Fluorescence Fragment Analysis (FFA120) Assay User Guide

For LabChip GX Touch/GXII Touch

CE

P/N CLS158447 Rev. B
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Specifications

Assay Specifications

The Fluorescence Fragment Analysis (FFA120) Assay is for use with the LabChip GX Touch™/GXII Touch™ instruments. The LabChip GX Touch/GXII Touch instruments are for Research Use Only. Not for Use in Diagnostic Procedures.

Table 1. Assay Specifications

Size Range[a]	20 - 120 nucleotides
Concentration Range[b]	4 nM – 2.5 µM Cy5-labeled DNA/RNA fragment
Sensitivity[b]	4 nM Cy5-labeled DNA/RNA fragment
Sizing Reproducibility	CV[c] < 5.0%
Sample Volume[d]	2 µL
Maximum Salt Concentration in Sample[e]	20 mM Tris, 100mM KCl
Run Time	60 seconds per well (~2.5 hours for 96 wells)
Compatible Plate Types	96-well[f]
Samples per Chip Prep	Up to 48 samples per HT[g] chip prep, up to 24 samples per LT[g] chip prep
Chip Preps per Reagent Kit	5 HT chip preps or 10 LT chip preps

[a] The upper marker is 120nt.

[b] Sample concentration before DMSO dilution. Dilute samples with nuclease-free H_2O first if sample concentration is above the maximum concentration.

[c] CV: Coefficient of Variation.

[d] Sample Volume:

Mix 2µL sample with 18µL DMSO for 96-well plate sample preparation.
If sample volume is plenty, mix 3µL sample with 27µL DMSO.
If sample volume is limited, mix 1.5µL sample with 13.5µL DMSO.
The on-plate sample volume should be ≥ 15µL to ensure successful sipping.

[e] Evaluated at the oligo template concentration close to detection sensitivity limitation level. The salt concentration tolerance can be different for oligo templates at a concentration higher than the sensitivity limitation level.

[f] See “Additional Items Required” for the recommended 96-well plates.

[g] HT: High throughput; LT: Low throughput.

Sample Conditions and Design Recommendations

To avoid run-to-run size-calling variation with small-sized fragments, Revvity recommends including appropriate run controls (fluorescently labeled oligos) in the same or similar buffer in each run.

Migration time of oligos smaller than 20nt is more impacted by dye, other modifications and buffers, and the size calling might be different from real oligo length (nt).

Table 2. Sample Conditions

Additives	Revvity recommends that BSA and detergents exceeding 0.05 mg/mL and 0.01% (v/v) respectively in concentration, not be used. Higher concentrations can result in chip failure. **Sample purification is strongly suggested to remove BSA or recombinant albumin from the sample before running it on the LabChip.** Refer to "Additional Items Required" for detailed recommendations. In addition, non-aqueous solvents are not compatible with LabChip protocols.
Particulates	Spin down all sample plates prior to analysis. Filter all buffers with a 0.22 µm cellulose acetate filter.
Salt Concentration	Total salt concentration in samples must not exceed 20 mM Tris, 100mM KCl. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity (peak height). However, samples can be run with more salt concentration if there are sufficient oligo templates in the samples. The shelf-life of the chip might be impacted.
Add EDTA to Improve Denaturing Efficiency	For double-stranded DNA/RNA samples in a buffer containing divalent ions such as Mg^{2+}, equal or high amount of EDTA (1:1 molar ratio of EDTA and Mg^{2+}) is recommended to be added to the sample before mixing with DMSO to ensure denaturing efficiency.

Reagent Kit Contents

Fluorescence Fragment Analysis (FFA120) Kit (P/N CLS158081) contains the reagents and consumables listed in the tables below.

Note: *Use only consumables that are within their expiration date.*

Storage: When not in use, store reagents at the temperatures specified in Table 3.

Table 3. Reagents

Reagent	Vial	Quantity	Storage Temperature
FFA Chip Storage Buffer	White ○	5 vials, 1.8 mL each	2-8°C
FFA Gel Matrix	Red 🔴	6 vials, 0.510 mL each	2-8°C
FFA Marker	Green 🟢	1 vial, 0.8 mL	2-8°C
FFA120 Ladder[1,2]	Yellow 🟡	1 vial, 0.12 mL	-15°C to -25°C
Sipper Wash Solution[3]		1 vial, 10mL	2-8°C

Table 4. Consumable Items

Item	Supplier and Catalog Number	Quantity
Spin Filters	Costar®, Cat. # 8160	10
Detection Window Cleaning Cloth	VWR®, Cat. # 21912-046	1
Swabs	ITW Texwipe®, Cat. # TX758B	3
Centrifuge Tubes, 2.0 mL	(Not sold separately)	10
Ladder Tubes, 0.2 mL	(Not sold separately)	20
Buffer Tubes, 0.75 mL	(Not sold separately)	20

[1] Ladder is Cy5-labeled. Sample size measurement might vary slightly due to DNA/RNA with different GC content, especially if DNA/RNA is labeled by fluorophores other than Cy5.

[2] FFA120 Ladder (P/N CLS158127) can be ordered separately.

[3] Sipper Wash Solution (P/N CLS158129) is not included in the kit, is ordered separately.

Table 5. Recommended oligonucleotide labeling dyes for end users on LabChip GX Touch Nucleic Acid Analyzer

Dye	Excitation (nm)	Emission (nm)	Label Site	Recommended Sample Concentration[a]
ATTO 633	635	653	5', Int, 3'	5 nM to 150 nM
Alexa 647	650	670	5', Int, 3'	10 nM to 200 nM
LC640	620	635	5', Int, 3'	10 nM to 200 nM
Cy5	648	668	5', Int, 3'	20 nM to 400 nM

[a] Before dilution with DMSO

DNA 5K/RNA/CZE LabChips

Storage: When not in use, store chips at 2-8°C. If using a prepared chip again within 24 hours, the chip can be stored at room temperature.

Table 6. DNA 5K/RNA/CZE LabChips

Item	Part Number	Samples per Chip
DNA 5K/RNA/CZE HT Chip (GX Touch/GXII Touch HT)	760435	2000[a]
DNA 5K/RNA/CZE 24 Chip (GX Touch/GXII Touch HT or 24)	CLS138949	750

[a] Based on the samples prepared in TE buffer only. It is subject to be different with different sample buffers.

Assay File

The fluorescence fragment analysis assay file "FFA120.asyx" is provided by one of the following approaches:

- Contact Customer Technical support "**BioprocessQC@revvity.com** with adding "FFA-" at the beginning of the subject line.
- Reach out to Field Application Scientist for the assay file and assay installation.
- Download the assay file directly from Revvity Website (https://www.Revvity.com) following the Readme file for installation instructions.

Safety and Usage

Safety Warnings and Precautions

WARNING!

We recommend that this product and components be handled only by those who have been trained in laboratory techniques and that products are used in accordance with the principles of good laboratory practice. All chemicals should be considered potentially hazardous. When handling chemical reagents, wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

Usage

The Assay is for use with LabChip GX Touch /GXII Touch instruments. LabChip GX Touch /GXII Touch instruments are for research use only. Not for use in diagnostic procedures.

Preparation Procedures

Additional Items Required

- 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent)
- 70% isopropanol solution in DI water
- Microseal 'B' Adhesive Sealing Film
- Revvity Hard-Shell thin-wall 96-well skirted PCR plate (blue), cat# 6008870 (recommended) or Bio-Rad Hard-Shell 96-well skirted PCR plate, cat# HSP-9601, cat# HSP-9631
- DMSO (Molecular Biology Grade)
- Nuclease-free water
- Sample purification recommendations (for the samples with high concentration of salts, BSA, or detergent): ZYMO RESEARCH ZR-96 Oligo Clean &Concentrator, cat# D4062 or equivalent.

