Protein Express Assay User Guide

For LabChip® GXII Touch

and LabChip® GXII

The protocols defined in this User Guide apply to all of the LabChip instrument products running the Protein Express Assay. The LabChip GXII Touch is used for general visual reference in most examples in this guide.

CE

PN CLS140156 Rev. F
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Specifications

Assay Specifications

Table 1. Assay Specifications

Sizing Range	P100 Assay: 14 kDa - 100 kDa P200 Assay: 14 kDa - 200 kDa
Sizing Resolution[a]	± 10% difference in molecular weight
Sizing Accuracy	± 20%
Linear Concentration Range	5.0 - 2000 ng/µL
Maximum Total Protein Concentration	10 mg/mL
Quantitation Reproducibility	30% CV up to 120 kDa. Above 120 kDa, quantitation is not specified.
Chip Lifetime	HT: 400 samples 24: 200 samples
Samples per Chip Prep	HT: up to 384 samples 24: up to 48 samples
Chip Preps per Reagent Kit	4 HT chip preps, or 8 LT chip preps

a. Resolution is defined as the height of the valley between two peaks to be no more than 50% of the maximum peak height. Actual separation performance can depend on the sample and application.

Sample Conditions

Table 2. Sample Conditions

Buffers, Salts and Additives	Refer to Compatible Buffers, Salts and Additives on page 34 for compatibility with specific buffers, salts and additives. If your conditions are not listed, contact PerkinElmer (see page 36) for more information on compatibility.
Particulates	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 1M

Storage Conditions

Chip Storage: Prior to use, store chips at 2 - 8°C. After use, store chips at room temperature (20 - 25°C) for up to 30 days.

Reagent Storage: Store reagents at 2 - 8°C. Protect the Dye, prepared Gel-Dye solution, and Lower Marker from light.

CRITICAL:
The chip and all reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.

Reagent Kit Contents

Protein Express Reagent Kit, P/N CLS960008

Table 3. Reagents

Reagent	Vial	Quantity
Dye Solution	Blue	1 vial, 0.13 mL
Sample Buffer	White	4 vials, 1.5 mL each
Gel Matrix	Red	3 vials, 1.7 mL each
Ladder	Yellow	2 vials, 0.08 mL each
Lower Marker	Green	2 vials, 0.5 mL each
Wash Buffer	Purple	5 vials, 1.8 mL each

Table 4. Consumable Items

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

Protein Express LabChips

Table 5. Protein Express LabChips

Item	Part Number
HT Protein Express LabChip (for use with GXII Touch HT or GXII)	760499
24 Protein Express LabChip (for use with GXII Touch 24 or HT)	CLS138950

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 it is 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 Solution contains DMSO. Avoid contact with skin and eyes.*
- *Dye Solution contains SDS. Avoid inhalation and contact with skin and eyes.*
- *Wash Buffer and Sample Buffer contain LDS. Avoid inhalation and contact with skin and eyes.*
- *Gel Matrix contains Methyl urea. Avoid contact with skin and eyes.*

Usage

The Protein Express Assay is for use with the LabChip GXII Touch instrument. LabChip GXII Touch instruments are for research use only and not for use in diagnostic procedures.

Preparation Procedures

CRITICAL:

- *The chip and all reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. Protect the Dye, prepared Gel-Dye solution, and Lower Marker from light.*
- *The assay requires exact and consistent adherence to the protocol as shown in this guide, or results may be compromised by increased variability.*
- *Fresh Milli-Q® water should be obtained the day of the assay.*
- *Adherence to the full vortex time is important for assay performance.*

Additional Items Required

- 0.6 mL centrifuge tubes and/or 96-well plates for denaturing protein samples.
- Means for heating samples to 100°C — 96-well PCR instrument or heating block.

Note: *Avoid using non-stick lab consumables. They may induce unexpected or erratic assay results caused by surface treatments leaching into dye or gel components.*

- 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent).
- 70% isopropanol solution in DI water.
- Reducing agents: BME (beta-mercaptoethanol), 1M DTT (dithiothreitol) or 100 mM TCEP.

