# DNA High Sensitivity Assay User Guide

## For LabChip GX Touch/GXII Touch

CE



PN CLS140158, Rev. D

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# Specifications

## Assay Specifications[1]

**Table 1. Assay Specifications**

| | |
|---|---|
| Sizing Range | 50 - 5000 bp |
| Sizing Resolution[a] | ± 5% from 100 - 500 bp<br>± 10% from 50 - 100 bp, 500 - 1000 bp<br>± 15% from 1000 - 3000 bp<br>± 22% from 3000 - 5000 bp |
| Sizing Accuracy | ± 10% |
| Sizing Precision | 5% CV |
| Linear Concentration Range | **Standard Sample Workflow**<br>10 pg/µL - 500 pg/µL **per fragment** from 50 bp to 2000 bp<br>50 pg/µL - 500 pg/µL **per fragment** from 2000 bp to 5000 bp<br>100 pg/µL - 5 ng/µL **for smears**<br><br>**Limited Sample Workflow (initial concentration)**<br>20 pg/µL - 500 pg/µL **per fragment** from 50 bp to 2000 bp<br>100 pg/µL - 500 pg/µL **per fragment** from 2000 bp to 5000 bp<br>200 pg/µL - 5 ng/µL **for smears** |
| | **Standard Workflow**<br>5 pg/µL **per fragment**<br>100 pg/µL **for smears**<br><br>**Limited Sample Workflow (initial concentration)**<br>10 pg/µL **per fragment**<br>200 pg/µL **for smears** |
| Maximum Total DNA Concentration | 5 ng/µL total, 500 pg/µL **per fragment** |
| Quantitation Accuracy | ± 30% |

1. All specifications pertaining to DNA fragments were determined using ladder as sample in TE buffer. All specifications pertaining to DNA smears were determined using Covaris sheared control genomic DNA (human male) in TE buffer. Shearing time was 30s or 240s.

**Table 1. Assay Specifications(Continued)**

| | |
|---|---|
| Sizing Range | 50 - 5000 bp |
| Quantitation Precision | 20% CV |
| Analysis Time | 68 seconds per sample (~2.5 hours for 96 samples) |
| Number of Samples per Chip Prep | 96 samples |

a. 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.

# Sample Conditions

**Table 2. Sample Conditions**

| | |
|---|---|
| Additives | PerkinElmer 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 DNA LabChip protocols. |
| Particulates | All sample plates should be spun down prior to analysis. All buffers should be filtered with a 0.22 µm cellulose acetate filter. |
| Salt Concentration | Total salt concentration must not exceed 10 mM Tris and 1 mM EDTA for the DNA High Sensitivity assay. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity. |

# Kit Contents

**Storage:** When not in use, store chips and reagents refrigerated at 4°C. Do not leave chips and reagents unrefrigerated overnight.

**DNA High Sensitivity Reagent Kit**
**Part Number CLS760672**

Each kit contains enough reagents for 20 Low-throughput (LT) or 10 High-throughput (HT) chip preparations. Up to 48 samples can be tested with a LT chip preparation. Up to 96 samples can be tested with a HT chip preparation.

**Table 3. Reagent Kit Contents, PN CLS760672**

| Reagent | Vial | Quantity |
|---|---|---|
| DNA Dye Concentrate | Blue | 1 vial, 0.09 mL |
| Chip Storage Buffer | White | 9 vials, 1.8 mL each |
| DNA HiSens Gel Matrix | Red | 5 vials, 1.1 mL each |
| 10X DNA HiSens Ladder | Yellow | 1 vial, 0.26 mL |
| DNA HiSens Marker | Green | 2 vials, 1.2 mL each |

**Table 4. Consumable Items**

| Item | Supplier and Catalog Number | Quantity |
|---|---|---|
| Spin Filters | Costar, Cat. # 8160 | 10 |
| Ladder Tubes | Genemate, Cat. # C-3258-1 | 20, 0.2 mL |
| Detection Window Cleaning Cloth | VWR, Cat. # 21912-046 | 1 |
| Swab | ITW Texwipe®, Cat. # TX758B | 3 |
| Buffer Tubes | E&K Scientific, Cat. # 697075-NC | 16, 0.75 mL |

**Table 5. DNA LabChips**

| Item | Catalog Number |
|---|---|
| DNA Extended Range Chip for use with GX Touch/GXII Touch HT | Cat. # 760517 |
| DNA Extended Range Chip for use with GX Touch/GXII Touch 24 | Cat. # CLS138948 |

# Safety Warnings and Precautions

### WARNING!

*For Research Use Only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.*

### 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. As all chemicals should be considered potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.*

### WARNING!

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

# Preparation Procedures

## Additional Items Required

- 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- 70% isopropanol solution in DI water.
- Bio-Rad Hard-Shell® 384-well Skirted PCR Plates, Cat # HSP-38XX (recommended).
- PerkinElmer Hard-Shell thin-wall 96-well skirted PCR plate (blue), Cat # 6008870 (recommended).

***Note:*** *Allow the chip and reagents to equilibrate to room temperature for about 20-30 minutes before use.*

## Preparing the Gel-Dye Solution

***Notes:*** *The Dye Solution contains DMSO and* ***must be thawed completely*** *before use.*

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

*Do not exceed 9300 rcf when filtering the Gel-Dye solution. Exceeding 9300 rcf will change the properties of the gel.*

*One vial of DNA HiSens Gel Matrix (red cap 🔴) is good for* ***4 Low-throughput chip preparations or 2 High-throughput chip preparations****. Up to 48 samples can be tested with a LT chip preparation. Up to 96 samples can be tested with a HT chip preparation.*

**1** Vortex the thawed DNA Dye Concentrate (blue cap 🔵) for 10-15 seconds before use.

**2** Transfer 13 µL of DNA Dye Concentrate (blue cap 🔵) to 1 vial of DNA HiSens Gel Matrix (red cap 🔴).

**3** Vortex the solution until it is well mixed and spin down for a few seconds.

**4** Transfer the mixture into two spin filters (approx. 550 µL each).

**5** Centrifuge at 9300 rcf for 10 minutes at room temperature.

**6** Discard filters, label and date the tubes.

**7** Store in the dark at 4°C. Use within 3 weeks.

# Preparing the DNA Samples, DNA Ladder and the Buffer Tube

***Notes:*** *Total salt concentration must not exceed 10 mM Tris and 1 mM EDTA. Higher salt concentrations and different ions may alter performance and reduce assay sensitivity.*