Preparing the Ladder Aliquots During First Time Use

Note: *Avoid multiple freeze-thaws of the FFA120 Ladder.*

1 Thaw the FFA120 Ladder ○ on ice or at 4ºC.

2 Vortex the thawed FFA120 Ladder for 10 - 15 seconds to mix and then spin down for a few seconds before each use.

3 Aliquot 65 µL into a nuclease-free tube, up to 5 cycles of freeze-thaw.

4 Store the FFA120 Ladder ○ aliquots at -20ºC until the expiration date.

Preparing the Instrument Before the First Run

1 Inspect the inside of the chip cartridge and O-rings for debris.

2 Use the provided lint-free swab dampened with water (Milli-Q® or equivalent) to clean the O-rings using a circular motion.

3 Clean the electrodes with the provided lint-free swab dampened with water (Milli-Q® or equivalent).

4 Run the Purge Pressure Line protocol.
If the O-rings stick to the chip or a pressure leak is detected, perform the more extensive monthly cleaning procedure.

Preparing the Gel Solution

Note: *The prepared 510 µL of Gel matrix is enough for one HT (High-Throughput) or two LT (Low-Throughput) chip preps.*

1. Allow the chip and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use.
2. Transfer 510 µL of Gel matrix (red cap) to a spin filter. Use a centrifuge tube filled with 510 µL of water to balance the centrifuge.
3. Centrifuge at 9300 rcf for 10 minutes at room temperature.
4. Discard the filter.
5. Label and date the tube. Store at 2-8°C.The filtered Gel matrix (stored at 2-8°C) is stable until it reaches Gel matrix's expiration date.

Preparing the Chip

1. Allow the chip to equilibrate to room temperature for at least 30 minutes before use.

2. Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells (Figure 1). For details on how to set up a vacuum line, see section "Chip Well Aspiration Using a Vacuum".

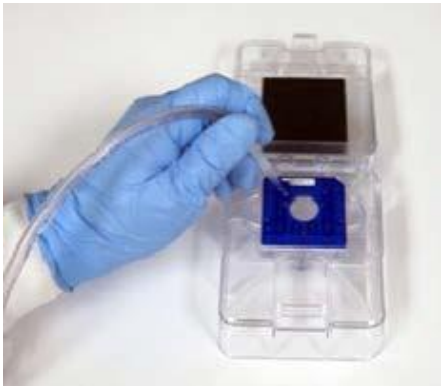

Figure 1. Using a vacuum to aspirate the chip wells is more effective than using a pipette

3. Rinse and completely aspirate each active chip well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.

4. If any water spills onto the top or bottom of the chip surfaces during rinsing, aspirate using the vacuum line. DO NOT run the tip over the detection window. Use the provided Detection Window Cleaning Cloth dampened with water (Milli-Q® or equivalent) or alcohol to clean the detection window as needed.

5. Using a reverse pipetting technique to avoid introducing any bubbles into the wells, add **Gel Matrix** to wells 3, 7, 8, and 10 as shown in Figure 2 (Low-throughput) or Figure 3 (High-throughput).

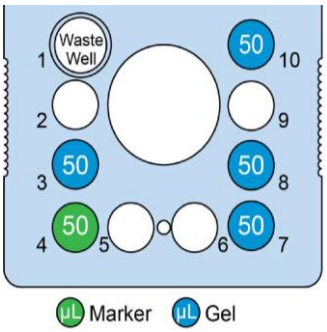

Figure 2. Low-Throughput Chip Preparation

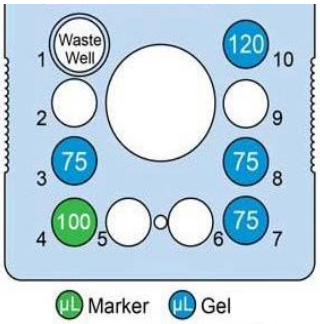

Figure 3. High-Throughput Chip Preparation

6 Add **50 µL** (Low-throughput) or **100 µL** (High-throughput) of **Marker** (green cap ●) to **chip well 4** as shown in Figure 2 or Figure 3 with reverse pipetting technique.

Note: *The marker well may need to be replenished if the chip is in idle mode on the instrument for an extended period of time.*

Table 7. Chip Preparation Volumes

Number of Wells*	Marker (µL)	Gel (µL)		Ladder Tube (µL)	Buffer Tube (µL)
		Chip wells 3, 7, 8	Chip Well 10		
24 (Limited Throughput)	25	25	25	100	750
48 (Low Throughput)	50	50	50	100	750
72	80	75	75	100	750
96 (High Throughput)	100	75	120	120	750

* Number of Wells includes Sample wells and Sipper Wash Solution wells.

If running 24 or 72 wells, use the volumes in Table 7 to prepare the chip. Make sure to wash the chip as soon as the run is complete to avoid any risk of evaporation. When running less than 24 wells over an eight-hour period, use the high-throughput (Figure 3) chip preparation volumes due to evaporation.

7 Make sure the rims of the chip wells are clean and dry.

8 **IMPORTANT:** Ensure **chip well 1** (waste well) is empty before placing the chip into the instrument.

Note: *Use the low-throughput protocol when running the LabChip GX Touch/GXII Touch 24 instrument.*

Use the low-throughput protocol when running the LabChip GX Touch/GXII Touch instrument with a 96-well plate with less than 48 wells selected (including sipper wash wells).

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Preparing the Sample Plate, Ladder Tube, and Buffer Tube

Notes: *To minimize well-to-well contamination, samples should be prepared in a 96-welll plate between Water or Sipper Wash Solution in the pattern shown in* Figure 4*.*

The total salt concentration of samples (before mixing with DMSO) must not exceed 20mM Tris, 100mM KCl. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity. To get good assay performance (e.g. sensitivity), sample plate should be ***freshly prepared*** *for each run.*

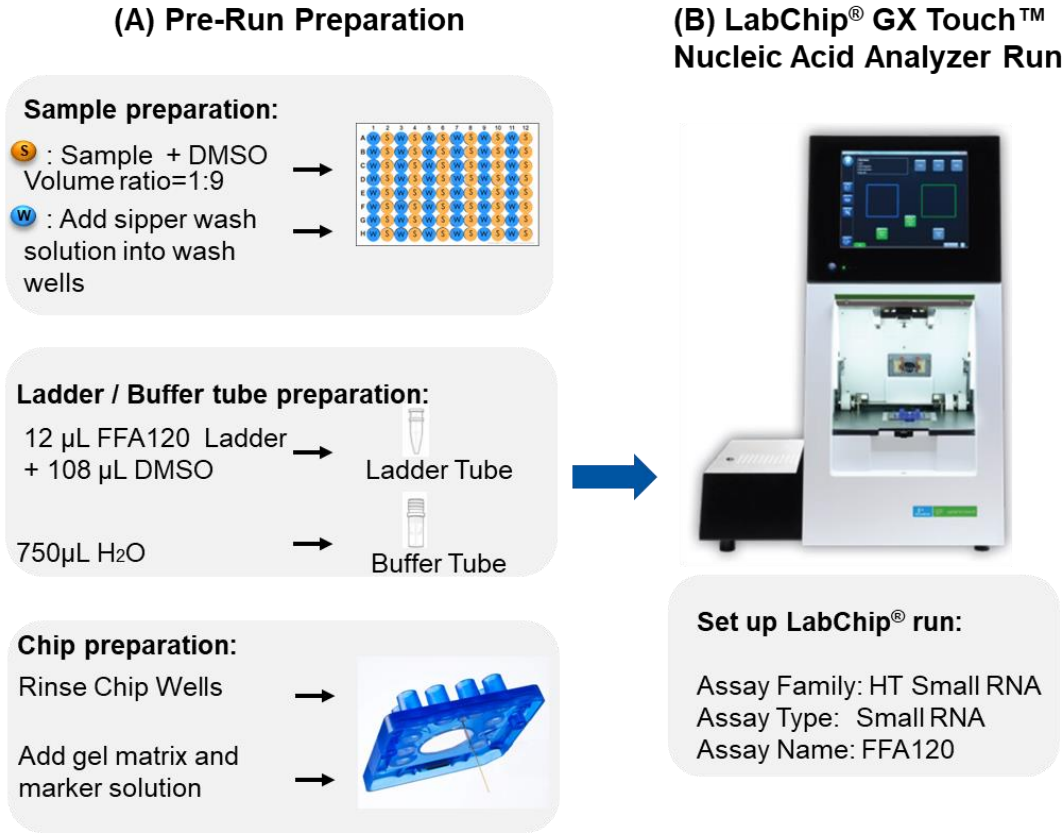

Figure 4. Sample plate, Ladder Tube, and Buffer Tube Preparation

1 Prepare sample plate:

a Determine sample run by row or by column. Every other row or column will contain samples.

