

DNA High Sensitivity Assay Guide

LabChip® GX Touch/GXII Touch

Chip Preparation

1. Allow the chip and reagents to equilibrate to room temperature for at least 30 minutes before use. **The Dye Concentrate must be completely thawed and vortexed before use.** One vial of DNA HiSens/NGS3K 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/NGS3K Gel Matrix.
3. Vortex and transfer mixture into two spin filters (approximately **550 µL** per spin filter).
4. Centrifuge at **9200 rcf for 7.5 minutes at room temperature.**
5. 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 2-8°C.**
6. Each active well (1, 3, 4, 7, 8, and 10) should be rinsed and aspirated twice with water (Milli-Q® or equivalent).
7. 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.**
8. Add HiSens DNA Marker to chip well 4 as shown in **Figure 1. Low-throughput** or **Figure 2. High-throughput.**
9. 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 into the LabChip GX Touch/GXII Touch.)**

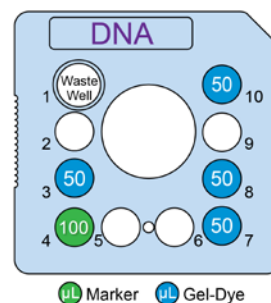


Figure 1. Low-throughput
(Up to 48 samples)

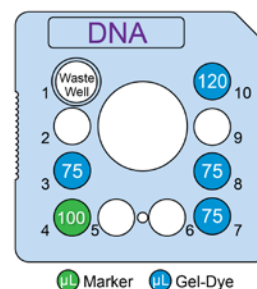
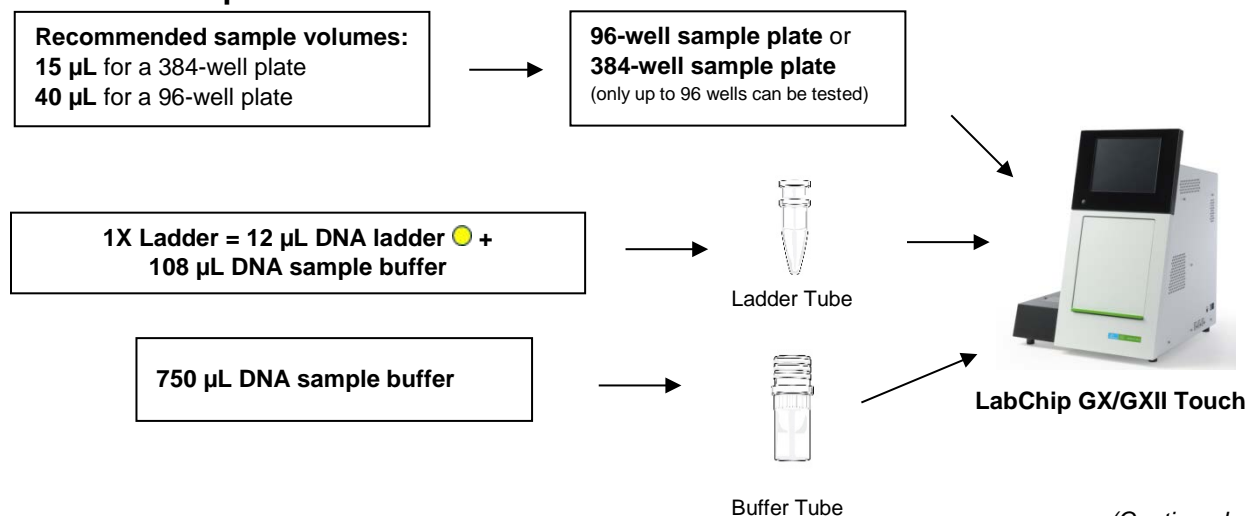


Figure 2. High-throughput
(Up to 96 samples)

