

# ssDNA 7K Assay User Guide

For LabChip GX Touch/GXII Touch



# **Table of Contents**

Specifications	. 3
Assay Specifications	. 3
Sample Conditions	. 3
ssDNA 7K Reagent Kit Contents	. 4
DNA 5K/RNA/CZE LabChips	. 5
Assay Program	. 5
Safety and Usage	. 6
Safety Warnings and Precautions	
Usage	
Preparation Procedures	
Additional Items Required	
Preparing the Ladder Aliquots	
Preparing the Gel-Dye Solution	
Preparing the Chip	
Preparing Samples, Ladder and Sample Buffer	
Preparing the Buffer Tube	
Inserting a Chip into the LabChip GX Touch/GXII Touch Instrument	
Operating Procedures	
Running the Assay	
Repriming Chips	
Washing and Repriming Chips	
Cleaning and Storing the Chip	
Chip Cartridge Cleaning	
Results	23
ssDNA 7K Assay Ladder Result	
Troubleshooting	
LabChip Kit Essential Practices	
General	
Reverse Pipetting Technique	
Reagents	
Chips	
Samples	
Chip Well Aspiration Using a Vacuum	
Customer Technical Support	
• •	33 21
LICARDE AND MIGHTE OF LICA	2 A

# **Specifications**

# **Assay Specifications**

**Table 1. Assay Specifications** 

Sensitivity <sup>1</sup>	500 pg/μL total fragments 19 pg/μL 1.1 kb fragment
	89 pg/µL 5.1 kb fragment
Quantitation Reproducibility	20% CV
Quantitation Accuracy	± 30% (for ladder as sample)
Size Range	Up to 7200 nucleotides
Sizing reproducibility	CV < 15%
Maximum Salt	10 mM Tris
Run Time	100 seconds per sample (About 3 hours for 96 samples)
Compatible Plate Types	384-well, 96-well plate
Chip Lifetime <sup>2</sup>	HT: 2000 samples 24: 750 samples
Samples per Chip Prep	Up to 96 samples per HT chip prep Up to 48 samples per LT chip prep
Chip Preps per Reagent Kit	5 HT chip preps or 10 LT chip preps

# **Sample Conditions**

**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. 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 must not exceed 10 mM Tris. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.

<sup>&</sup>lt;sup>1</sup> Estimated by using ssDNA 7K Ladder as sample diluted in TE buffer

<sup>&</sup>lt;sup>2</sup> Estimated from RNA Pico Assay Reagent (P/N CLS960012)

# ssDNA 7K Reagent Kit Contents

The ssDNA 7K Reagent Kit (P/N CLS158169) 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
ssDNA Dye Concentrate	Blue		1 vial, 0.5 mL	2-8°C
ssDNA Chip Storage Buffer	White	$\bigcirc$	5 vials, 1.8 mL each	2-8°C
ssDNA Gel Matrix	Red		5 vials, 0.510 mL each	2-8°C
ssDNA Marker	Green		1 vial, 0.8 mL	2-8°C
10X Sample Buffer Concentrate	Purple		3 vials, 2.0 mL each	2-8°C
ssDNA 7K Ladder <sup>a</sup>	Yellow		1 vial, 0.04 mL	-25 to -15°C

a. Additional ssDNA 7K Ladder can be ordered separately using Part Number CLS157950.

Table 4. Consumable Items

Item	Supplier and Catalog Number	Quantity
Spin Filters	Costar <sup>®</sup> , Cat. # 8160	10
Detection Window Cleaning Cloth	VWR <sup>®</sup> , Cat. # 21912-046	1
Swab	ITW Texwipe <sup>®</sup> , 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

# 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 5. DNA 5K/RNA/CZE LabChips

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

## **Assay Program**

The ssDNA 7K assay program "ssDNA 7K.asyx" are provided by one of the following approaches:

- Contact Customer Technical support "BioprocessQC@revvity.com with adding "ssDNA-" 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**

#### **CAUTION**

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.

#### **WARNING!**



ssDNA Dye contains DMSO. S24/25: Avoid contact with skin and eyes.