b Add **27 µL DMSO** to each sample well.

c Add **3 µL sample** to each sample well containing 27 µL DMSO. Refer to Table 2. Sample Conditions for recommendations on sample preparation for samples with high salt, protein, or additives.

d Add **30 µL Sipper Wash Solution** or nuclease-free water to the rows or

columns between the sample wells.

e After all samples are plated, seal the plate, vortex the plate for 10 seconds, then spin the plate at 350xg for 5 minutes.

f Carefully remove the seal from the plate, ensuring there are no air bubbles at the bottom of the sample/wash wells.

g Place the sample plate onto the LabChip GX Touch /GXII Touch instrument.

2 Prepare the Ladder Tube

a Before adding **FFA120 Ladder** (yellow cap) to the Ladder Tube, vortex and spin down the **FFA120 Ladder**.

b Add **108 µL DMSO** to the provided Ladder Tube.

c Add **12 µL FFA120 Ladder** (yellow cap) to the Ladder Tube.

d Mix thoroughly by pipetting up and down. Ensure there are no air bubbles in the Ladder Tube.

e Insert the Ladder Tube into the LabChip GX Touch /GXII Touch instrument as shown below (Figure 5).

3 Prepare the Buffer Tube

a Add **750 µL nuclease-free water** to the Buffer Tube provided with the reagent kit. Ensure there are no air bubbles in the Buffer Tube.

b Insert the Buffer Tube into the buffer slot on the LabChip GX Touch /GXII Touch instrument.

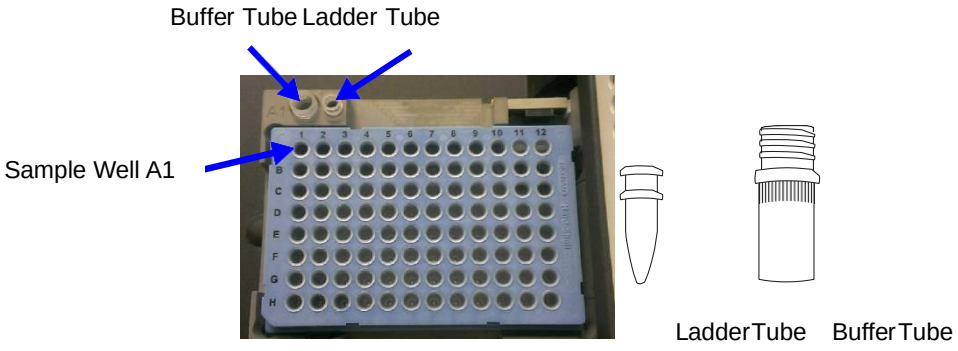

Figure 5. Locations of the Buffer Tube and Ladder Tube in the LabChip GX Touch /GXII Touch instrument

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Inserting a Chip into the LabChip GX Touch/GXII Touch Instrument

1 Remove the chip from the chip storage container and inspect the detection window. Clean BOTH sides of the detection window with the Revvity-supplied Detection Window Cleaning Cloth dampened with a 70% isopropanol solution in DI water.

2 Touch the **Unload Chip** button on the Home screen (Figure 6).

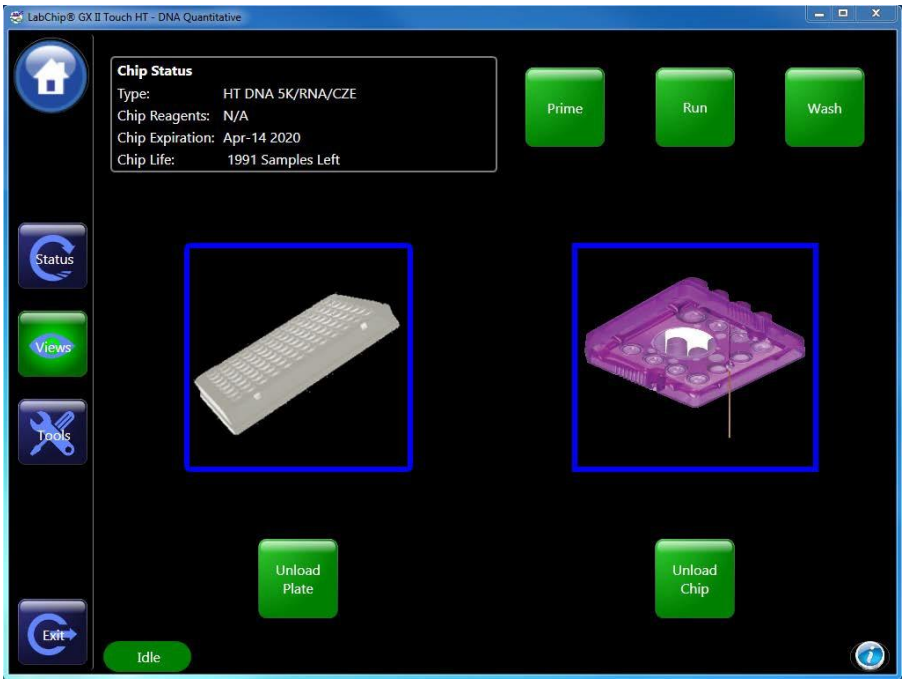


Figure 6. Home Screen

3 Insert the chip into the LabChip GX Touch /GXII Touch instrument (Figure 7) and close the chip door securely.

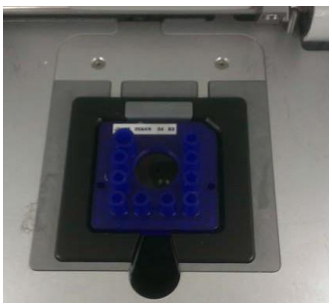


Figure 7. Chip in the LabChip GX Touch /GXII Touch instrument

4 Touch the **Load Plate** button on the Home screen (Figure 6) to retract the sample plate. The Assay Choice screen opens (Figure 8).

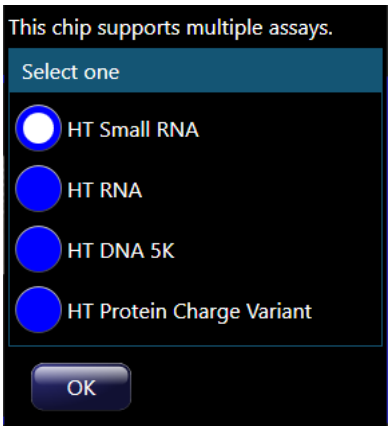


Figure 8. Assay Choice Screen

5 Select **HT Small RNA** and then touch **OK**.

Notes: *If performing multiple chip preps in one day, wash the chip in between chip preparations using the instrument and Chip Storage Buffer as described in "*Washing and Repriming Chips*".*

Be sure to periodically clean the O-rings on the top plate of the chip interface on the LabChip GX Touch /GXII Touch. Use the provided lint-free swab dampened with water to clean the O-rings using a circular motion. Allow the O-rings to dry before inserting a chip.