Preparing the Gel-Dye Solution

Notes: *The Dye contains DMSO and* ***must be thawed completely*** *before use. Thaw the dye at room temperature for at least 30 minutes, and then we recommend rolling the vial vigorously between gloved hands to ensure it is fully thawed.*

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.*

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

For High Throughput Chip Preparation (> 48 samples)

1. Vortex the thawed Dye Solution for 20 seconds and quickly spin down before use.
2. Using a reverse pipetting technique, transfer 520 µL of Protein Express Gel Matrix (red cap 🔴) to the top “basket” of a provided spin filter.

Note: *Gel matrix is extremely viscous. It is important to use a reverse pipetting technique, as described on page 29, to accurately transfer the correct amount of gel.*

3. Add 20 µL of Protein Express Dye Solution (blue cap 🔵) to the 520 µL Gel Matrix in the spin filter.
4. Cap the spin filter, invert, and vortex in the inverted orientation until the Gel-Dye solution is a uniform blue color.
5. For Destain Solution, transfer 250 µL of Protein Express Gel Matrix (red cap 🔴) to a second spin filter.
6. Spin the Gel-Dye solution and the Destain solution at 9300 rcf for 8 minutes at room temperature. Ensure that all the solution has passed through the filter (spin longer if necessary), then discard the filter baskets and cap the tubes. Store the Gel-Dye solution in the dark until ready to use.

For Low Throughput Chip Preparation (≤ 48 samples)

1. Vortex the thawed Dye Solution for 20 seconds and quickly spin down before use.
2. Using a reverse pipetting technique, transfer 280 µL of Protein Express Gel Matrix (red cap 🔴) to the top “basket” of a provided spin filter.

Note: *Gel matrix is extremely viscous. It is important to use a reverse pipetting technique, as described on page 29, to accurately transfer the correct amount of gel.*

3. Add 10.7 µL of Protein Express Dye Solution (blue cap 🔵) to the 280 µL Gel Matrix in the spin filter.
4. Cap the spin filter, invert, and vortex in the inverted orientation until the Gel-Dye solution is a uniform blue color.
5. For Destain Solution, transfer 180 µL of Protein Express Gel Matrix (red cap 🔴) to a second spin filter.
6. Spin the Gel-Dye solution and the Destain solution at 9300 rcf for 8 minutes at room temperature. Ensure that all the solution has passed through the filter (spin longer if necessary), then discard the filter baskets and cap the tubes. Store the Gel-Dye solution in the dark until ready to use.

Preparing the Sample Denaturing Solution

Note: *The volumes shown here are sufficient for preparing Sample Denaturing Solution for 96 samples. If running in Low Throughput mode (48 samples or fewer), prepare the Sample Denaturing Solution using 1/2 the indicated volumes of Sample Buffer and reducing agent.*

1 Pipette 700 µL of Protein Express Sample Buffer (white cap ◯). into a 2.0 mL centrifuge vial.

2 If samples need to be reduced, add 24.5 µL of BME or 1 M DTT or 3.75 µL of 100 mM TCEP.

3 Vortex for 10 seconds.

Preparing the Protein Samples and Ladder

Note: *Samples can be prepared in either a 96-well PCR plate or in 0.6 mL microcentrifuge tubes (and subsequently pipetted into a 96-well or 384-well plate).*

1 For each sample to be analyzed, pipette 7 µL of Denaturing solution into the wells of a microtiter plate or into individual 0.6 mL microcentrifuge tubes.

2 Pipette 2 µL of each protein sample into the wells of the 96-well plate or microcentrifuge tube. When finished, cover the plate with a foil seal to minimize evaporation. (If running the High Sensitivity Assay, pipette 5 µL of each protein sample.)

3 Ensure the Protein Express Ladder has been warmed to room temperature, then vortex gently for 10 seconds. Briefly spin the ladder vial. Ensure no precipitate is visible in the solution. If precipitate is present, let the vial sit at room temperature for a little longer then repeat the gentle vortex and spin.

4 Pipette 12 µL of Protein Express Ladder into a microcentrifuge tube or into the well of the microtiter plate.

Do not add denaturing solution to the ladder.