## **Usage**

The ssDNA 7K 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.

# **Preparation Procedures**

# **Additional Items Required**

- 18 megohm, 0.22-µm filtered water (Milli-Q<sup>®</sup> or equivalent)
- 70% isopropanol solution in DI water
- DEPC-treated water (nuclease-free)
- PCR cap strips
- Bio-Rad Hard-Shell<sup>®</sup> 384-well Skirted PCR Plates, Cat # HSP-38XX (recommended)
- Revvity Hard-Shell thin-wall 96-well skirted PCR plate (blue), Cat # 6008870 (recommended)

# **Preparing the Ladder Aliquots**

There is no need of heat denature ssDNA 7K Ladder before use. Aliquot the Ladder to several aliquots for individual use after thawing the vial for the first time. Additional ssDNA 7K Ladder can be ordered separately using Part Number CLS157950.

**Note:** Store ssDNA 7K Ladder (yellow cap ) at -25 to -15°C. Keep thawed Ladder on ice. Avoid multiple freeze-thaws.

- 1 Thaw ssDNA 7K Ladder (yellow cap ) on ice.
- 2 Spin down the Ladder.
- 3 Prepare 4-10 μL aliquots in nuclease-free tubes.
- 4 Store unused aliquots at -25 to -15°C.

## **Preparing the Gel-Dye Solution**

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

Warning: The dye is light sensitive. Do not expose the Dye or Gel-Dye solution to light for any length of time. Keep the prepared Gel-Dye solution in the dark.

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

**Note:** The ssDNA Dye contains DMSO and **must be thawed completely** before use.

- 2 Vortex the thawed **ssDNA Dye Concentrate** (blue cap ) for 10 15 seconds before use.
- 3 Add 90 μL of ssDNA Dye Concentrate (blue cap ) to one vial of ssDNA Gel Matrix (red cap ).
- **4** Vortex until the Gel-Dye solution is well mixed and spin down for a few seconds.
- 5 Transfer the Gel-Dye solution to a spin filter. Use a centrifuge tube filled with 600 μL of water to balance the centrifuge.
- **6** Centrifuge at 9300 rcf for 10 minutes at room temperature.
- 7 Discard the filter.
- 8 Label and date the tube.
- **9** Store in the dark at 2-8°C. Use within 5 days.

# **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 (see Figure 1). For details on how to set up a vacuum line, see Chip Well Aspiration Using a Vacuum. Using a vacuum to aspirate the chip wells is more effective than using a pipette.



Figure 1. Aspirate Chip wells with Vacuum

- **3** Rinse and completely aspirate each active chip well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q<sup>®</sup> or equivalent). Do not allow active wells to remain dry.
- 4 If any water spills onto the top or bottom 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, add **Gel-Dye solution** to chip wells 3, 7, 8, and 10 as shown in Figure 2 (Low-throughput) or Figure 3 (High-throughput).

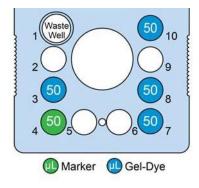


Figure 2. Low-Throughput Chip Preparation

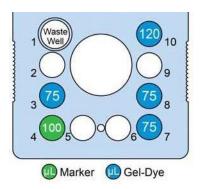


Figure 3. High-Throughput Chip Preparation

6 Add 50 μL (Low-throughput) or 100 μL (High-throughput) of ssDNA Marker (green cap ) to chip well 4 as shown in Figure 2 or Figure 3.

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.

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

# Preparing Samples, Ladder and Sample Buffer

**Notes:** Higher salt concentrations and different ions may alter performance and reduce assay sensitivity. For good performance, please control final total salt concentration of samples at ~10 mM Tris.