Operating Procedures

Running the Assay

Note: *The chip can be primed independently from running assays. Touch the Prime button on the Home screen (Figure 6). Select the desired assay from the Assay drop-down list (Figure 11). Make sure the Buffer Tube is in the instrument. Touch the Prime button on the Chip Priming screen (Figure 9).*

Figure 9. Chip Priming Screen

To run an assay:

1 Touch the **Run** button on the Home screen (Figure 6), the Select Wells Tab opens (Figure 10).

2 Select the wells based on the experiment design by the following methods (the selected wells are highlighted in blue):

- select a whole row by clicking the row letter on the left, or
- select a whole column by clicking the column number on the top, or
- select an individual well by clicking the well.

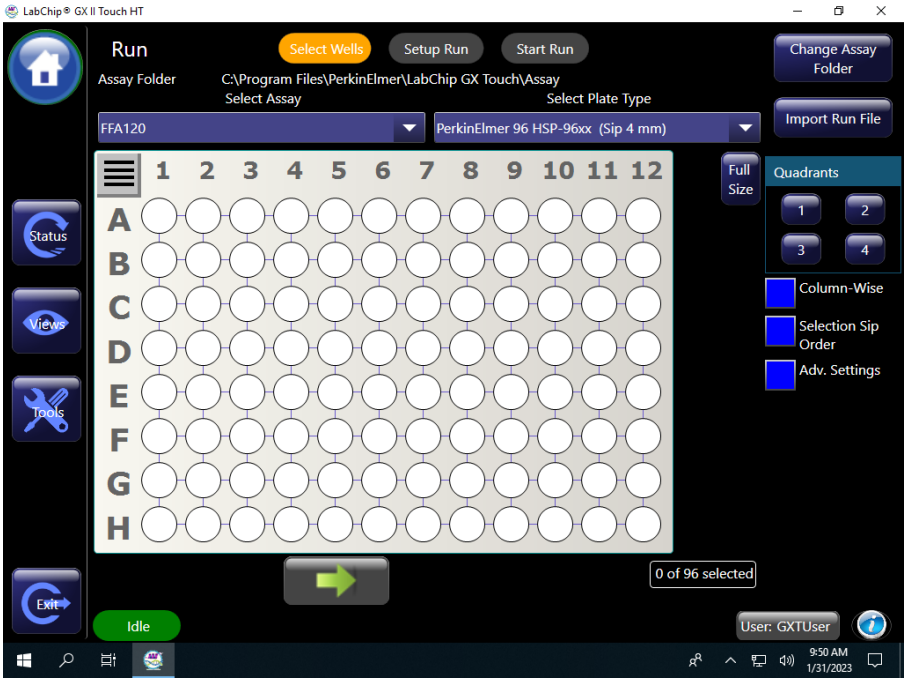


Figure 10. Select Wells Tab

3 Select the **FFA120** assay in the Assay Type list (Figure 11).

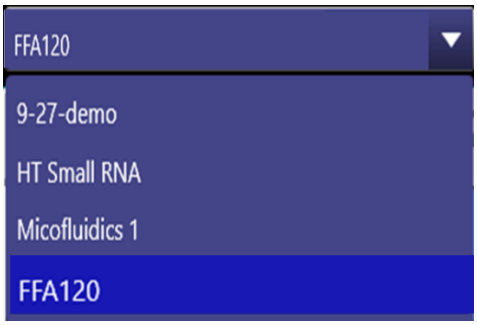


Figure 11. Assay Type Drop-Down List

4 Select the plate type, well pattern, and sip order (column or row).

The default **Plate Type** is Revvity 96 HSP-96xx (Sip 4mm). If only 15µL of sample/DMSO mixture is in the plate well, Revvity 96 HSP-96xx (Sip 2mm) is recommended.

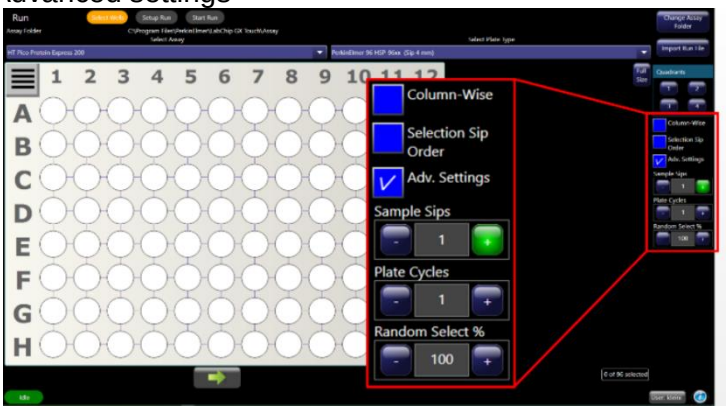
The default **Well Pattern** is by row; if sample wells and wash wells are prepared by column, click **Column-Wise** to run by column. If desired, touch **Adv. Settings** to specify the repeat settings, either using sample sips or plate cycles (Figure 12A shows the default settings to sip each well once by row).

- **Sample sips**: All samples are sipped sequentially first and then are repeated according to the setting; All sample sip data is saved in one plate run file. For example, if there is one row of samples (A1-A12) and sample sips = 3, the results are saved as A1-A12 (first round of sipper result), B1-B12 (second round of sipper result), C1-C12 (third round of

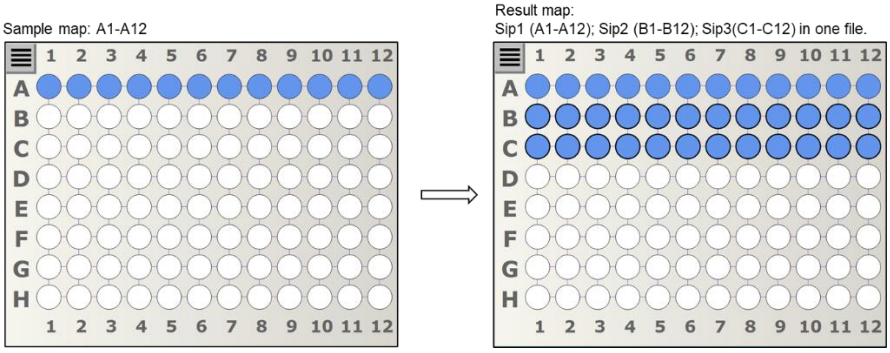
sipper result) in one result file (Figure 12B).

- **Plate Cycles**: All samples are sipped sequentially first and then are repeated according to the setting; Each plate cycle repeat data is saved as an individual plate run file. For example, if with one row of samples (A1-A12), plate cycles = 3, it will save results as A1-A12 (first round of sipper result), A1-A12 (second round of sipper result), A1-A12 (third round of sipper result) in three separate result files (Figure 12C).