*DNA Ladder should be prepared in the same buffer as your DNA samples. A buffer mismatch between sample and ladder may lead to inaccurate quantitation and sizing.*

*DNA sample buffer is the user's DNA buffer such as the PCR buffer, etc.*

## Standard Sample Workflow

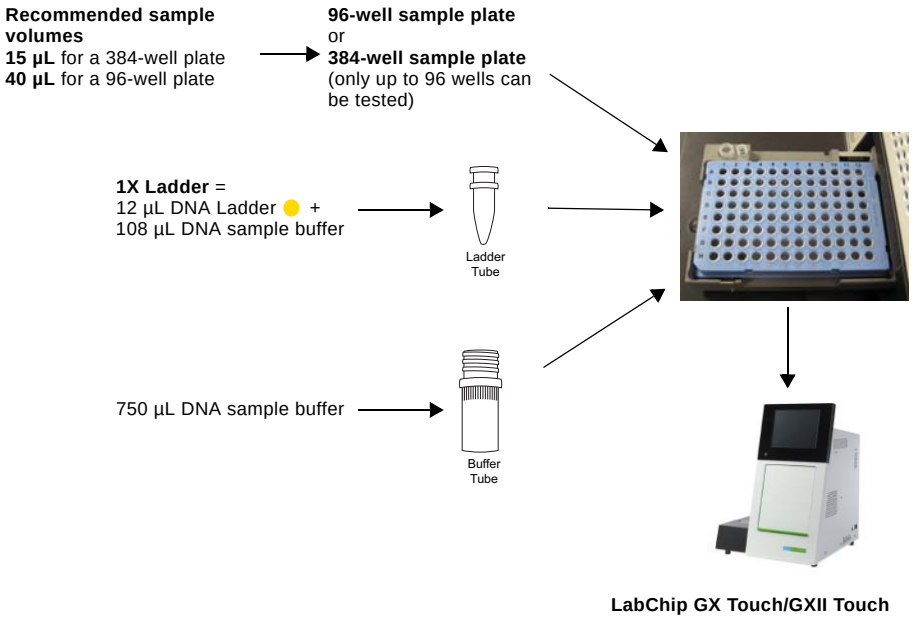


**Figure 1. Standard Sample Workflow.**

1. Gently vortex DNA Ladder (yellow cap) for 10 seconds. Briefly spin the ladder vial.
2. In the provided 0.2 mL Ladder Tube, add 12 µL of DNA Ladder to 108 µL of 1X DNA sample buffer solution. Mix thoroughly by pipetting the solution up and down several times. Ensure there are no air bubbles in the Ladder Tube.
3. Insert the Ladder Tube into the ladder slot on the LabChip GX Touch/GXII Touch instrument.

4 Pipette samples into a 384-well or 96-well plate. Recommended sample volumes are 15 µL for a 384-well plate or 40 µL for a 96-well plate. Seal and spin the plate at 1000 x g for 2 minutes prior to placing on the instrument.

5 Add 750 µL of 1X DNA Sample Buffer solution to the 0.75 mL Buffer Tube provided with the reagent kit. Ensure there are no air bubbles in the Buffer Tube.

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

## Limited Sample Workflow

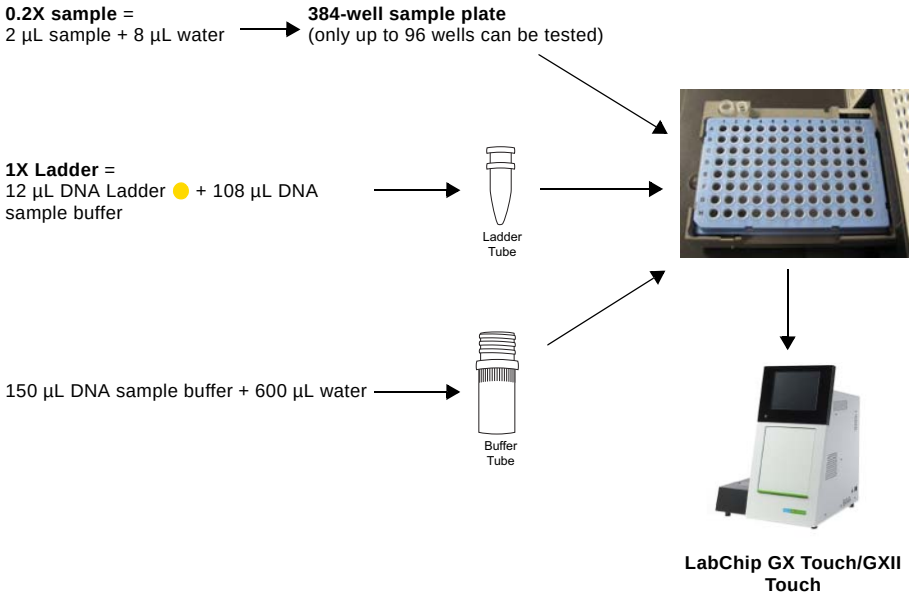


**Figure 2. Limited Sample Workflow.**

1 Gently vortex DNA Ladder (yellow cap) for 10 seconds. Briefly spin the ladder vial.

2 In the provided 0.2 mL Ladder Tube, add 12 µL of DNA Ladder (yellow cap) to 108 µL of diluted DNA sample buffer solution. Ensure there are no air bubbles in the Ladder Tube.

***Note:*** *Dilute 1X DNA sample solution at the same ratio as your samples with water.*

3 Insert the Ladder Tube into the ladder slot on the LabChip GX Touch/GXII Touch instrument.

4 In a 384-well plate pipette 8 µL of water to an equivalent number of wells as samples being tested (but to no more than 96 wells). Add 2 µL of sample to each well and mix by pipetting up and down few times. Avoid creating air bubbles. Seal and spin the plate at 1000 x g for 2 minutes.

5 In the provided Buffer Tube add 150 µL of 1X DNA sample buffer solution to 600 µL of water.

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

***Notes:*** *This workflow requires only 2 µL of sample with a minimum initial concentration of* ***20 pg/µL per fragment*** *or* ***200 pg/µL for smears****. Before testing, the 2 µL of sample is diluted to 0.2X in water for a total volume of 10 µL.To ensure a buffer match, the sample buffer solution should be diluted in the same manner as your samples. Prepare Ladder with the diluted sample buffer solution. Use diluted sample buffer solution in the Buffer Tube.*

*The sample plate should be tested soon after preparation to minimize evaporation. Sipping samples more than once is not recommended.*

*Use this workflow if testing LabChip XT fractionated samples.*

*Quantitation given by the LabChip GX Touch software should be multiplied by the dilution factor (i.e. your sample dilution ratio).*

## Preparing the Chip

1 Allow the chip to come to room temperature.

2 Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells (see Figure 3). For more details on how to set up a vacuum line see page 34.