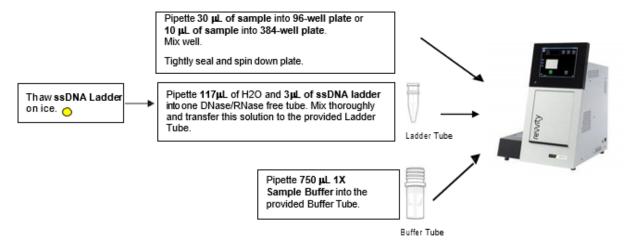


Figure 4. Sample, Ladder Tube, and Buffer Tube Preparation

Prepare 1X Sample Buffer by adding 200 µL Sample Buffer Concentrate (purple cap ) to 1800 µL DEPC-treated or nuclease-free water.

**Note:** The Sample Buffer Concentrate is a 10X solution. Sample Buffer is stable after dilution, but to avoid DNase RNase contamination, sample buffer should be prepared fresh.

- 2 Define/determine the sample preparation (for example, denature treatment) and dilutions based on the sample concentration, storage buffer, etc.
- 3 Add 30  $\mu$ L of sample to each well in 96-well plate (or 10  $\mu$ L in 384-well plates) and mix by pipetting up and down a few times. Avoid introducing bubbles.

- 4 Cover samples in microtiter plates with PCR cap strips or plate film. Foil is not recommended because the adhesive may contaminate the samples.
- 5 Spin down the plate at 3000 rpm for 5 minutes at room temperature to remove air bubbles.
- **6** Prepare the ssDNA 7K Ladder:
  - Thaw ssDNA 7K Ladder (yellow cap ) on ice.
  - b Add 117 µL of nuclease-free water into one RNase/DNase free tube
  - c Add 3 µL ssDNA 7K Ladder (yellow cap ) into water.
  - **d** Mix thoroughly by vortex and spin-down, transfer entire volume (~120 µL) to the provided Ladder Tube. Ensure there are no air bubbles in the Ladder Tube.
  - Insert the Ladder tube into the LabChip GX Touch/GXII Touch instrument.

## **Preparing the Buffer Tube**

- Add **750 µL** of prepared **1X Sample Buffer** to the 0.75 mL Buffer Tube provided with the reagent kit. Ensure there are no air bubbles in the Buffer Tube.
- 2 Insert the Buffer Tube into the LabChip GX Touch/GXII Touch instrument.

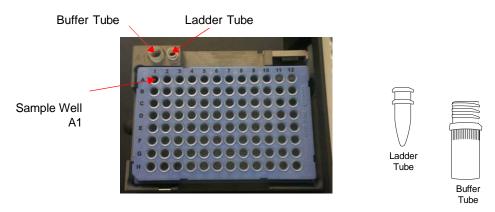


Figure 5. Locations of the Buffer Tube and Ladder Tube in the GX **Touch/GXII Touch instrument** 

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

- Remove the PCR cap strips and plate seal. Place the sample plate, Buffer Tube, and Ladder Tube into the instrument.
- 2 Remove the chip from the chip storage container and inspect the detection window. Clean BOTH sides of the detection window with the Revvity-supplied clean-room cloth dampened with a 70% isopropanol solution in DI water.
- 3 Touch the **Unload Chip** button on the Home screen (Figure 6).

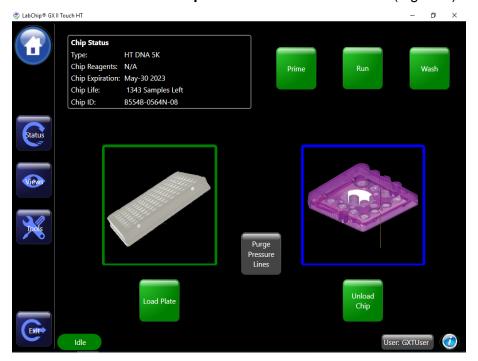


Figure 6. Home Screen

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

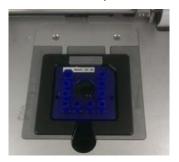


Figure 7. Chip in the LabChip GX Touch/GXII Touch instrument

5 Touch the Load Plate button on the Home screen (Figure 6) to retract the sample plate and move the sipper to the Buffer Tube. The Assay Choice screen opens (Figure 8).

