





HT DNA High Sensitivity Assay Quick Guide

LabChip® GX/GXII Touch

Chip Preparation

1. 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.** One vial of DNA HiSens Gel Matrix  is good for **4 Low-throughput chip preparations (for up to 48 samples) or 2 High-throughput chip preparations (for up to 96 samples).**
2. Prepare Gel-Dye by adding **13 µL** DNA Dye Concentrate  to 1 vial of DNA HiSens Gel Matrix . Vortex and transfer mixture into **two spin filters** (approximately 550 µL per spin filter). Centrifuge at **9200 rcf for 10 minutes at room temperature.** Ensure that all of the gel has passed through the filter and then discard the filter. (**Note: Gel-Dye can be stored for up to 3 weeks in the dark at 4°C.**)
3. Rinse and aspirate each active well (1, 3, 4, 7, 8 and 10) twice with molecular biology grade water.
4. Using a Reverse Pipetting Technique add gel-dye to chip well 3, 7, 8 and 10 as shown in **Figure 1 (Low-throughput) or Figure 2 (High-throughput).**
5. Add DNA HiSens Marker  to chip well 4 as shown in **Figure 1 (Low-throughput) or Figure 2 (High-throughput).**
6. Clean both sides of the chip window with the supplied clean room cloth dampened with 70% isopropanol. (**Note: Ensure chip well 1 is empty before placing the chip on the LabChip GX/GXII Touch.**)

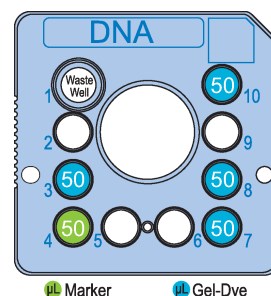


Figure 1. Low-throughput
(Up to 48 samples)

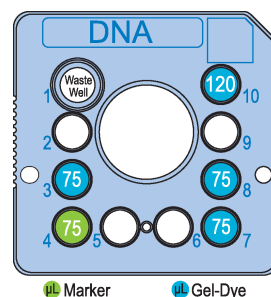


Figure 2. High-throughput
(Up to 96 samples)

DNA Sample, Ladder and Buffer Preparation

Standard Sample Workflow

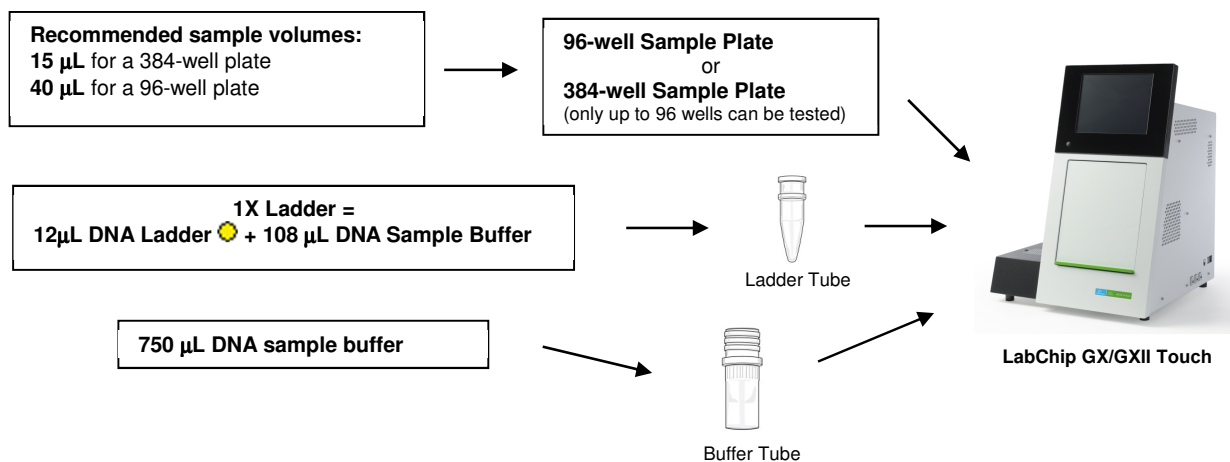


Figure 3. Standard Sample Workflow

Preparation Notes:

- DNA sample buffer is the user's DNA buffer such as the PCR buffer, etc.
- The sample buffer and the buffer used to dilute the ladder must be closely matched. A buffer mismatch between sample and ladder may lead to inaccurate quantitation and sizing.
- The number of samples per chip prep is 96. A 384-well plate may be used to conserve sample volume but only 96 wells of the 384 can be tested.