5 Denature samples and ladder at 100°C for 5 minutes. Optimum denaturing conditions may vary by sample type.

6 Tap or spin the sample plate to move the fluid to the bottom of the wells.

7 Add 35 µL of water (Milli-Q® or equivalent) to each sample well or sample tube (if running the High Sensitivity assay, add 32 µL of water). If using a plate, mix by pipetting up and down a few times or by placing the plate on a shaker. If using sample tubes, mix by vortexing gently. Avoid creating air bubbles. Water should not be added to the denatured samples more than one hour before starting the assay.

8 Add 120 µL of water (Milli-Q® or equivalent) to the Protein Express Ladder. Vortex the ladder mixture for a few seconds to achieve good mixing.

9 Spin the Protein Express Ladder (and sample tubes if used) for 15 seconds using a mini-centrifuge.

10 If the samples are prepared in tubes, transfer 44 µL of each sample onto a 96-well plate.

11 Spin the sample plate at 3000 rpm for 5 minutes to eliminate bubbles and move the fluid to the bottom of the wells.

12 Place the sample plate onto the instrument's plate holder.

13 Transfer 120 µL of prepared ladder to the provided 0.2 mL Ladder Tube. Ensure there are no air bubbles in the Ladder Tube.

14 Insert the Ladder Tube into ladder slot on the LabChip GXII Touch or GXII instrument.

Preparing the Buffer Tube

1 Add 750 µL of Protein Express Wash Buffer (purple cap ●) 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 buffer slot on the LabChip GXII Touch or GXII instrument.

Note: *Replace the Buffer Tube with a freshly prepared tube every 8 hours when the chip and instrument are in use.*

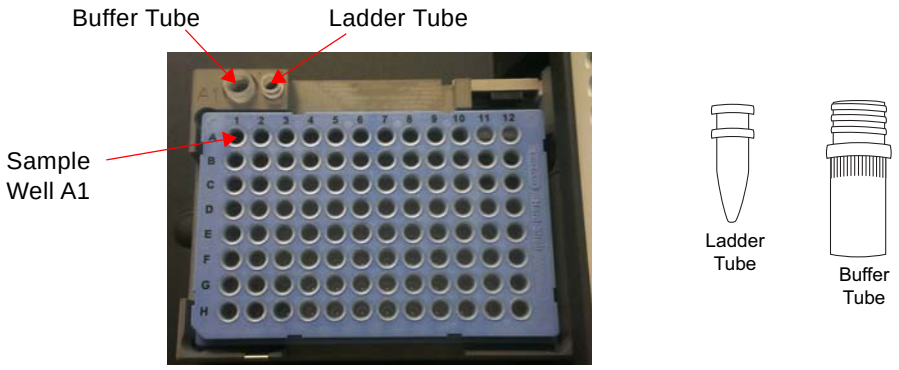

Figure 1. Locations of the Buffer Tube and Ladder Tube in the GXII Touch Instrument

Preparing the Chip

1 Allow the chip to equilibrate to room temperature (20 - 25°C) 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 2). For more details on how to set up a vacuum line see page 35.

3 Rinse and completely aspirate each active chip well (1, 2, 3, 4, 7, 8, 9, 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 central region of the detection window. Use the provided Detection Window Cleaning Cloth to clean the chip detection window.

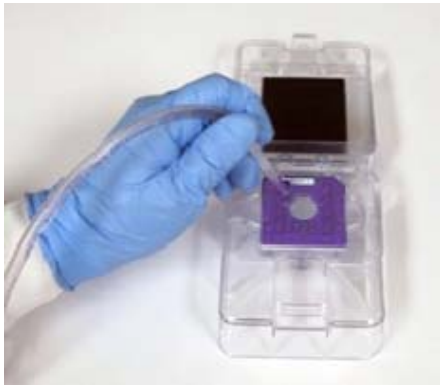

Figure 2. Using a vacuum to aspirate the chip wells is more effective than using a pipette. See page 35 for more details.

5 Using a reverse pipetting technique, add Gel-Dye solution to chip wells 3, 7, 8, and 10 as shown in Figure 3 (High-throughput) or Figure 4 (Low-throughput).