**Note:** Do not keep the chip door open for any length of time. Dye is sensitive to light and can be photobleached.

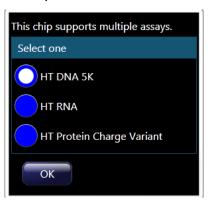


Figure 8. Assay Choice Screen

Touch the desired assay 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 lintfree 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 (see Figure 11). Make sure the Buffer Tube is placed on the instrument. Touch the Prime button on the Chip Priming screen (Figure 9).



Figure 9. Chip Priming Screen

To run an assay:

Touch the Run button on the Home screen (see Figure 6). The Select Wells tab opens (see Figure 10).

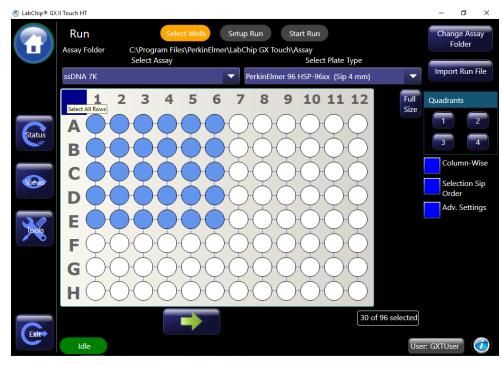


Figure 10. Select Wells Tab

- Select the desired assay type (see Figure 11). For ssDNA 7K assay, the valid assay types are:
  - ssDNA 7K: For analysis of ssDNA ranged from 1.1kb to 7.2kb.



Figure 11. Assay Type Drop-Down List

- Select the plate type, well pattern, and sip order (column or row). If necessary, click Adv. Settings to specify the number of times each well is sampled.
- Touch the **Green Arrow** button. The Setup Run tab (Figure 12) opens.



Figure 12. Setup Run Tab

- Specify the operator 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.
- Touch the **Green Arrow** button. The Start Run tab (Figure 13) opens.

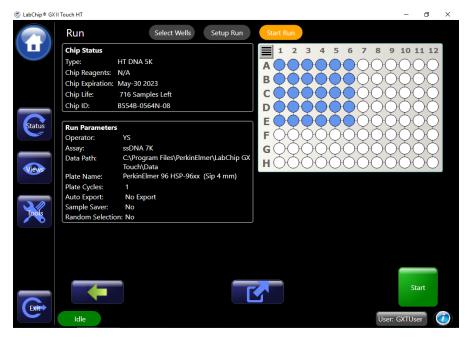


Figure 13. Start Run Tab

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 1X Sample Buffer solution or water into the instrument while priming or washing chips.

- 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.
- Place the chip and Buffer Tube into the instrument.
- 3 Close the chip door securely and choose the corresponding assay.
- Touch the **Prime** button on the Home screen. The Prime screen opens.
- 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 residue.

- 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.
- 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<sup>®</sup> 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.
- Place a Buffer Tube with **750 µL** of prepared **1X Sample Buffer** or water in the instrument.
- Close the chip door securely.
- Touch the **Wash** button on the Home screen. The Wash screen (Figure 14) opens.



Figure 14. 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 Chip Storage Buffer, then suction the sipper with a vacuum line as shown in Figure 15 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.

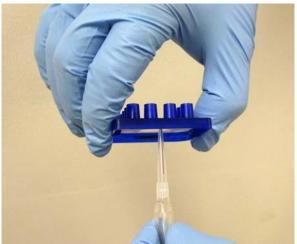


Figure 15. Removing an air bubble or clog by suctioning the sipper with a vacuum

# 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.

- 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<sup>®</sup> or equivalent).
- 4 Add 120 μL of Chip Storage Buffer (white cap Ο) to the active wells.
- 5 Place the chip in the LabChip GX Touch/GXII Touch instrument. Place a buffer tube with 750 µL of Chip Storage Buffer in the Buffer slot.
- Touch the Wash button on the Home screen. The Wash screen (Figure 16) opens.