A. Advanced settings



B. Sample sips



C. Plate cycles

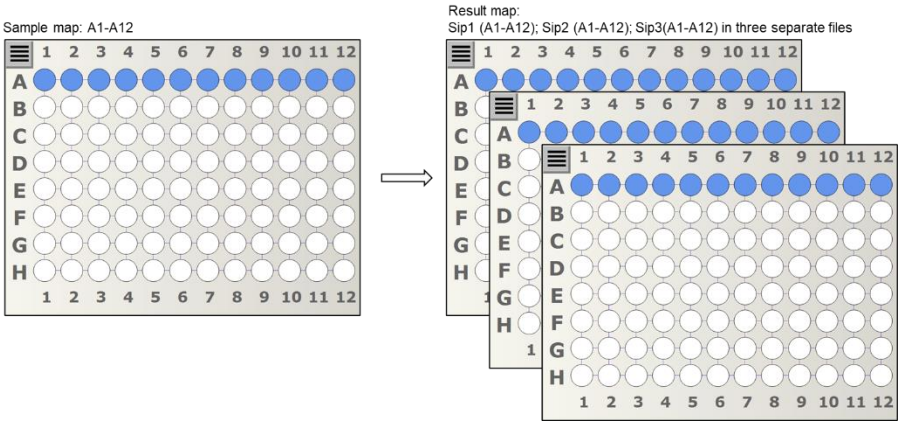


Figure 12. Advanced Settings

5 Select the sample wells. Sample wells selected should include both sample wells and wash wells.

6 Touch the **Green Arrow** button (Figure 10). The Setup Run tab (Figure 13) opens.

Figure 13. Setup Run Tab

7 Specify the operator's name, the option to read the barcode, the destination of the file, the use of sample names, expected peaks, excluded peaks, the filename convention, and the auto export settings.

8 Touch the **Green Arrow** button. The Start Run tab (Figure 14) opens.

Figure 14. Start Run Tab

9 Touch **Start** to begin the run. If the chip has not been primed since the last time the chip door was opened, the chip is primed automatically at the start of the run.

Repriming Chips

If air bubbles or clogs in the chip channels are suspected, the chip can be reprimed to help remove air bubbles or clogs.

Note: *Place a Buffer tube with prepared nuclease-free water into the instrument while priming chips.*

1. Touch the **Unload Chip** button on the Home screen to open the instrument door. The software automatically resets to require priming prior to running the chip again.
2. Place the chip and Buffer tube into the instrument.
3. Close the chip door securely and choose the corresponding assay.
4. Touch the **Prime** button on the Home screen. The Prime screen opens.
5. Touch the **Prime** button on the Prime screen to reprime the chip.

Washing and Repriming Chips

Washing the chip clears all reagents from the chip channels. The chip can be immediately reprimed to help remove air bubbles, clogs, particulates, or residues.

1. Touch the **Unload Chip** button on the Home screen to open the instrument door.
2. Remove the chip from the instrument. Place the chip in the chip storage container, ensuring the sipper is submerged in fluid.
3. Thoroughly aspirate all fluid from the chip wells using a vacuum.
4. Rinse and completely aspirate each active well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
5. Add **120 µL** of **Chip Storage Buffer** to each active well (1, 3, 4, 7, 8, and 10).
6. Place the chip in the LabChip GX Touch/GXII Touch instrument.
7. Place a Buffer Tube with **750 µL** of nuclease-free water in the instrument.
8. Close the chip door securely.
9. Touch the **Wash** button on the Home screen. The Wash screen (Figure 15) opens if the plate holder is extended. Otherwise, the wash starts automatically.

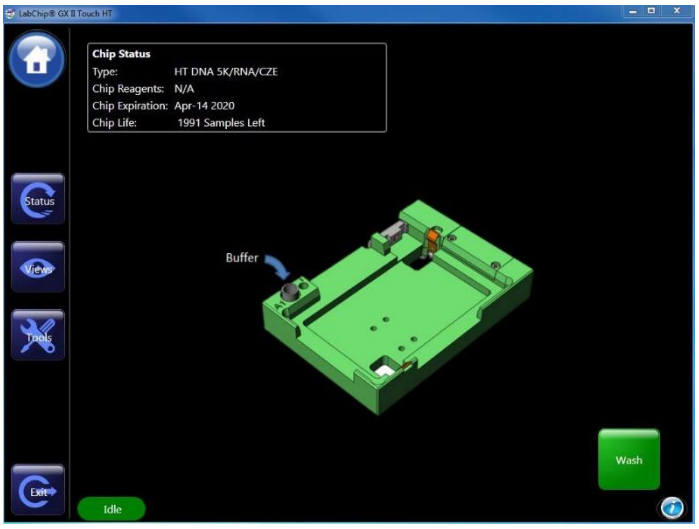


Figure 15. Wash Screen

10 Touch the **Wash** button on the Wash screen to start the chip wash.

11 After the completion of the wash cycle, touch the **Unload Chip** button on the Home screen to open the instrument door.

12 Return the chip to the chip storage container. Verify the sipper is submerged in fluid.

13 Thoroughly aspirate all fluid from the chip wells using a vacuum.

14 Prepare the chip as described in "Preparing the Chip".

15 Place the chip into the LabChip GX Touch /GXII Touch instrument.

16 Close the chip door securely.

17 Touch the **Run** or **Prime** button on the Home screen.

18 If air bubbles are not dislodged after a reprime: Fill all active wells with 100 µL of FFA Chip Storage Buffer, then suction the sipper with a vacuum line as shown in Figure 16 until droplets of fluid flow out from the sipper. When suctioning the sipper, be careful not to bend or break the sipper. To facilitate this, cut the end of the pipette tip attached to the vacuum line to widen the mouth.

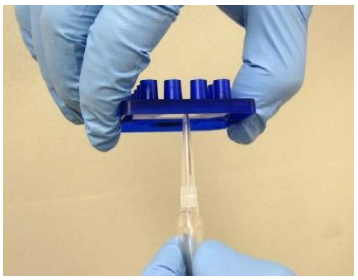


Figure 16. Removing an air bubble or clog by suctioning the sipper with a vacuum

Cleaning and Storing the Chip

After use, the chip must be cleaned and stored in the chip container. The chip can be washed the following morning when running overnight.

1 Place the chip into the chip storage container. Verify the sipper is submerged in the fluid reservoir.

2 Remove the reagents from each well of the chip using vacuum.

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

4 Add **120 µL Chip Storage Buffer** (white cap ◯) to the active wells.

5 Place the chip in the LabChip GX Touch /GXII Touch instrument. Ensure a buffer tube with **750 µL** water is in the Buffer slot.

6 Touch the **Wash** button on the Home screen. The Wash screen (Figure 17) opens.

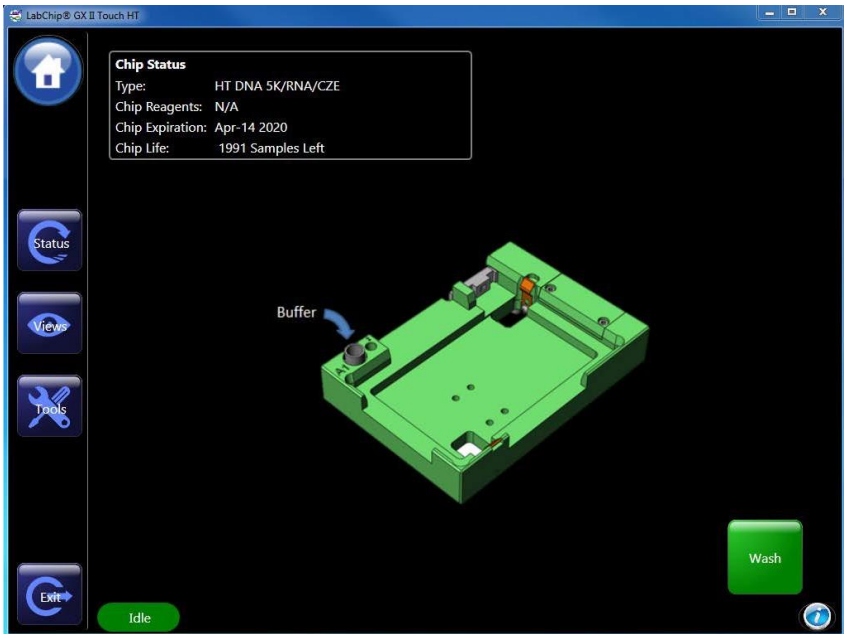


Figure 17. Wash Screen

7 Touch the **Wash** button on the Wash screen to start the chip wash.

8 When the chip wash is complete, touch the **Unload Chip** button, remove the chip from the instrument and place the chip in the chip storage container.

