Chip Preparation

WARNING: cfDNA Dye contains DMSO. Avoid contact with skin and eyes.

NOTES:

• The cfDNA assay requires one of the chip types below. Note that the HT chips are not compatible with GXII Touch 24 instruments.

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

- Unlike other DNA assays, the cfDNA 1X Marker Solution 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 cfDNA Dye and cfDNA Marker are sensitive to light. Avoid prolonged exposure to light during chip and sample preparation.
- 1 Allow the chip and reagents to equilibrate to room temperature for at least 30 minutes before use. The cfDNA Dye Concentrate must be completely thawed and vortexed for 10 15 seconds before use. One vial of cfDNA 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 96 samples each).
- Prepare Gel-Dye by adding 13 µL of cfDNA Dye Concentrate to 1 vial of cfDNA Gel Matrix ●.
- **3** Vortex and transfer the Gel-Dye solution into **two spin filters** (approximately **550 μL** per spin filter).
- 4 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.
- **5** Rinse and completely aspirate each active well (1, 3, 7, 8, and 10) twice with water (Milli-Q[®] or equivalent).
- 6 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**.
- 7 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



cfDNA Assay Quick Guide LabChip[®] GX Touch/GXII Touch

cfDNA 1X Marker Solution Preparation

Prepare fresh cfDNA 1X Marker Solution just before running the assay for best results. To prepare enough cfDNA 1X Marker Solution for 96 samples:

- 1 Prepare cfDNA 1X Marker by adding 0.3 mL of cfDNA 10X Marker to 2.7 mL of water. Mix thoroughly.
- **2** Add 60 μL of cfDNA Marker Booster **•** to 3 mL of cfDNA 1X Marker prepared in step 1.
- **3** Vortex again briefly and protect from light.

To prepare other volumes of cfDNA 1X Marker Solution, scale the volumes of cfDNA 10X Marker, water, and cfDNA Marker Booster accordingly.

cfDNA Sample, Ladder, and Buffer Preparation

NOTE: Samples must be diluted with cfDNA 1X Marker Solution at 1:10 dilution.

Sample Preparation



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** μ L of DNA Chip Storage Buffer (white cap \bigcirc) 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

NOTE: The cfDNA 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 - 7000 bp
Sizing Resolution ¹	50 - 800 bp
Sizing Accuracy	± 10% for mono-nucleosomal cfDNA
	± 15% for di- and tri-nucleosomal cfDNA
Sizing Precision	5% CV
Starting Sample Linear Concentration Range	50 - 1000 pg/μL
Linear Concentration Range (on plate after	5 - 100 pg/µL
dilution with marker at 1:10 dilution)	
Sample Volume	1 μL for 384-well plates
	3 μL for 96-well plates
Sensitivity	50 pg/µL
Quantitation Accuracy ²	± 20%
Quantitation Precision	15% CV
Carry-Over	< 0.5%
Analysis Time	40 seconds per sample (~1.5 hours for 96 samples)
Samples per Chip Prep	Up to 48 samples per LT chip prep
	Up to 96 samples per HT chip prep
Chip Preps per Reagent Kit	10 HT chip preps or 20 LT chip preps
Chip Lifetime ³	500 samples per chip (24 chip)
	1000 samples per chip (HT chip)

¹ Resolution is defined as baseline separation between mono-nucleosomal and di-nucleosomal smears. 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.

 2 ±20% for concentration over 75pg/uL, ±40% for concentration 50-75 pg/uL.

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

cfDNA Assay Quick Guide LabChip[®] GX Touch/GXII Touch

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LabChip Chip QC test data portal: <u>https://www.revvity.com/tools/LabChipQCSearch</u> LabChip Reagent CoA: <u>https://www.revvity.com/tools/COASearch</u> For the complete cfDNA Assay User Guide, go to: <u>https://www.revvity.com/</u>

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