Figure 16. Wash Screen

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 ssDNA 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**

- Inspect the inside of the chip cartridge and O-rings for debris.
- 2 Use the provided lint-free swab dampened with water (Milli-Q<sup>®</sup> 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.
- 3 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. Remove the O-rings from the top plate of the chip interface on the LabChip GX Touch/GXII Touch instrument. 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.
- 2 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).
- 3 Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

# **Results**

# ssDNA 7K Assay Ladder Result

The electropherogram of a typical ssDNA 7K ladder using the Standard Sample Workflow is shown in Figure 17. Following the lower marker, ladder fragments in order of increasing migration time correspond to 1100, 2100, 3200, 4000, 5100, and 7200 nt.

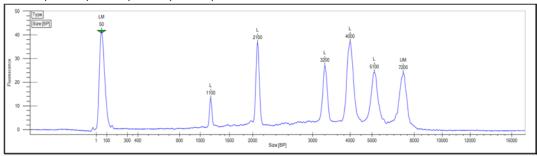


Figure 17. Typical ssDNA 7K Ladder

# **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:

1 Air bubble in sipper introduced during chip priming.

#### What to do:

Reprime the chip. See "LabChip Kit Essential Practices" for instructions on how to reprime the chip.

#### Symptom: Missing sample, ladder, and marker peaks.

#### Possible causes:

1 Clog in sipper or marker channel of chip.

#### What to do:

Reprime the chip. See "LabChip Kit Essential Practices" for instructions on how to reprime the chip.

#### Symptom: Ladder detected but no sample peaks.

#### Possible causes:

- 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.

- Add more sample to the well.
- 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:

1 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:

- No ssDNA Marker added to chip well 4. The ssDNA 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 ssDNA Marker in chip well 4, the marker channel may be clogged.

- 1 Add or replenish the ssDNA 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<sup>®</sup> or equivalent).
  - e Add ssDNA Marker (green cap ) to chip well 4.
  - Insert the chip back into the instrument.
  - 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).

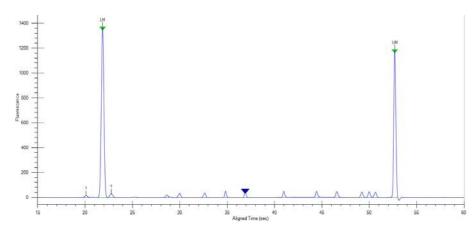


Figure 18. Small ladder peaks in sample well caused by delayed sip

#### Possible causes:

- Separation channel overloaded with sample.
- 2 Partial clog in the separation channel.

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

#### Symptom: Unexpected sharp peaks.

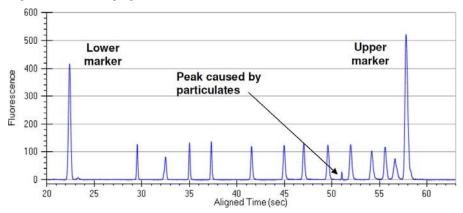


Figure 19. Unexpected sharp peak

#### 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- $\mu m$  filtered water (Milli-Q $^{\rm @}$  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.

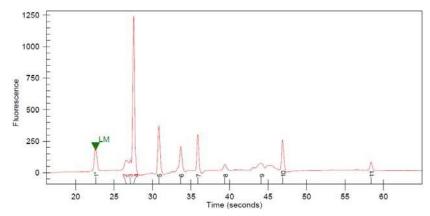


Figure 20. Humps in several electropherograms

#### Possible causes:

Electrode 7 is dirty and has contaminated the Gel-Dye 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:

ssDNA 7K Assay Lower Marker: 31 - 37 seconds

#### Possible causes:

1 Incorrect Gel-Dye ratio. Migration time is sensitive to dye concentration and peaks will migrate too fast or too slow if the dye concentration in the gel is too low or too high, respectively.

**Note:** Excess dye in the separation channel will slow down migration, and less dye in the separation channel will speed up migration.

- 2 Particulates from the samples may be clogging the separation channel (this will slow down migration).
- **3** Gel-Dye solution was not primed properly into the chip.
- **4** A pressure leak or current leak can slow peak migration.