DNA Sample, Ladder, and Buffer Preparation

Standard Sample Workflow



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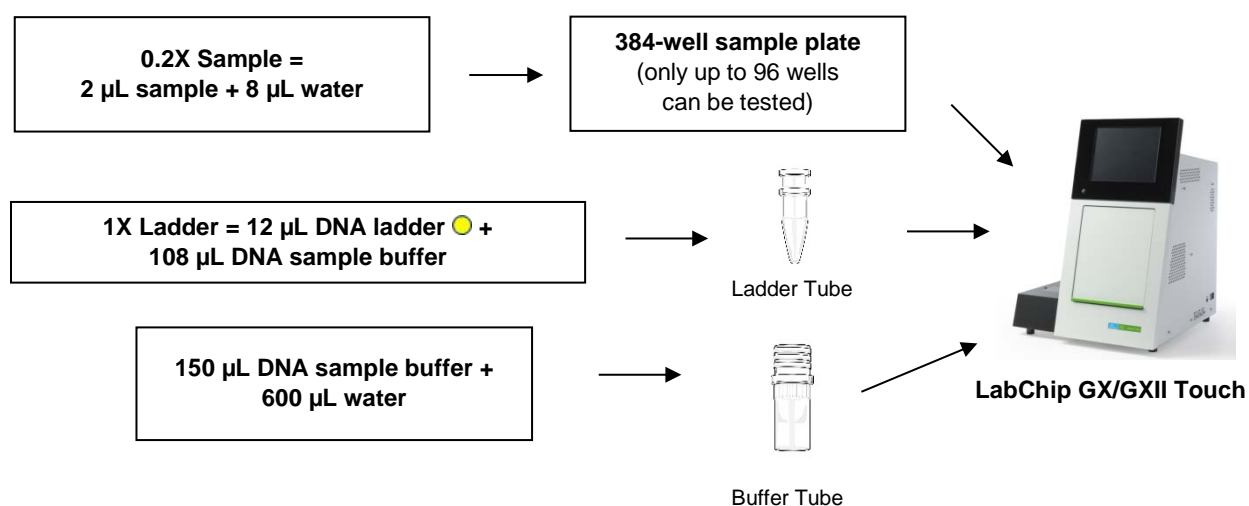
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Standard Sample Preparation Notes

- 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.

Limited Sample Workflow



Limited Sample Workflow

Dilute ladder with diluted DNA sample solution

Limited Sample Preparation Notes

- This workflow requires only 2 μ L of sample with a **minimum initial concentration of 20 μ g/ μ L per fragment or 200 μ g/ 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., the sample dilution ratio).**

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Chip Cleaning and Storage

After use, the chip must be cleaned and stored in the chip container.

1. Place the chip into the plastic storage container. The sipper should be submerged in the fluid reservoir.
2. Remove reagents from each chip well using vacuum.
3. Each active well (1, 3, 7, 8, and 10) should be rinsed and aspirated twice with water (Milli-Q® or equivalent).
4. Add **100 µL** of DNA Chip Storage Buffer (white cap ○) to the active wells.
5. Place the chip back on the LabChip GX/GXII Touch. Ensure that a Buffer Tube with **750 µL** of water (Milli-Q® or equivalent) is in the buffer slot.
6. Touch the **Wash** button.
7. Remove the chip from the instrument and place it into the plastic storage container.
8. Add an additional 50 µL of DNA Storage Butter to well 1.
9. Cover the wells with Parafilm® to prevent evaporation and store at 2-8°C until next use. If using the chip again within 24 hours it may be left at room temperature. Allowing chip wells to dry may lead to changes in chip performance.

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Assay Specifications¹

The DNA High Sensitivity 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.

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	Standard Sample Workflow 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 Limited Sample Workflow (initial concentration) 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	Standard Workflow 5 pg/μL per fragment 100 pg/μL for smears Limited Sample Workflow (initial concentration) 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
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

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.

For the complete DNA High Sensitivity Assay User Guide, go to: <http://www.perkinelmer.com>