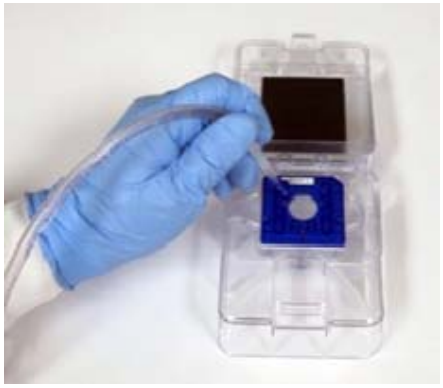

**Figure 3. Using a vacuum to aspirate the chip wells is more effective than using a pipette.**

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

**4** If any water spills onto the top and bottom chip surfaces during rinsing, aspirate using the vacuum line. DO NOT run the tip over the central region of the detection window. Use the provided Detection Window Cleaning Cloth dampened in water or alcohol to clean the chip 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 4 (Low-throughput) or Figure 5 (High-throughput).

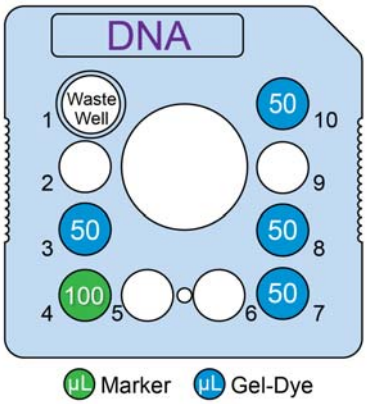


**Figure 4. Reagent placement for Low-throughput (up to 48 samples).**

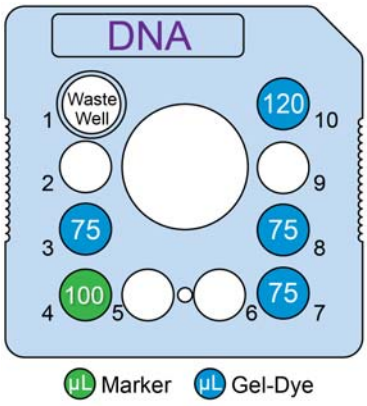


**Figure 5. Reagent placement for High-throughput (up to 384 samples).**

6 Add DNA HiSens Marker (green cap ●) to chip well 4 as shown in Figure 4 (Low-throughput) or Figure 5 (High-throughput). Please note that the marker well may need to be replenished if the chip is in idle mode on the instrument for an extended period of time.

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.*

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

1 Before inserting the chip, close the instrument door and touch the **Purge Pressure Lines** button on the **Home** screen to prevent any potential liquid or particles on the chip interface from getting into the chip during priming.

2 Check that the sample plate, Buffer Tube, and Ladder Tube are placed on the instrument properly.

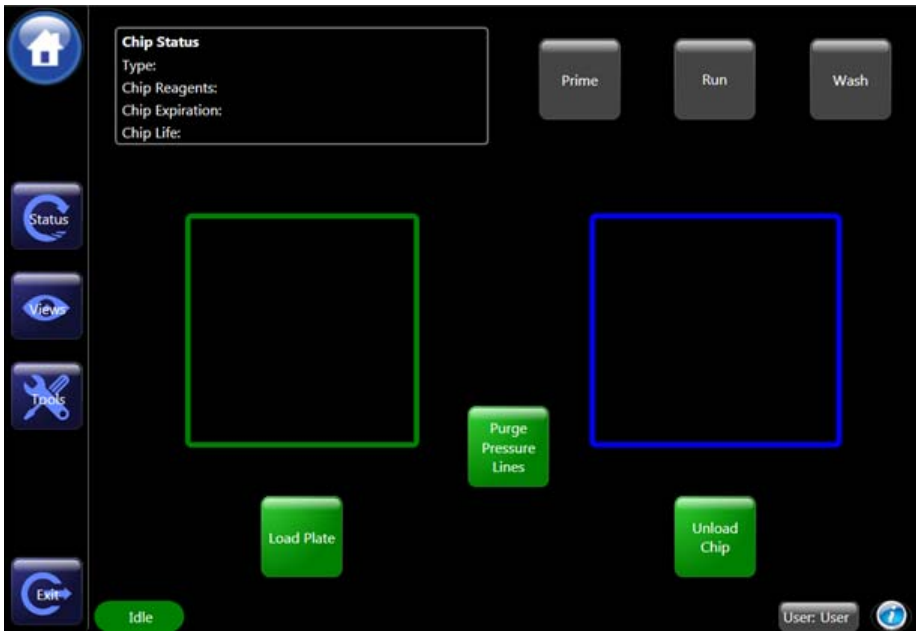


**Figure 6. *Home* screen.**

3 Once the purge is complete, touch the **Unload Chip** button on the **Home** screen.

4 Clean the electrodes (seen in Figure 7) using provided lint-free swabs dampened with DI water. Do not leave any visible droplets of water on the electrodes.

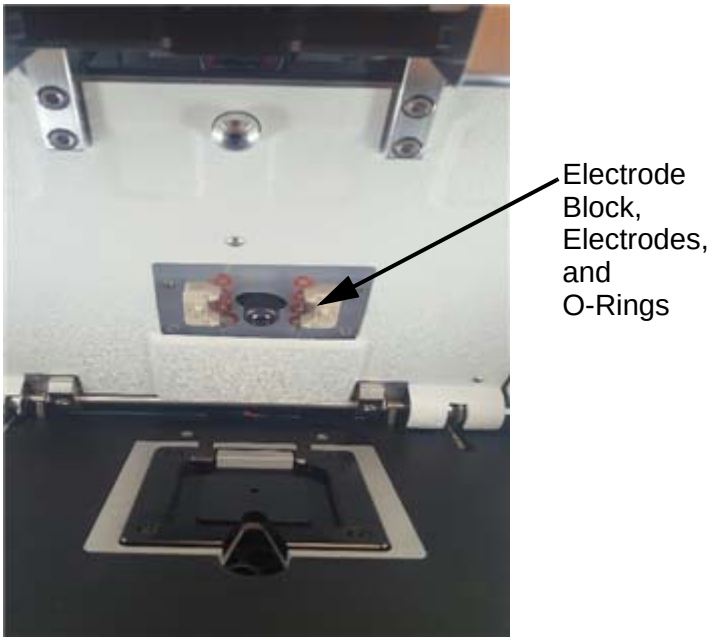


**Figure 7. Chip Loading Area**

5 Remove the chip from the chip storage container and inspect the chip window. Clean BOTH sides of the chip window with the PerkinElmer-supplied clean-room cloth dampened with a 70% isopropanol solution in DI water.

