

DNA NGS 3K Assay Quick Guide

LabChip® GX Touch/GXII Touch

Chip Preparation

WARNING: DNA NGS 3K Dye contains DMSO. Avoid contact with skin and eyes.

NOTES:

- The DNA NGS 3K assay requires one of the chip types below. Note that the HT chips are not compatible with GX Touch/GXII Touch 24 instruments.

Chip	Part Number
HT X-Mark LabChip (GX Touch/GXII Touch HT)	CLS144006
24 X-Mark LabChip (GX Touch/GXII Touch 24 or HT)	CLS145331

- Unlike other DNA assays, the DNA NGS 3K Marker is used as the sample diluent when preparing the sample plate and is NOT pipetted into chip well 4. For the X-Mark chip, well 4 is not an active well.
- For accurate concentration readings, thoroughly mix the sample and marker during the dilution step.
- The DNA NGS 3K Dye and the DNA NGS 3K Marker are sensitive to light. Avoid prolonged exposure to light during chip and sample preparation.

- Allow the chip and reagents to equilibrate to room temperature for at least 30 minutes before use. **The DNA NGS 3K Dye Concentrate must be completely thawed and vortexed for 10 – 15 seconds before use.** One vial of DNA Hi Sens/NGS3K Gel Matrix ● is enough for **4 Low-Throughput chip preparations (for up to 48 samples each) or 2 High-Throughput chip preparations (for up to 192 samples each).**
- Prepare Gel-Dye by adding 13 µL of DNA NGS 3K Dye Concentrate ● to 1 vial of DNA Hi Sens/NGS3K Gel Matrix ●.
- Vortex and transfer the Gel-Dye solution into **two spin filters** (approximately 550 µL per spin filter).
- Centrifuge at **9300 rcf for 7.5 minutes at room temperature.** Ensure all of the gel/dye passes 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.
- Rinse and completely aspirate each active well (1, 3, 7, 8, and 10) twice with water (Milli-Q® or equivalent).
- Use a reverse pipetting technique to add gel-dye to chip wells 3, 7, 8, and 10 as shown in **Figure 1. Low-throughput** or **Figure 2. High-throughput.**
- Clean both sides of the detection window with the supplied clean room cloth dampened with 70% isopropanol. **Note:** Ensure chip well 1 and chip well 4 are empty before placing the chip on the LabChip GX Touch.

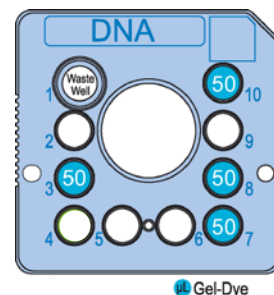


Figure 1. Low-throughput (Up to 48 samples)

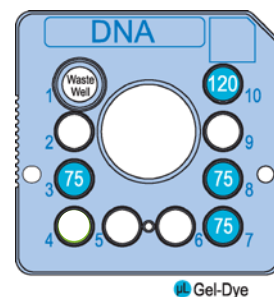


Figure 2. High-throughput (Up to 192 samples)

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DNA Sample, Ladder, and Buffer Preparation

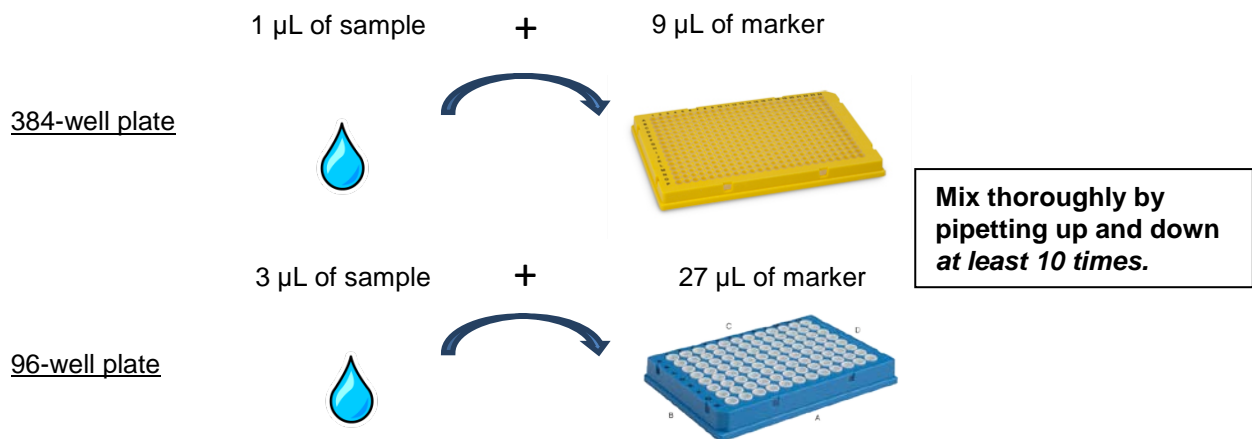
NOTE: Samples must be diluted with DNA NGS 3K Marker at 1:10 ratio. New: DNA NGS 3K reagent kits now include two vials of NGS 3K Marker Booster, which acts as an additive for the DNA NGS 3K Marker. It is recommended that NGS 3K Marker Booster be used for customers running extracted cell-free DNA samples on the X-Mark LabChip with the DNA NGS 3K reagent kit. NGS 3K Marker Booster may also be used to correct the loss of the Upper Marker in NGS library samples. (See Troubleshooting in the DNA NGS 3K Assay User Guide.)

