Plasmid DNA Assay Quick Guide
LabChip™ GX/GXII Touch

Section 1. Chip Preparation

WARNING: *Plasmid DNA Dye contains DMSO. Avoid contact with skin and eyes.*

NOTES:

- *The Plasmid DNA Dye and Plasmid DNA Marker are sensitive to light. Avoid prolonged exposure to light during chip and sample preparation.*
- *The Plasmid DNA assay requires the HT chip type below.*
- *NaOH solution (1N) is required for chip preparation. For preparing NaOH information, details are provided in the user guide (page 19).*

LabChip	Part Number
HT Plasmid DNA LabChip	CLS160538

1 Allow the chip and reagents to equilibrate to room temperature for at least 30 minutes before use. ***The Plasmid DNA Dye Concentrate must be completely thawed and vortexed for 10 – 15 seconds before use***. **Follow the chip cleaning protocol in section 3 before each run.**

2 Prepare the Working Gel by diluting **133 µl Plasmid DNA Gel Concentrate (reverse pipetting)** 🔴 with **1867 µl of Gel Dilution Buffer** (final 2 mL volume). Mix it well. Amounts of DNA gel concentrate, and dilution buffer can be modified proportionally to desired final volume.

Note: *The Working Gel (without dye concentrate) can be stored for up to 3 weeks at 2-8°C.*

3 Transfer 500 uL of the **Working Plasmid DNA Gel** (step 2) to a spin filter. Add 15 uL of **DNA dye** 🔵 into the solution. **Vortex and mix well.**

Note: *Gel-Dye should be prepared fresh on the day of the experiment.*

4 Centrifuge at **9300 rcf for 9.0 minutes at room temperature.** Ensure all the gel-dye passes through the filter and then discard the filter.

5 Rinse and completely aspirate each active well (1, 3, 4, 7, 8, and 10) with water (Milli-Q® or equivalent).

6 Repeat the water rinse (step 5).

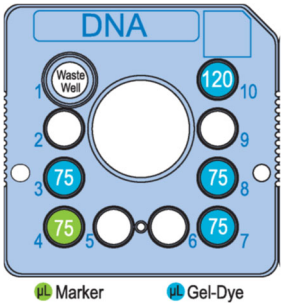

Figure 1. Up to 24 samples

7 Use a reverse pipetting technique to add gel-dye to chip wells 3, 7, 8, and 10 shown in Figure 1**. Add plasmid DNA Marker** 🟢 to chip well 4, as shown in Figure 1.

8 Clean both sides of the detection window with the supplied clean room cloth dampened with 70% isopropanol.

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Section 2. Plasmid DNA Sample, Ladder, and Buffer Preparation

NOTES:

- Aliquot the ladder into 12 µL fractions using 0.2 mL PCR tubes, store aliquots at -20 °C.
- When handling the pDNA ladder and supercoiled plasmid samples, mix by pipetting up and down. Do not vortex.

1. Dilute samples using pDNA sample buffer to a final on plate concentration of 500 pg/µL.
2. Transfer samples to 96 or 384 well plate.
3. Spin down the plate for 9300 rcf for 5 minutes at room temperature.

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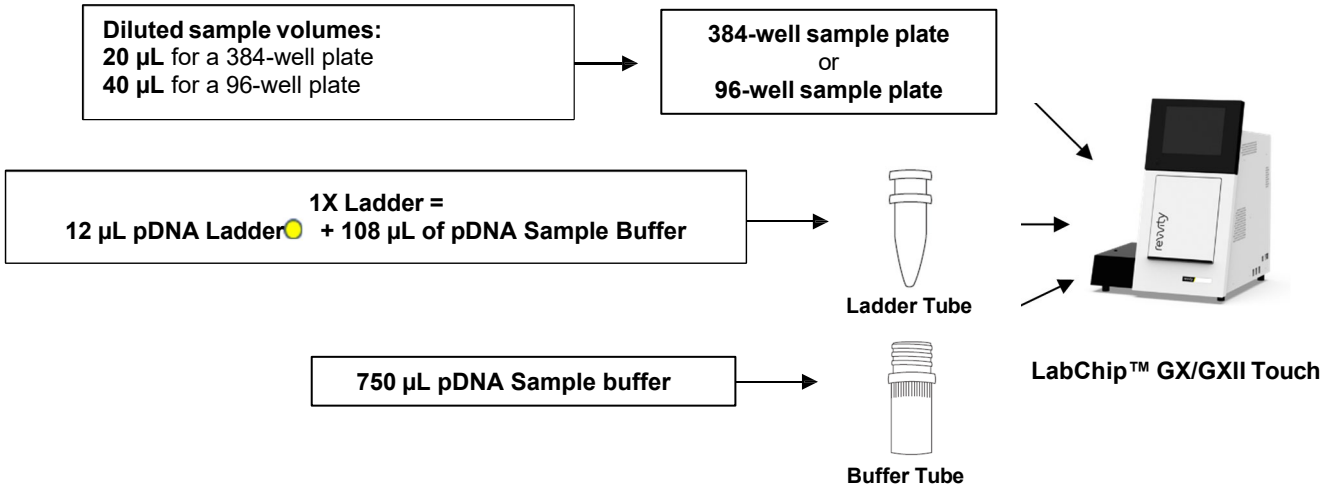
Sample Workflow