6 Place the chip on the chip heater block in the LabChip GX/GXII Touch instrument (Figure 8) and close the chip door securely.

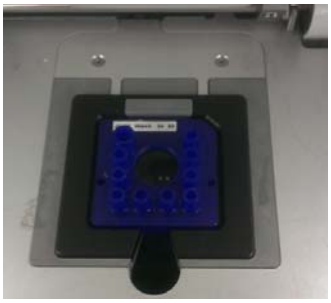

**Figure 8. Chip in the LabChip GX Touch/GXII Touch instrument.**

**7** Touch the *Load Plate* button on the *Home* screen (Figure 6) to retract the sample plate and send the sipper to the Buffer Tube.

***Note:*** *After placing the chip on the heater block, close the instrument door promptly. Dye is sensitive to light and can be photobleached if the door is left open.*

**8** The *Assay Choice* window will appear (Figure 9). Touch the desired assay and then touch *OK*.

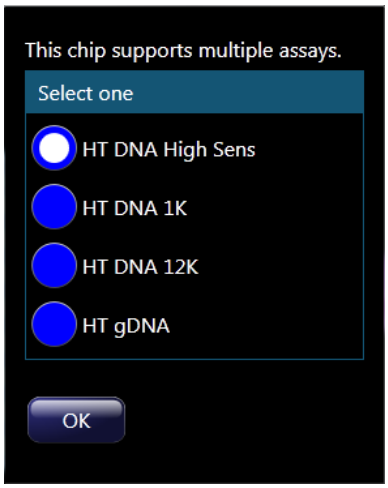


**Figure 9. *Assay Choice* menu.**

# Running the Assay

***Note:*** *Chips can be primed independently from running assays. Touch the Prime button on the Home screen.* ***Make sure the Buffer Tube is placed on the instrument.***

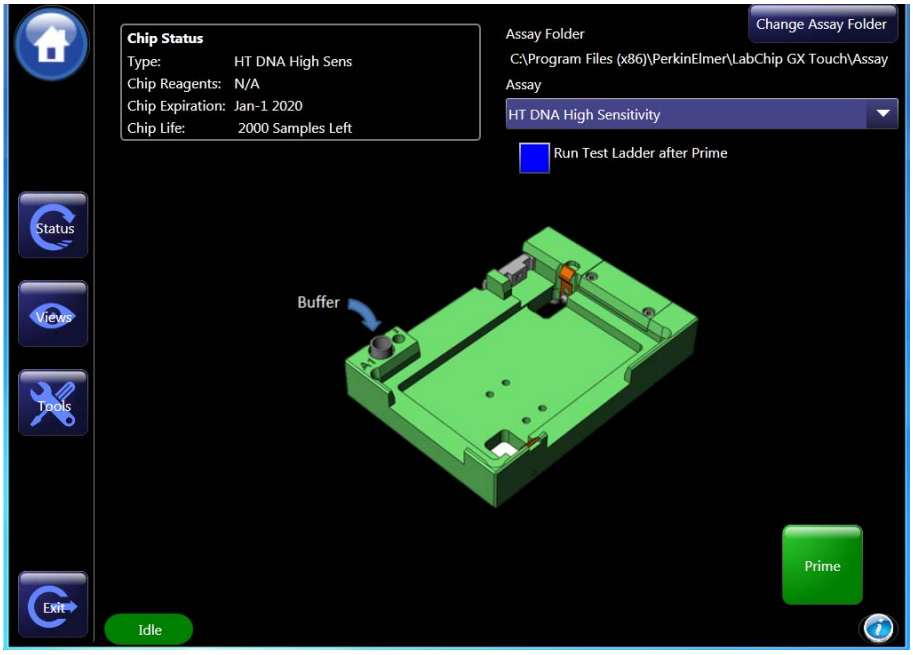


**Figure 10. Chip priming screen.**

**1** Touch the *Run* button (see Figure 10).

**2** Select the appropriate assay type (see Figure 9), plate name, well pattern, and whether to read wells in columns or rows. Select number of times each well is sampled under *Adv. Settings* (Figure 11). Touch the *green arrow* button.

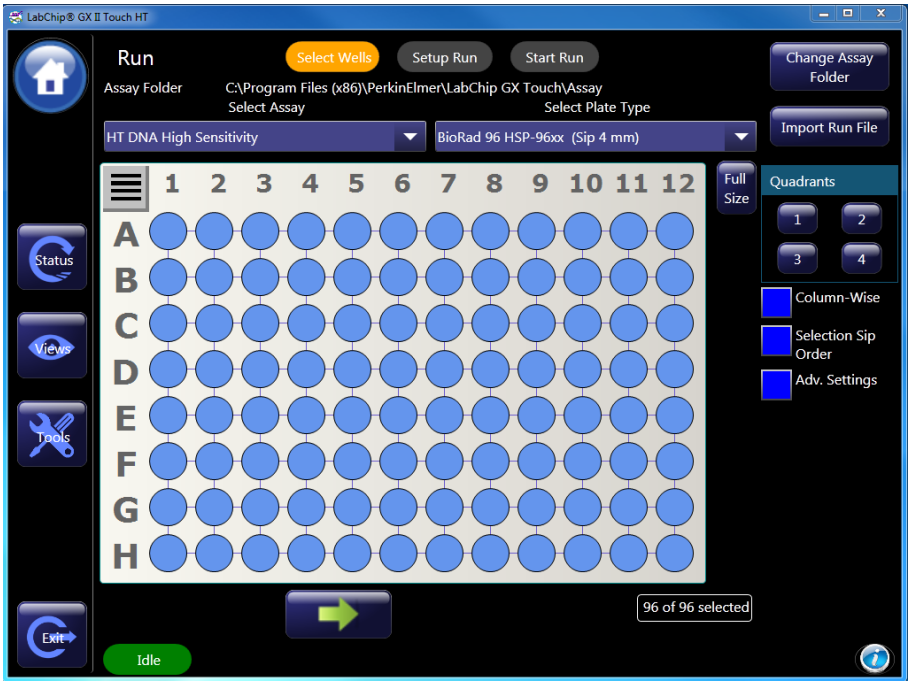


**Figure 11. Selecting wells.**

**3** In the *Setup Run* tab, select the operator name, the option to read barcode, the destination of the file, the inclusion of sample names, expected peaks, and excluded peaks and the filename convention. Select *Auto Export* to export results tables automatically (Figure 12). Touch the *green arrow* button.