9 Add an additional **50 µL** of **Chip Storage Buffer** to well 1.

10 Cover the wells with Parafilm® to prevent evaporation and store at 2-8°C. Storing a chip with dry wells may clog the chip. If using the chip again within 24 hours, the chip can be stored at room temperature.

Chip Cartridge Cleaning

Daily

1. Before the first run and loading of the day, run the Purge Pressure Line assay.
2. Inspect the inside of the chip cartridge and O-rings for debris.
3. Use the provided lint-free swab dampened with water (Milli-Q® or equivalent) to clean the O-rings using a circular motion. If the O-rings stick to the chip or a pressure leak is detected, perform the more extensive monthly cleaning procedure.
4. Clean the electrodes with the provided lint-free swab dampened with water (Milli-Q® or equivalent).

Monthly

To reduce pressure leaks at the chip interface, clean the O-rings frequently.

Soak O-rings in water (Milli-Q® or equivalent) for a few minutes. Clean the O-ring faces by rubbing between two fingers. Wear gloves.

1. Remove the O-rings from the top plate of the chip interface on the LabChip GX Touch/GXII Touch instrument.
2. Soak O-rings in water (Milli-Q® or equivalent) for a few minutes.
3. Clean the O-ring faces by rubbing between two fingers. Wear gloves.
4. To reduce the occurrence of current leaks, clean the chip interface frequently. Clean the top plate of the chip interface using the provided lint free swab dampened with water (Milli-Q® or equivalent).
5. Allow the O-rings and chip interface to air dry. Reinsert the O- rings into the chip cartridge.

Data Analysis

LabChip GX Reviewer Software: Free download from (https://www.Revvity.com)

Data analysis: Refer to data analysis guide for details.

For the relative peak percentage quantification analysis, use either aligned area or %purity as the data points.

Results

FFA120 Assay Ladder Result

The electropherogram of a typical FFA120 ladder using the Standard Sample Workflow is shown in Figure 18. Following the lower marker, the ladder fragments in order of increasing migration time correspond to 20, 40, 60, 80 and 120 nt.

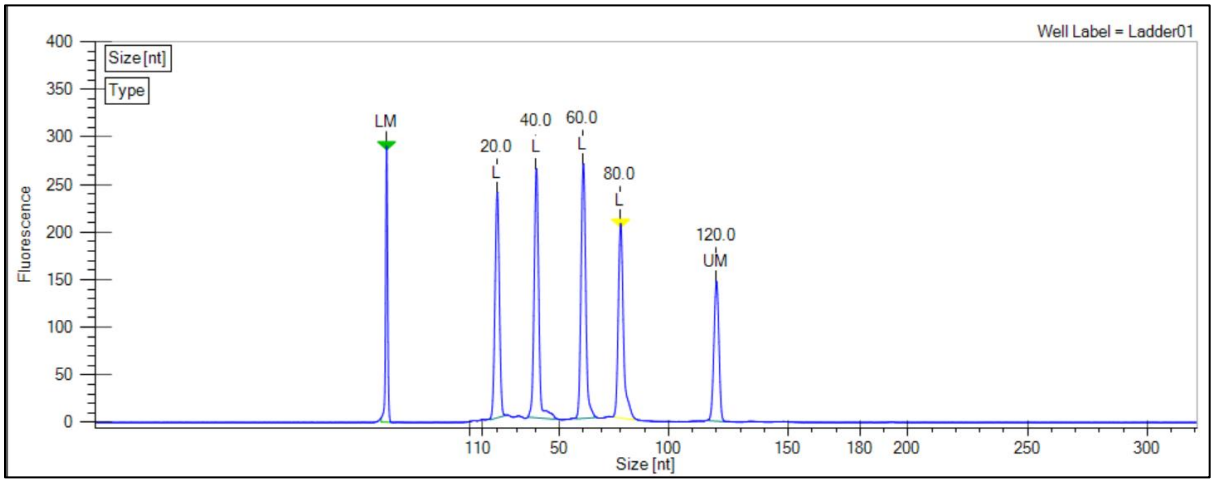


Figure 18. Typical FFA120 Ladder Electropherogram

Sample Result

Figure 19 shows the electropherogram for a fluorescence fragment (20 nt, IDT laboratories) measured using the FFA120 Assay. The peak (measured 19.9 nt) can be quantified using the LabChip GX Reviewer software.

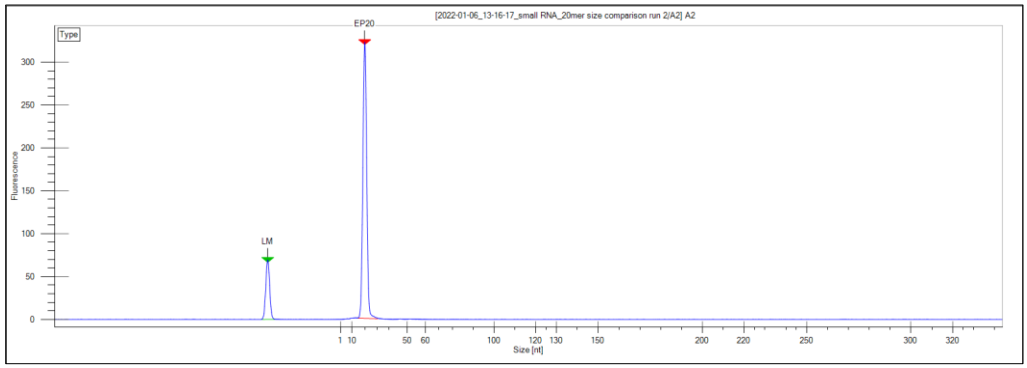


Figure 19. Electropherogram of an example sample result

Troubleshooting

Note: *Some of the data examples shown in this section were generated with assays other than the assay described in this user guide.*

Symptom: No ladder or sample peaks but marker peaks detected.

Note: *The lower marker peak height will most likely be greater than normal height.*

Possible causes:

- Air bubble in sipper introduced during chip priming.

What to do:

- Reprime the chip. See "Repriming Chips" for instructions on how to reprime the chip.

Symptom: Missing sample, ladder, and marker peaks.

Possible causes:

- Clog in sipper or marker channel of chip.

What to do:

- Reprime the chip. See "Repriming Chips" for instructions on how to reprime the chip.

Symptom: Ladder detected but no sample peaks.

Possible causes:

1. The sipper is not reaching the sample due to low sample volume in the well of the plate.
2. If the missing sample peaks occurred only in a few wells of the plate, check those wells for air bubbles.
3. The sipper is not reaching the sample due to an incorrect capillary height setting or incorrect plate definition.
4. If the plate has been uncovered for some time, sample evaporation might have occurred.
5. Debris from the sample or sample prep is clogging the sipper.

What to do:

1. Add more sample to the well.
2. Manually insert a larger volume pipette tip (~100 µL) into the sample well and dislodge the bubble. Rerun these sample wells.
3. Check the plate definitions.
4. Check the sample wells, especially around the edge of the plate

where evaporation is fastest, and make a fresh plate if volumes are low.

5 If there may be debris in the samples, spin the sample plate down in a centrifuge (e.g. 3000 rpm for 5 minutes). Unclog the sipper by repriming the chip. See "Repriming Chips" for instructions on how to reprime the chip.

Symptom: No ladder peaks but sample peaks and marker peaks are present.

Possible causes:

- Low or no ladder volume in the Ladder Tube.

What to do:

- Add more ladder to the Ladder Tube and restart the run. Recommended standard ladder volume is 120 µL (minimum volume is 100 µL).

Symptom: No marker peaks but sample peaks are present.

