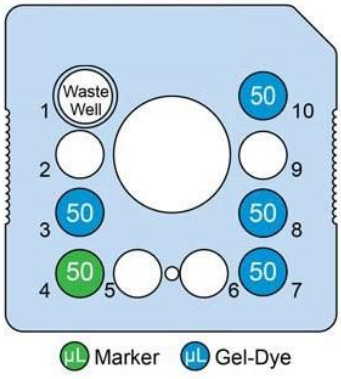

Figure 1. Low-Throughput Chip Preparation

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

1. Allow the chip to equilibrate to room temperature for at least 30 minutes before use.
2. Rinse and completely aspirate each active well (1, 3, 4, 7, 8, and 10) twice with nuclease-free water.
3. Using a Reverse Pipetting Technique, add Gel 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) FFA Marker** to chip well 4 as shown in **Figure 1 (LT)** or **Figure 2 (HT)**.
5. Clean both sides of the chip window with the supplied clean room cloth dampened with 70% isopropanol.
6. Make sure the rims of the chip wells are clean and dry.
7. **IMPORTANT**: Ensure chip well 1 (waste well) is empty before placing the chip into the LabChip GX Touch/GXII Touch.

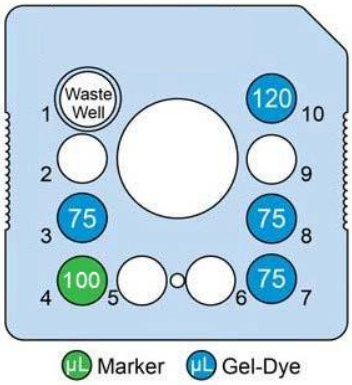

Figure 2. High-Throughput Chip Preparation

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RNA Sample, Ladder and Buffer Preparation

1. Prepare sample, Ladder Tube, and Buffer Tube according to **Figure 3**.

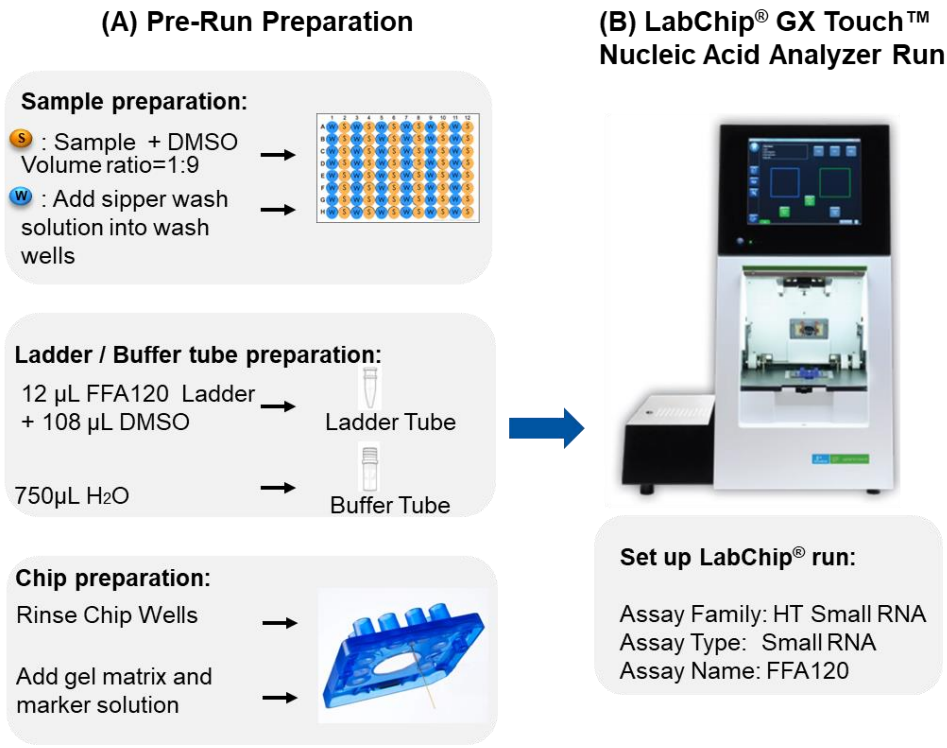

Figure 3. Sample, Ladder Tube and Buffer Tube Preparation

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 FFA 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 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 FFA 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.

FFA120 Reagent Kit	
Size Range	20-120 nt
Concentration Range	4nM to 2.5µM before DMSO dilution
Sensitivity[1]	4nM before DMSO dilution
Sizing Reproducibility	CV < 5.0% or 4 nt
Sample Volume[2]	2 µL
Maximum Salt Concentration	20 mM Tris, 100mM KCl
Run Time	60 seconds per sample (~ 2.5 hours for 96 samples)
Compatible Plate Types	96-well
Chip Lifetime	2000[3] samples (DNA 5K/RNA/CZE HT LabChip 760435) 750 samples (DNA 5K/RNA/CZE 24 LabChip CLS138949)
Samples per Chip Prep	Up to 48 samples per HT chip prep Up to 24 samples per LT chip prep
Chip Preps per Reagent Kit	5 HT chip preps or 10 LT chip preps

[1] Maximum sensitivity is 4nM for samples (in TE buffer, 10 mM Tris, pH 8.0) before diluted in DMSO.

[2]Sample Volume:

Mix 2µL sample with 18µL DMSO for 96-well plate sample preparation.

If sample volume is available, you can mix 3µL sample with 27µL DMSO.

If sample volume is limited, you can mix 1.5µL sample with 13.5µL DMSO.

The on-plate sample volume should be ≥ 15 µL to ensure successful sipping.

[3] Based on the samples prepared in TE buffer only.

Contact Revvity

Revvity, Inc. 68 Elm Street, Hopkinton, MA 01748-1668 USA

Revvity Technical Support
Phone: (USA Toll Free) 800-762-4000; (Worldwide) +1 203-925-4602
Email: BioprocessQC@revvity.com

For the complete *FFA120 Assay User Guide* (P/N CLS158447), go to: http://www.Revvity.com/

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