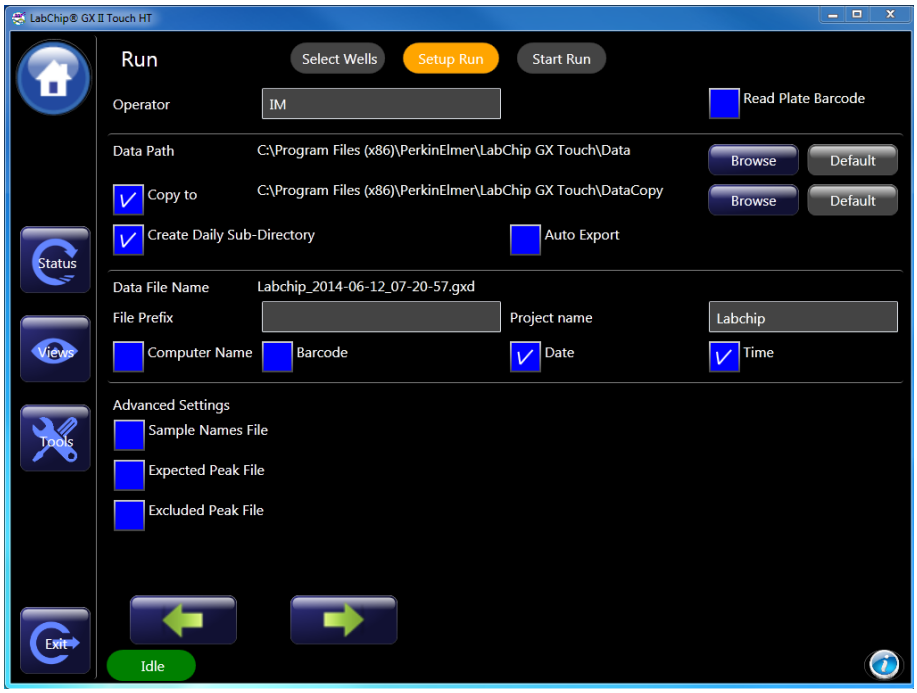


**Figure 12. Run setup screen.**

**4** Touch *Start* to begin the run (Figure 13).


**Figure 13. Starting a run.**

## Storing the Chip

After use, the chip must be cleaned and stored in the chip container. The procedure below can be conducted the following day when running overnight.

1. Remove the chip from the instrument and place into the storage container.
2. Wipe the electrodes with a lint-free swab to remove any droplets of gel.
3. Remove the reagents from each well of the chip using vacuum.
4. Each active well (1, 3, 4, 7, 8, and 10) should be rinsed and aspirated twice with water molecular biology grade water.
5. Add **100 µL** of DNA Chip Storage Buffer (white cap ◯) to the active wells.
6. Place the chip back on the LabChip GX/GXII Touch. Ensure that a Buffer Tube with **750 µL** sample buffer or molecular biology grade water is in the buffer slot.
7. Touch the **Wash** button.
8. Remove the chip from the instrument and place into the storage container.
9. Add an additional **50 µL** of DNA Chip Storage Buffer to well 1.
10. Cover the wells with Parafilm® to prevent evaporation and store the chip at room temperature until next use. Allowing chip wells to dry may cause clogging.

## Chip Cartridge Cleaning

1. **Daily**
   a. Inspect the inside of the chip cartridge and O-rings for particles or debris.
   b. If a long run has been performed, or if the chip has been left on the instrument overnight, touch the **Purge Pressure Lines** button. The purge is complete when the Run Status displays “Purge successfully completed” and the instrument status returns to Idle.

**c** Use the provided lint-free swab dampened with DI water to clean the electrodes and 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.

**2 Monthly**

**a** 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 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.

**b** 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).

**c** Allow the O-rings and chip interface to air dry. Reinsert the O-rings into the chip cartridge.

# Results

## DNA High Sensitivity Ladder Result

The electropherogram of a typical DNA High Sensitivity ladder using the Standard Sample Workflow is shown in Figure 14. Between the upper and lower markers, peaks in order of increasing migration time correspond to ladder fragments of 50, 100, 150, 200, 300, 400, 500, 700, 1100, 1900, 2900 and 4900 bp.

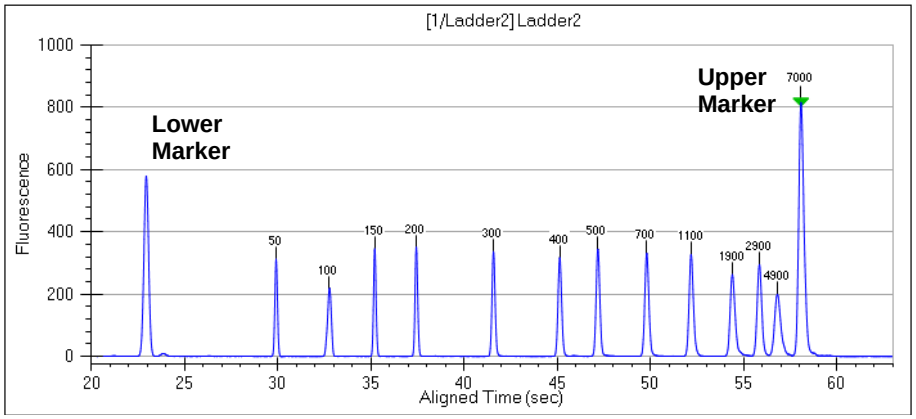


**Figure 14. DNA High sensitivity ladder electropherogram produced using the Standard Sample Workflow.**

The electropherogram of a typical DNA High Sensitivity ladder using the Limited Sample Workflow is shown in Figure 15.