Possible causes:

1 No Marker added to chip well 4. The marker may not have been put into the marker well during chip prep or the chip may have remained idle in the instrument for an extended period of time.

2 If there is Marker in chip well 4, the marker channel may be clogged.

What to do:

1 Add or replenish the Marker in the chip:

- **a** Touch the **Unload Chip** button on the Home screen to open the chip door.
- **b** Return the chip to the chip storage container, ensuring the sipper is submerged in fluid.
- **c** Thoroughly aspirate all fluid from chip well 4 using a vacuum.
- **d** Rinse and completely aspirate chip well 4 twice with water (Milli-Q® or equivalent).
- **e** Add Marker (green cap) to chip well 4.
- **f** Insert the chip back into the instrument.
- **g** Restart the run.

2 Reprime the chip to unclog the marker channel. See "Repriming Chips" for instructions on how to reprime the chip.

Symptom: Ladder traces show up in the lanes following the ladders (delayed sip, Figure 20).

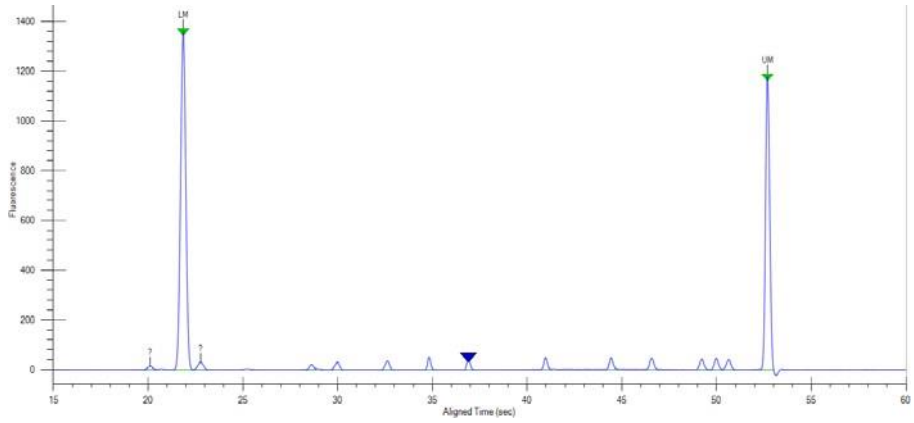


Figure 20. Small ladder peaks in sample well caused by delayed sip

Possible causes:

1 Separation channel overloaded with sample.

2 Partial clog in the separation channel.

What to do:

- Lower the starting sample concentration.
- Reprime the chip. See "Repriming Chips" for instructions on how to reprime the chip.

Symptom: Unexpected sharp peaks.

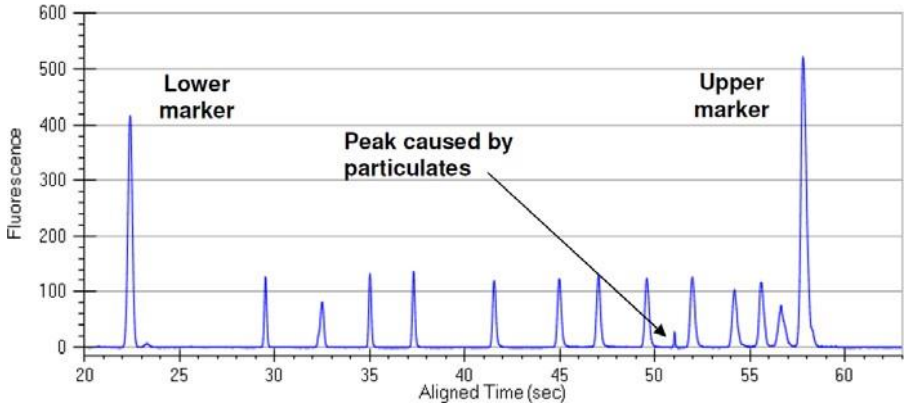


Figure 21. Unexpected sharp peak

Possible causes:

- Dust or other particulates introduced through sample or reagents.

What to do:

- Do one or all of the following:
  - Replace the 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent) used for chip preparation.
  - Replace the buffer used for sample and reagent preparation.
  - Use a 0.22-micron filter for all water and buffers used for chip, sample, and reagent preparation.
  - Spin down sample plate to pellet any particulates.

Symptom: Humps in several electropherograms which do not correspond to sample data.

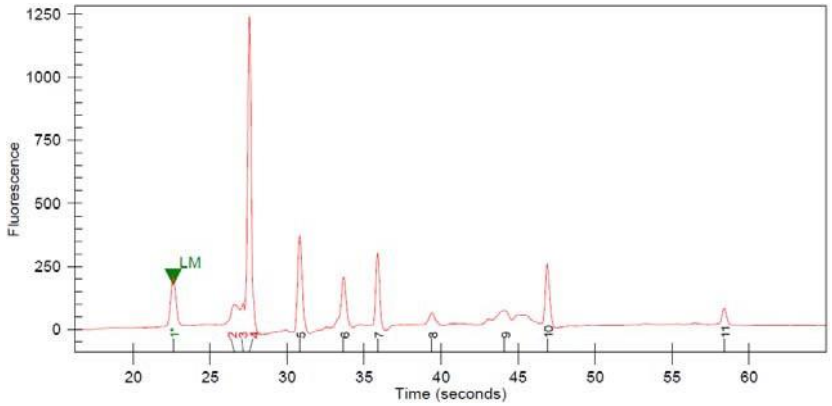


Figure 21. Humps in several electropherograms

Possible causes:

- Electrode 7 is dirty and has contaminated the Gel solution in well 7.

What to do:

- Before restarting the run, clean electrode 7. Remove the chip and follow the electrode cleaning procedure. We recommend using the provided swab and isopropanol to manually clean electrode 7.

Symptom: Peaks migrating much faster or slower than expected.

Note: *Some migration time variance between chips or within a plate is considered normal chip performance. All chips are QC tested at Revvity prior to shipment.*

Normal migration time windows for the markers are:

- *FFA120 Assay Lower Marker: 19-24 seconds.*

Possible causes:

1 Particulates from the samples may be clogging the separation channel (this will slow down migration).

2 Gel matrix was not primed properly into the chip.

3 A pressure leak or current leak can slow peak migration.

What to do:

1 Prepare fresh Gel matrix. Wash and reprime the chip with the new Gel matrix. See “Washing and Repriming Chips” for instructions on how to wash and reprime the chip.

2 If fast or slow migration is observed repeatedly on a new chip, contact technical support to arrange for return of the chip to Revvity. Please send a data file showing the failure along with the return request.

3 Minimize the loading of particulates in the sample by spinning the sample plate (e.g., 3000 rpm for 5 minutes) before starting a new run. The debris can be flushed out of the chip by washing and re-priming the chip. See “Washing and Repriming Chips” for instructions on how to wash and reprime the chip.

4 Check the O-rings on the top surface of the chip interface and clean if necessary.

Symptom: Loss of fragments above 100 nucleotides in sample or Ladder

Possible causes:

1 The chip being used for the assay has expired or passed its lifetime use.

2 Fluorescence label on sample has degraded and is no longer detectable by the LabChip GX Touch.

What to do:

1 Prepare a new chip that has not expired or has gone past its lifetime usage.

2 Freshly prepare a sample plate using FFA120 assay kit. Make sure to run the plate as soon as possible to avoid degradation of fluorescent label.

LabChip Kit Essential Practices

To ensure proper assay performance, please follow the important handling practices described below. Failure to observe these guidelines may void the LabChip Kit product warranty.[1]

Note: *It is important to keep particulates out of the chip wells, channels, and capillary. Many of the following guidelines are designed to keep the chips particulate-free.*

General

For assay and instrument troubleshooting, refer to the LabChip GX Touch Software Help file or call Revvity "Customer Technical Support".