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Limited Sample Workflow

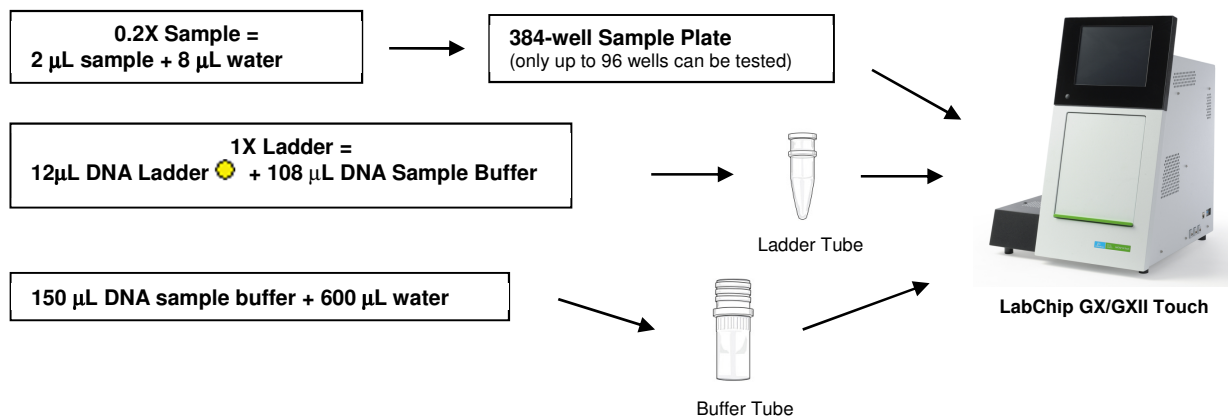


Figure 4. Limited Sample Workflow


Dilute Ladder with diluted DNA sample solution

Preparation Notes:

- This workflow requires only 2 μ L of sample with a **minimum initial concentration of 20 pg/ μ L per fragment or 200 pg/ μ L for smears**. Before testing, the 2 μ L of sample is diluted to 0.2X in water for a total volume of 10 μ L. To ensure a buffer match, the buffer for the Buffer Tube is diluted in a similar manner as the sample. Then Ladder is diluted in the diluted buffer.
- Sample plate should be tested soon after preparation to minimize evaporation. Sipping samples more than once is not recommended.
- Use this workflow if analyzing LabChip XT fractionated samples.
- **Quantitation given by the Labchip GX software should be multiplied by the dilution factor. (i.e. your sample dilution ratio).**

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 μ L** of Storage Buffer  to active wells.
4. Place the chip back on the LabChip GX/GXII Touch. Ensure a Buffer Tube with **750 μ L** sample buffer or molecular biology-grade water is in the buffer slot and click the **Wash** button.
5. Remove the chip from the LabChip GX/GXII Touch and place in container.
6. Make sure to cover all wells with Parafilm® and store at 4°C.

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Assay Specifications¹

Sizing Range	50 – 5000 bp
Sizing Resolution²	± 5% from 100 – 500 bp ± 10% from 50 – 100 bp, 500 – 1000 bp ± 15% from 1000 – 3000 bp ± 22% from 3000 – 5000 bp
Sizing Accuracy	± 10%
Sizing Precision	5% CV
Linear Concentration Range	<u>Standard Sample Workflow</u> 10 pg/μL – 500 pg/μL per fragment from 50 bp to 2000 bp 50 pg/μL – 500 pg/μL per fragment from 2000 bp to 5000 bp 100 pg/μL – 5 ng/μL for smears <u>Limited Sample Workflow (initial concentration)</u> 20 pg/μL – 500 pg/μL per fragment from 50 bp to 2000 bp 100 pg/μL – 500 pg/μL per fragment from 2000 bp to 5000 bp 200 pg/μL – 5 ng/μL for smears
Sensitivity	<u>Standard Workflow</u> 5 pg/μL per fragment 100 pg/μL for smears <u>Limited Sample Workflow (initial concentration)</u> 10 pg/μL per fragment 200 pg/μL for smears
Maximum Total DNA Concentration	5 ng/μL total, 500 pg/μL per fragment
Quantitation Accuracy	± 30%
Quantitation Precision	20% CV
Carry-Over	< 0.25%
Maximum Salt Concentration³	10 mM Tris, 1 mM EDTA
Analysis Time	68 seconds per sample (~2.5 hours for 96 samples)
Number of Samples per Chip Prep	96 samples
Chip Lifetime⁴	2000 samples per chip

¹ All specifications pertaining to DNA fragments were determined using ladder as sample in TE buffer. All specifications pertaining to DNA smears were determined using Covaris sheared control genomic DNA (human male) in TE buffer. Shearing time was 30s or 240s.

² Resolution is defined as half height or better separation of two peaks. Actual separation performance can depend on the sample and application. Peaks that are resolved less than half height can still be accurately identified by the system software.

³ Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.

⁴ Expected chip lifetime is based on use under normal laboratory conditions and adherence to Caliper preparation protocols, sample guidelines and storage conditions. Individual results may vary.

For complete DNA High Sensitivity Assay User Guide go to:

<http://www.perkinelmer.com/tools/technicallibrarylandingpage.asp>