Marker Preparation with NGS 3K Marker Booster (Optional)

It is recommended to mix the NGS 3K Marker Booster immediately prior to running the assay (with scaling to the required total volume of marker solution). If not using the optional NGS 3K Marker Booster, the DNA NGS 3K Marker is used undiluted as provided in the reagent kit.

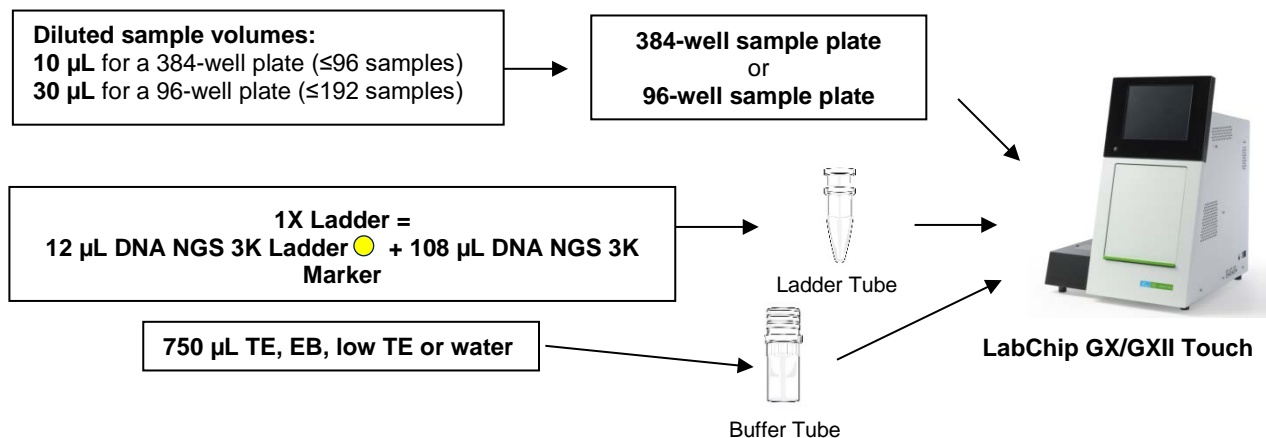
Add 1 mL of NGS 3K Marker Booster to each 27 mL of DNA NGS 3K Marker and mix well. Vortex well and protect from light. Continue with Sample Preparation below.

Sample Preparation



See the *DNA NGS 3K Assay User Guide* for additional mixing ratios.

Sample Workflow




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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 chip storage container. Verify the sipper is submerged in the fluid reservoir.
- 2 Remove the reagents from each chip well using vacuum.
- 3 Rinse and completely aspirate each active well (1, 3, 7, 8, and 10) 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 into the LabChip GX/GXII Touch.
- 6 Place a Buffer Tube with **750 µL** of water (Milli-Q® or equivalent) in the buffer slot.
- 7 Touch the **Wash** button on the Home screen.
- 8 Touch the **Wash** button on the Wash screen.
- 9 When the chip wash is complete, remove the chip from the instrument and place the chip into the chip storage container.
- 10 Add an additional **50 µL** of DNA Chip Storage Buffer to well 1.
- 11 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.

Assay Specifications

NOTES:

- 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.
- The DNA NGS 3K assay is for use with LabChip GX Touch/GX II Touch instruments. LabChip GX Touch/GX II Touch instruments are for research use only and not for use in diagnostic procedures.

Sizing Range	50 - 3000 bp
Sizing Resolution¹	± 10% from 200 - 1000 bp ± 15% from 50 - 200 bp, 1000 - 2000 bp ± 20% from 2000 - 3000 bp
Sizing Accuracy	± 10%
Sizing Precision	5% CV
Starting Sample Linear Concentration Range	50 - 5000 pg/µL for smears 5 - 500 pg/µL per fragment from 50 to 2000 bp 20 - 500 pg/µL per fragment from 2000 to 3000 bp
Linear Concentration Range (on plate after dilution with marker at 1:10 ratio)	5 - 500 pg/µL for smears 0.5 - 50 pg/µL per fragment from 50 to 2000 bp 2 - 50 pg/µL per fragment from 2000 to 3000 bp
Sensitivity	25 pg/µL for smears 2 pg/µL per fragment

¹ 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.

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Maximum Starting DNA Concentration	5000 pg/μL total 500 pg/μL per fragment
Quantitation Accuracy	± 30% for smear samples and fragment peaks > 2 pg/μL (concentration on plate after 1:10 dilution)
Quantitation Precision	20% CV
Carry-Over	< 0.5%
Maximum Salt Concentration²	10 mM Tris, 1 mM EDTA
Analysis Time	68 seconds per sample (~2.5 hours for 96 samples)
Samples per Chip Prep	Up to 48 samples per LT chip prep Up to 192 samples per HT chip prep
Chip Preps per Reagent Kit	10 HT chip preps or 20 LT chip preps
Chip Lifetime³	500 samples per chip (24 chip) 1000 samples per chip (HT chip)

Contact PerkinElmer



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LabChip Chip QC test data portal: <https://www.perkinelmer.com/tools/LabChipQCSearch>

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

For the complete DNA NGS 3K Assay User Guide, go to: <http://www.perkinelmer.com/>

² Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.

³ Expected chip lifetime is based on use under normal laboratory conditions and adherence to PerkinElmer chip preparation protocols, recommended sample composition, instrument maintenance procedures, and recommended chip and reagent storage. Individual results may vary.