- Allow the chip, sample plate, and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use.
- Use only consumables that are within their expiration date.
- Clean the O-rings in the chip interface weekly and the electrodes daily. Refer to the Instrument Users Guide *Maintenance and Service* section for procedures.
- Avoid use of powdered gloves. Use only non-powdered gloves when handling chips, reagents, sample plates, and when cleaning the instrument electrodes and electrode block.
- Calibrate laboratory pipettes regularly to ensure proper reagent dispensing.
- Only the Revvity-supplied clean room cloth can be used on the chip to clean the detection window.
- Water used for chip preparation procedures must be 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- Using the Reverse Pipetting Technique will help avoid introducing bubbles into the chip when pipetting the gel.

1. Revvity, Inc. warrants that the LabChip Kit meets specification at the time of shipment and is free from defects in material and workmanship. LabChip Kits are warranted for 90 days from the date of shipment. All claims under this warranty must be made within thirty days of the discovery of the defect.

Reverse Pipetting Technique

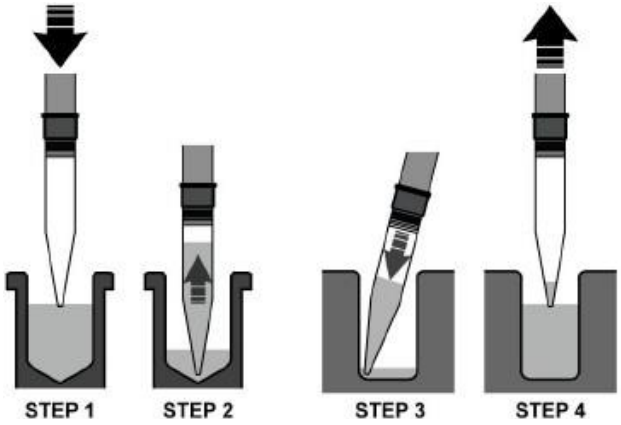


Figure 20. Reverse pipetting

1 Depress the pipette plunger to the second stop.

2 Aspirate the selected volume plus an excess amount from the tube.

3 Dispense the selected volume into the corner of the well by depressing plunger to the first stop.

4 Withdraw the pipette from the well.

Reagents

- Store reagents at 2-8°C when not in use. All refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.
- Gently vortex all kit reagents before use.
- Dispense reagents into chip wells slowly without introducing air bubbles. Insert the pipette tip vertically and to the bottom of the chip well.
- Filter Gel matrix before use. Store Gel matrix at 2-8°C when not in use.

Chips

- Store chips at 2-8 °C prior to first use.
- After use, cover the active chip wells with Parafilm® and store at 2-8°C. If using the chip again within 24 hours, store the chip at room temperature (20 - 25°C).
- Do not allow the liquid in the chip container to freeze, as this may lead to poor chip performance. Do not submerge the chip in any solution.
- The entire chip surface must be thoroughly dry before use.
- Keep the sipper submerged in fluid at all times and do not expose to an open environment for long periods of time.
- Use care in chip handling to prevent sipper damage. Damage to the sipper can result in inconsistent sampling.
- Avoid exposing the chip to dust by keeping the chip in a closed environment such as in the chip storage container or in the instrument before and after chip preparation.
- Chips can be prepared and left idle on the instrument for up to 8 hours. This workflow allows analysis of samples as needed throughout the day without having to re-prep the chip, as long as the maximum number of samples per chip prep is not exceeded.
- Revvity recommends the chip be re-prepared after it has been idle for 8 hours.

Samples

- Prepared sample plates should be free of gas bubbles and particulate debris, both of which may inhibit sipper flow.
- Spin down sample plates containing gas bubbles and/or particulate debris at 3000 rpm (1250 rcf) for 5 minutes prior to analysis.
- With the FFA120 assay kit, up to 48 total samples can be processed with an HT chip prep. Up to 24 total samples can be processed with an LT chip prep.

Chip Well Aspiration Using a Vacuum

Aspirating with a pipette can leave used reagents in the chip wells. For this reason, Revvity recommends vacuuming the wells instead. This can be accomplished by attaching a permanent pipette tip to a house vacuum line with trap (Figure 23). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 24).

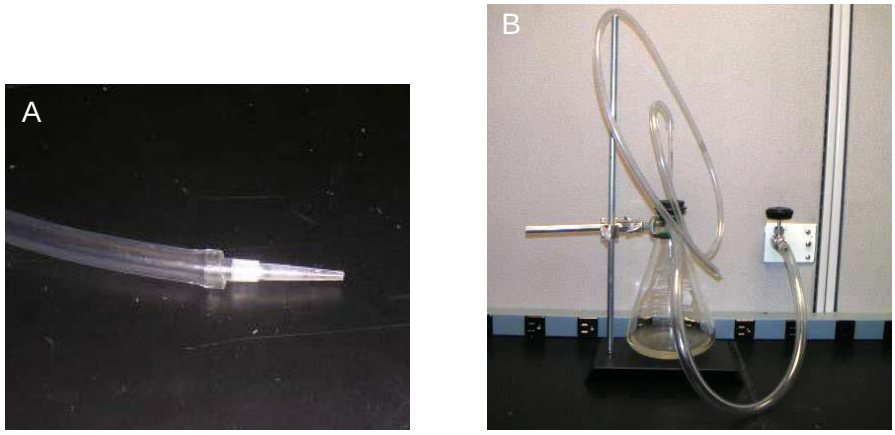


Figure 23. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap

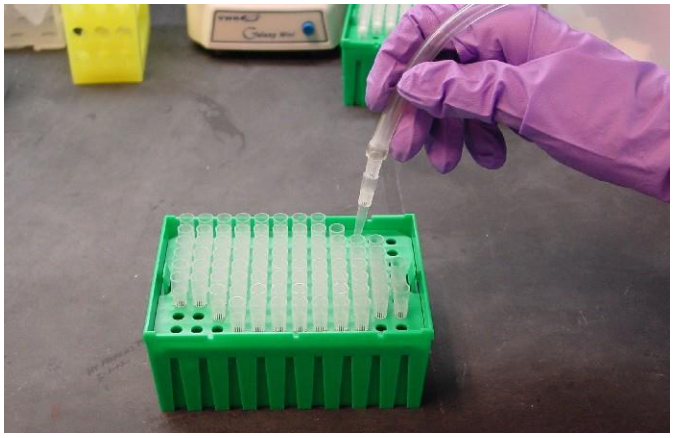


Figure 24. Replacing the disposable pipette tip

Ordering Information

Table 8. Ordering Information

Product	Part Number
FFA120 Reagent Kit	CLS158081
DNA 5K/RNA/CZE HT Chip (GX Touch/GXII Touch HT)	760435
DNA 5K/RNA/CZE 24 Chip (GX Touch/GXII Touch HT or 24)	CLS138949
Detection Window Cleaning Cloth	VWR®, Cat. # 21912-046
Swab	ITW Texwipe®, Cat. # TX758B
Revvity 96-well PCR Plate	6008870

Customer Technical Support

Revvity, Inc.
68 Elm Street
Hopkinton, MA 01748-1668

Revvity Technical Support

Phone (USA Toll Free): 800-762-4000
Phone (Worldwide): +1 203-925-4602
Fax: +1 203-925-4602
Email: BioprocessQC@revvity.com
And add “FFA-“ at the beginning of the subject line.
Internet: https://www.Revvity.com

For additional assay and instrument troubleshooting, refer to the LabChip GX Touch Software Help file or the *LabChip GX Touch User Manual*.

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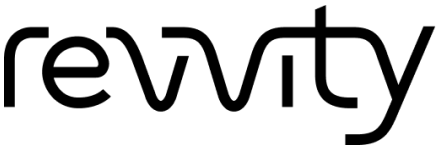

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