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Section 3: Chip Cleaning and Storage

Cleaning the pDNA chip before a run

1. Rinse and completely aspirate each well (1, 2 3, 4, 7, 8, 9 and 10) with water (Milli-Q® or equivalent)
2. Repeat the water rinse of wells (1, 2, 3, 4, 7, 8, 9 and 10).
3. Add **100 µL of 1N NaOH solution to the active wells** (1, 3, 4, 7, 8, and 10)**.**
4. **Place the chip into the** LabChip™ GX/GXII Touch.
5. Place a Buffer Tube with **750 µL** of water (Milli-Q® or equivalent) in the buffer slot.
6. Touch the wash button on the home screen.
7. When the wash is complete, remove the chip from the instrument and place the chip into chip storage container.
8. Rinse and completely aspirate each active well (1, 3, 4, 7, 8, and 10) with water (Milli-Q® or equivalent).
9. Repeat the water rinse of wells (1, 3, 4, 7, 8 and 10).
10. Add 120 µL of water (Milli-Q® or equivalent) to each active well, place the chip back into the LabChip™ GX/GXII Touch, and wash chip. Once done, repeat wash step by pressing the wash button once more.
11. When the wash is complete, remove the chip from the instrument and proceed to chip preparation steps (Section 1). Discard the used wash buffer tube.

Cleaning the chip after each run

1. Place the chip into the chip storage container. Verify the sipper is submerged in the fluid reservoir. Remove the reagents from each chip well using vacuum.
2. Rinse and completely aspirate each active well (1, 3, 4, 7, 8, and 10) with water (Milli-Q® or equivalent).
3. Repeat the water rinse of wells (1, 3, 4, 7, 8 and 10).
4. Completely aspirate each active well (1, 3, 4, 7, 8, and 10) and add **120 µL** of DNA Chip Storage Buffer (white cap ○) to the active wells.
5. Place the chip back into the LabChip™ GX/GXII Touch, and wash chip.
6. When the wash is complete, remove the chip from the instrument, cover the wells with Parafilm® to prevent evaporation and store at 2-8°C. If using the chip again within 24 hours, the chip can be stored at room temperature. Storing a chip with dry wells may clog the chip.

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Plasmid DNA Assay Specifications

Minimum diluted sample volume on plate	20uL (384 well plate) 40uL (96 well plate)
Plasmid size range*	3 -13 kbp
Sizing Accuracy*	< 15% (3-13 kbp, SC) < 20% (3-4 kbp, Linear) < 15% (4-10 kbp, Linear) < 20% (10-13 kbp, Linear)
Input sample Concentration range	25 pg/µL – 500 pg/µL diluted in pDNA sample buffer
Maximum sample concentration	500 pg/µL
Linear concentration range	50 pg/µL – 500 pg/µL (Plasmid DNA)
Sensitivity (LOD)	25 pg/µL
Percent purity (CV)	< 10%
Carryover	< 0.5%
Time per sample	157 seconds
Samples per chip prep	24
Chip Lifetime	480 samples
Chip preps per reagent kit	20 Chip preps

**Note: 2-3 kbp plasmid isoforms can be analyzed for percent purity analysis*

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