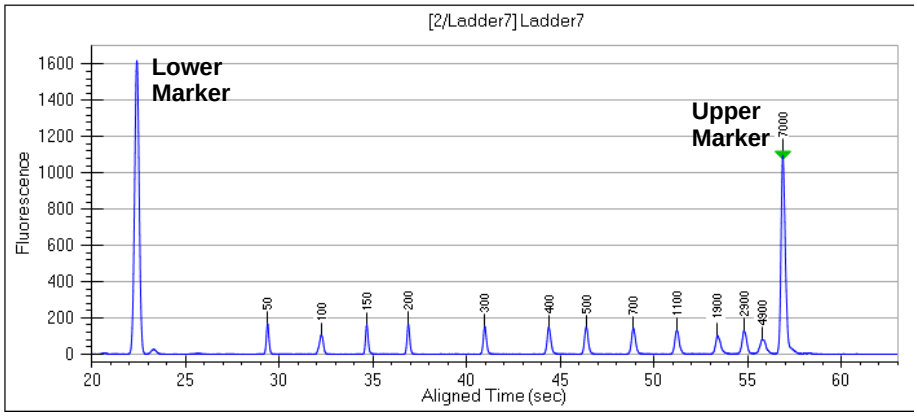


**Figure 15. DNA High sensitivity ladder electropherogram produced using the Limited Sample Workflow.**

# 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:**

**1** Reprime the chip. See “LabChip Kit Essential Practices” on page 28 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:**

**1** Reprime the chip. See “LabChip Kit Essential Practices” on page 28 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 you suspect there may be debris in your samples, spin the sample plate down in a centrifuge (e.g. 3000 rcf for 5 minutes). Unclog the sipper by repriming the chip. See "LabChip Kit Essential Practices" on page 28 for instructions on how to reprime the chip.

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

**Possible causes:**

1 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:**

1 No marker added to chip well 4.

2 If there is marker solution in chip well 4, the problem may be due to a marker channel clog.

**What to do:**

1 This may be due to not filling marker well or chip remaining idle on instrument for extended period of time. Add or replenish the marker solution in the chip using the following procedure:

- Touch the *Unload Chip* button on the Home screen to open the chip door.
- Return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from chip well 4 using a vacuum line.
- Ensure that chip well 4 is rinsed and completely aspirated twice with water (Milli-Q® or equivalent).
- Add Marker Solution (green cap ●) to chip well 4.

- Reinsert the chip back into the instrument.
- Restart the run.

2 Perform a marker channel unclogging procedure by repriming the chip. See “LabChip Kit Essential Practices” on page 28 for instructions on how to reprime the chip.

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

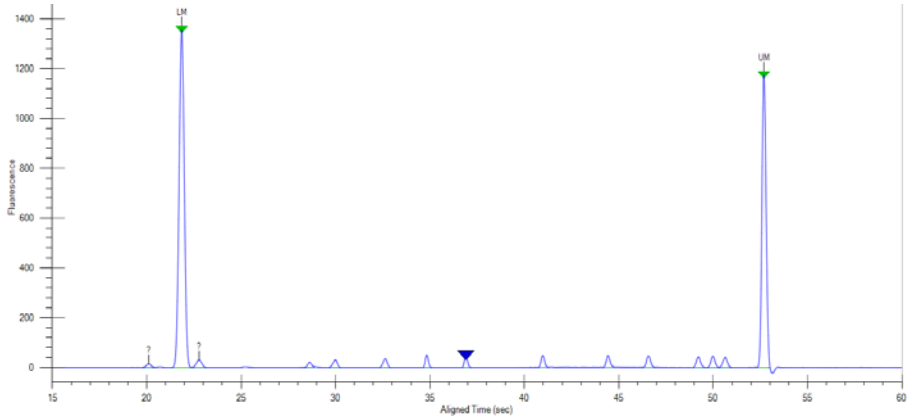


**Figure 16. Small ladder peaks in sample well caused by delayed sip.**

**Possible causes:**

1 Separation channel overloaded with sample.

2 Partial clog in the separation channel.

**What to do:**

1 Lower the starting sample concentration.

2 Reprime the chip. See “LabChip Kit Essential Practices” on page 28 for instructions on how to reprime the chip.

## Symptom: Unexpected sharp peaks.

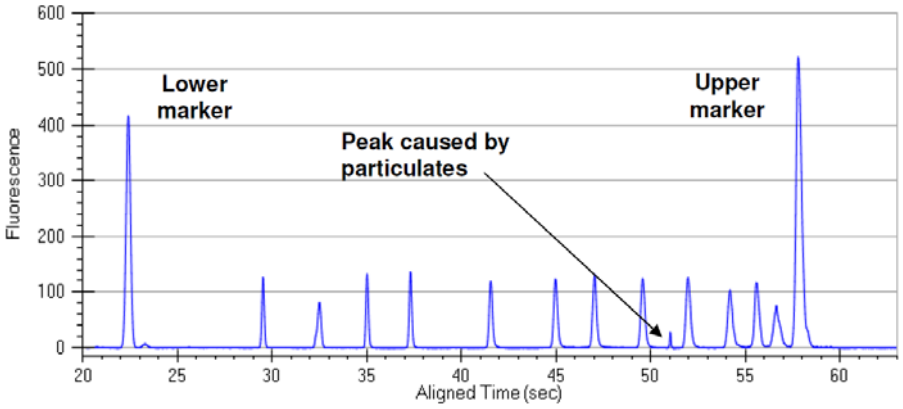


**Figure 17. Unexpected sharp peak.**

**Possible causes:**

- Dust or other particulates introduced through sample or reagents.

**What to do:**

**1** Do one or all of the following:

- Replace the 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent) water 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.

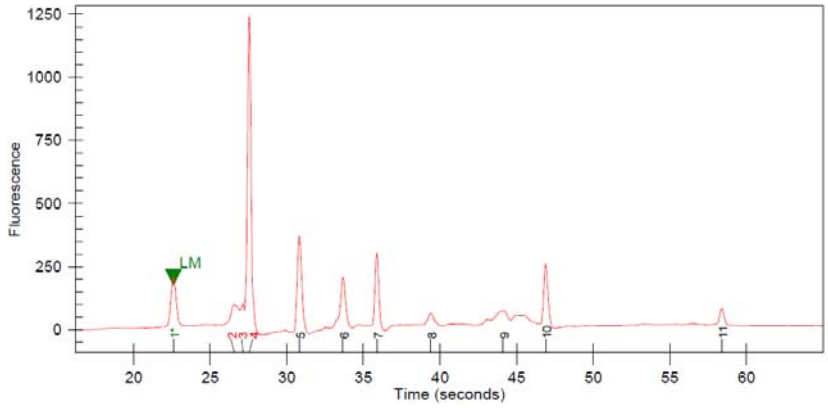


**Figure 18. Humps in several electropherograms.**

**Possible causes:**

1 Electrode 7 is dirty and has contaminated the Gel-Dye mixture in well 7.

**What to do:**

1 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 PerkinElmer prior to shipment.*

*Normal migration time windows for the markers are:*

- *DNA High Sensitivity assay Lower Marker (21-25 seconds)*
- *DNA High Sensitivity assay Upper Marker (54-64 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 within the separation channel will slow down migration, and less dye in the separation channel will make peaks migrate faster.*

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

3 Gel-Dye was not primed properly into the chip.

**What to do:**

1 Prepare a fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye mixture. See “LabChip Kit Essential Practices” on page 28 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 return of the chip to PerkinElmer. Please send a data file showing the failure along with the return request.