- Prepare fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye solution. 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.
- Check the O-rings on the top surface of the chip interface and clean if necessary.

# **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.<sup>1</sup>

**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.

For assay and instrument troubleshooting, refer to the LabChip GX Touch User Manual or Help file or call Revvity Technical Support

#### General

- Allow the chip, sample plate, and all refrigerated reagents to equilibrate to room temperature for at least 30 minutes before use. Thaw the ssDNA Ladder on ice. (It is recommended to aliquot for individual use after thawing the vial for the first time.)
- 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, 0.22µm filtered water (Milli-Q® or equivalent).
- Using the Reverse Pipetting Technique will help avoid introducing bubbles into the chip when pipetting the gel.
- 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**

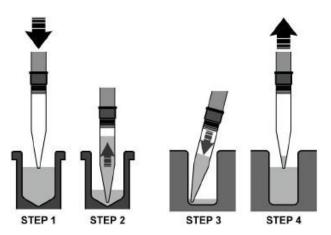


Figure 21. Reverse pipetting

- 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. Store ssDNA Ladder at -20°C when not in use.
- All refrigerated reagents must equilibrate to room temperature (20 -25°C) for at least 30 minutes before use.
- Thaw the ssDNA Ladder on ice. Aliquot ssDNA Ladder for individual use after thawing for the first time.
- The ssDNA Dye contains DMSO and must be thawed completely before use. It is recommended that you prepare aliquots to reduce the time required for thawing.
- 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
- Protect the Dye and Gel-Dye solution from light. Store in the dark at 2-8°C when not in use.
- The Gel-Dye solution expires 5 days after preparation.

## **Chips**

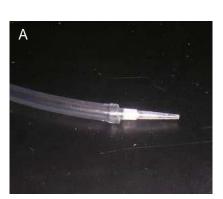
- Store chips at 2-8 °C prior to first use.
- After use, cover the active chip wells with Parafilm® and store at 2-8°C. If using the chip again within 24 hours, store the chip at room temperature (20 - 25°C).
- Do not allow the liquid in the chip container to freeze, as this may lead to poor chip performance. 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.
- Up to 96 samples can be processed with an HT chip prep. Up to 48 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 22). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 23).



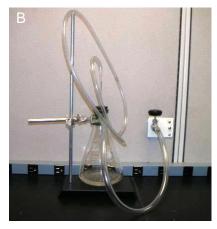


Figure 22. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap



Figure 23. Replacing the disposable pipette tip

# **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 Internet: <a href="https://www.Revvity.com">https://www.Revvity.com</a>

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

Manual.

# Licenses and Rights of Use

#### **Label Licenses**

This product is provided under an intellectual property license from Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the data for internal research and training purposes solely in combination with Revvity, Inc. instrumentation, and not to generate revenue, which may include, but is not limited to use of the product: (1) in manufacturing or in quality assurance or quality control of a commercial product;

(2) to provide a service, information, or data for a fee or other consideration; (3) for therapeutic or prophylactic purposes; (4) for diagnostic use; and (5) for resale, whether or not such items are resold for use in research. For information on purchasing a license to this product for any other purposes, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 USA or outlicensing@lifetech.com.

#### Rights of Use

The chip and reagents supplied with this kit are sold with limited rights of use. The chip may only be used with the specific quantity of reagents supplied with this kit. The purchaser has no right or license to refurbish, reuse, remanufacture, or otherwise use the chip with any other reagents than those specifically supplied in this kit. For more information on the terms and conditions of use of these chips and reagents, please read your LabChip GX Touch User Guide and refer to the applicable label license. The reagent kit contains materials that are provided under a license from Life Technologies Corporation, for research use only.

Revvity is a registered trademark of Revvity, Inc. All other trademarks and registered trademarks are the property of their respective holders.

© 2023 Revvity, Inc. https://www.Revvity.com



Revvity, Inc.

68 Elm Street
Hopkinton, Massachusetts 01748 U.S.A.
TEL 508-435-9500
FAX 508-435-3439
<a href="https://www.revvity.com">https://www.revvity.com</a>