3 Minimize the loading of particulates in the sample by performing a centrifuge spin of the sample plate (e.g. 3000 rcf for 5 minutes) before starting a new run. The debris may be flushed out of the chip by washing and re-priming the chip. See "LabChip Kit Essential Practices" on page 28 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.

# Frequently Asked Questions

**1** Why is 120 µL of gel-dye needed in Chip Well 10 for the DNA High Sensitivity assay (High-throughput workflow) and not the other DNA assays?

*As a result of the formulation of the DNA High Sensitivity assay, additional ions are needed.*

**2** Why is 75 µL of Marker solution needed to run 96 samples using the DNA High Sensitivity assay (High-throughput workflow) and only 50 µL of Marker solution for the other DNA assays?

*For the DNA High Sensitivity assay, more marker and sample are sipped compared to the other DNA assays.*

**3** If my DNA samples fall within the sizing range and linear concentration range of both the DNA 1K and the DNA High Sensitivity assays, which assay should I use?

*The DNA High Sensitivity assay should be used since less sample is required to run this assay. If the sample exceeds the assay concentration limits of the DNA High Sensitivity assay, then it can be further diluted and re-tested.*

**4** Why can only 96 samples be tested per High-throughput chip preparation for the DNA High Sensitivity assay and up to 384 samples for the other DNA assays?

*For the DNA High Sensitivity assay, ion depletion occurs past 96 samples. Additionally, since more marker and sample are sipped compared to the other DNA assays, the waste well in the chip fills up more quickly.*

**5** If using the DNA High Sensitivity assay to test samples fractionated by the LabChip XT, what buffer do I use to prepare the ladder and buffer for the Buffer Tube?

*To test LabChip XT fractionated samples use the Limited Sample Workflow. To prepare the ladder and buffer for the Buffer Tube use LabChip XT Running Buffer as the DNA Sample Buffer. After removing the extracted collection from LabChip XT chip, pipette out 300 µL of Running Buffer from source well.*

# 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.*

For assay and instrument troubleshooting, refer to the LabChip GX Touch software Help file or call PerkinElmer Technical Support at 1-800-762-4000.

## General

- Allow the chip, sample plate and all reagents to equilibrate to room temperature before use (approximately 20 to 30 minutes).
- 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 PerkinElmer-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” (described next) will help avoid introducing bubbles into the chip when pipetting the gel.

1. PerkinElmer 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

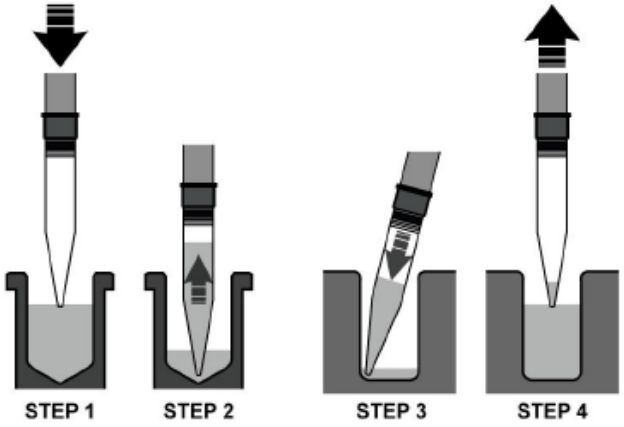


**Figure 19. Reverse pipetting.**

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 4°C when not in use.
- The LabChip dye contains DMSO and should 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 well.
- Protect the dye and Gel-Dye mixture from light. Store in the dark at 4°C when not in use.
- The Gel-Dye mixture expires 3 weeks after preparation.

# Chips

## Repriming Chips

***Note:*** *Buffer tubes filled with 1X DNA sample buffer or water should be placed into the instrument while priming or washing chips.*

- Touch the *Unload Chip* button on the *Home* screen to open the instrument door. Place the chip into the instrument.
- Close the chip door securely and choose the corresponding assay.
- Touch the *Prime* button on the *Home* screen to reprime the chip.

## Washing and Repriming Chips

- Touch the *Unload Chip* button on the *Home* screen to open the instrument door.
- Return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from the chip wells using a vacuum line.
- Ensure that each active well (1, 3, 4, 7, 8 and 10) is rinsed and completely aspirated twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
- Add 120 µL of Chip Storage Buffer to each active well (1, 3, 4, 7, 8 and 10).
- Place the chip in the LabChip GX Touch/GXII Touch instrument.
- Close the chip door securely.
- Touch the *Wash* button on the *Home* screen (Figure 20).

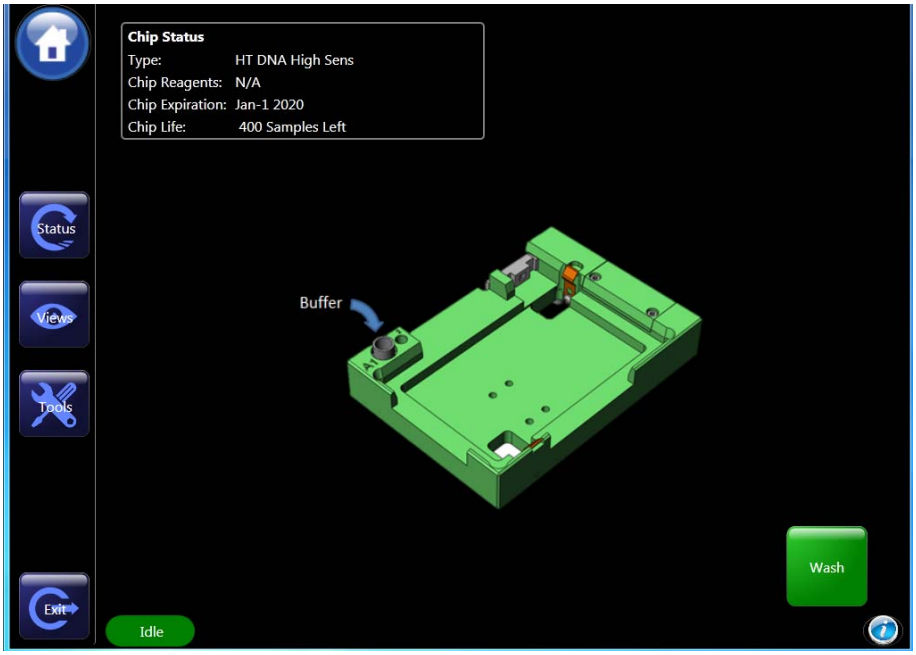


**Figure 20. *Wash* screen.**

- After the completion of the wash cycle return the chip to the chip container ensuring the sipper is immersed in fluid.
- Thoroughly aspirate all fluid from the chip wells using a vacuum line.
- Ensure that each active well (1, 3, 4, 7, 8 and 10) is rinsed and completely aspirated twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
- Add Gel-Dye solution to chip wells 3, 7, 8 and 10 using a Reverse Pipetting Technique as shown in Figure 19.
- Add 50 µL (Low-throughput) or 75 µL (High-throughput) DNA Marker (green cap ●) to chip well 4. Please note that the marker well may need to be replenished if the chip is in idle mode on the instrument for an extended period of time.
- Place the chip into the LabChip GX Touch/GXII Touch instrument.
- Close the chip door securely.
- Touch the *Run* or *Prime* button on the *Home* screen.

- If air bubbles are not dislodged after a reprime, apply a vacuum to the sipper. Perform this by filling all active wells with 100 µL of Chip Storage Buffer. Then suction the sipper with a vacuum line as shown in Figure 21 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.

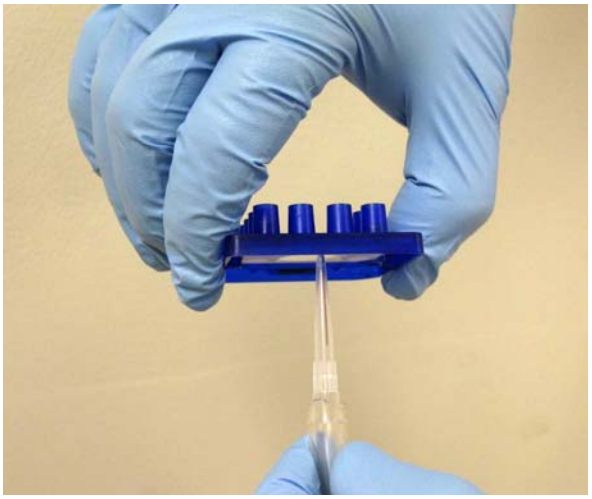

**Figure 21. Removing an air bubble or clog by suctioning the sipper with a vacuum line.**

## Other Considerations:

- Chips should be stored refrigerated prior to first use.
- Cover the active wells on the chip with Parafilm® and store at 4°C. If using the chip again within 24 hours it may be left at room temperature.
- 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.
- The sipper must be kept immersed in fluid at all times and should not be exposed 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 chips to dust by keeping them in a closed environment such as in the chip 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.
- PerkinElmer 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.
- Sample plates containing gas bubbles and/or particulate debris should be spun down at 3000 rpm (1250 rcf) prior to analysis.
- Up to 96 samples in a 96-well or 384-well plate can be run with a single chip preparation.

***Note:*** *The number of samples per chip prep is 96. A 384-well plate may be used to conserve sample volume but only 96 wells of the 384 can be tested.*

# Chip Well Aspiration Using a Vacuum

Aspirating with a pipette can leave used reagents in the chip wells. For this reason, PerkinElmer 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).

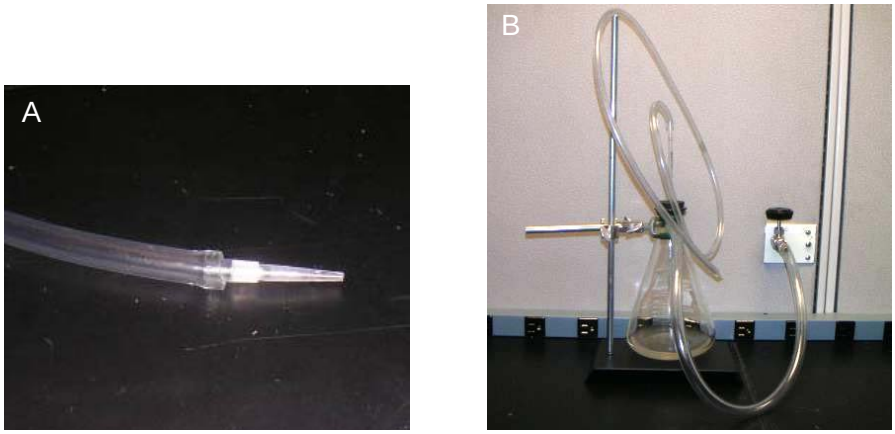


**Figure 22. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap.**

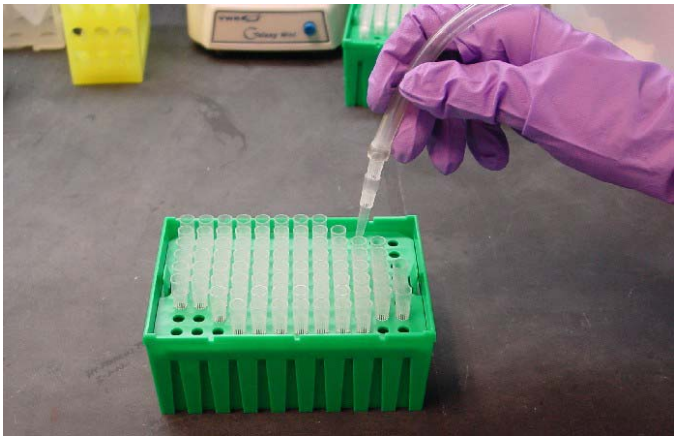

**Figure 23. Replacing the disposable pipette tip.**

# Customer Technical Support

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

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