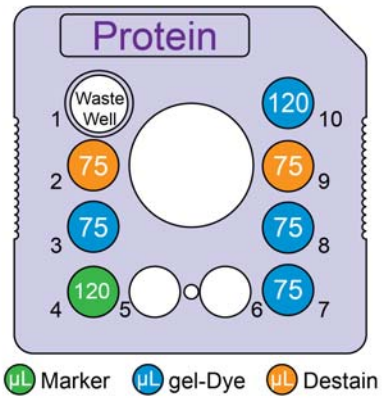

Figure 3. Reagent placement for High-throughput (up to 384 samples)

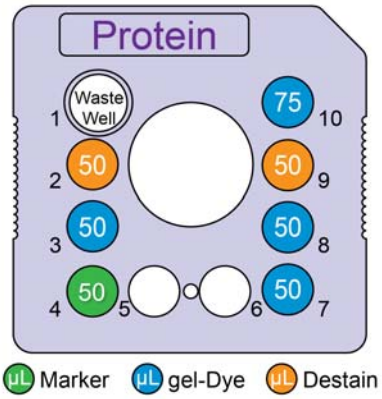

Figure 4. Reagent placement for Low-throughput (up to 48 samples)

Note: *Up to 48 samples can be analyzed in LT mode.*

6 Using a reverse pipetting technique, add Destain Solution from spin filter tube to chip wells 2 and 9 as shown in Figure 3 (High-throughput) or Figure 4 (Low-throughput).

7 Add Protein Express Lower Marker (green cap ●) to chip well 4 as shown in Figure 3 (High-throughput) or Figure 4 (Low-throughput). Make sure the marker volume is pipetted accurately. If there is not enough marker in chip well 4, the marker will deplete and will not be added to subsequent samples on-chip. Data collected without marker peaks cannot be analyzed by the software.

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

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

Inserting a Chip into the LabChip GXII Touch or GXII Instrument

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

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

3 Touch the *Unload Chip* button on the *Home* screen. (For GXII, press the *Chip* button.)

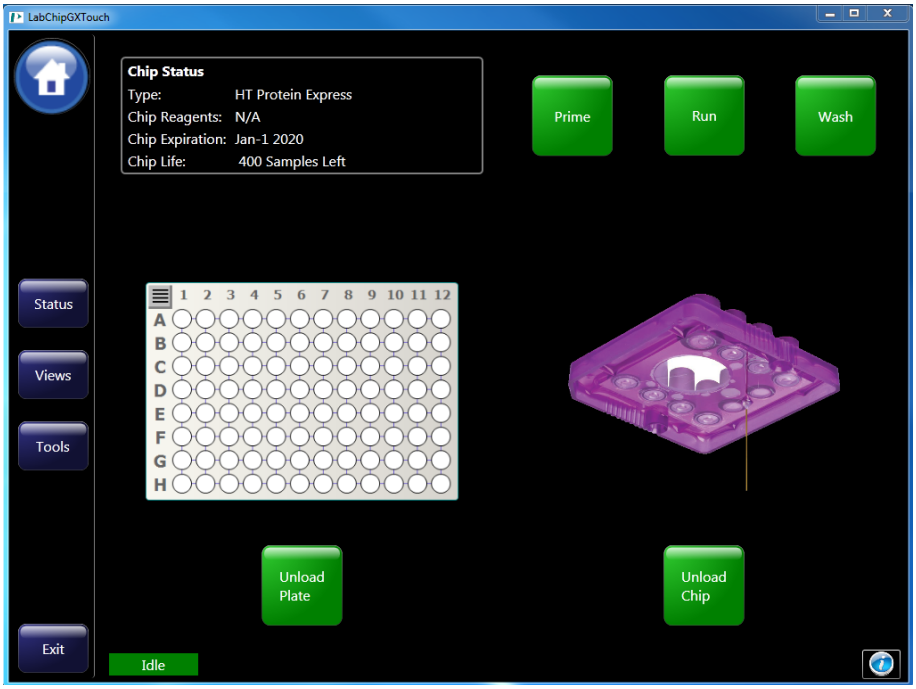

Figure 5. *Home* Screen

4 Insert the chip into the LabChip GXII Touch instrument (Figure 6) and close the chip door securely. (For GXII, release the latch, insert the chip, latch the chip cartridge, and push in the cartridge.)

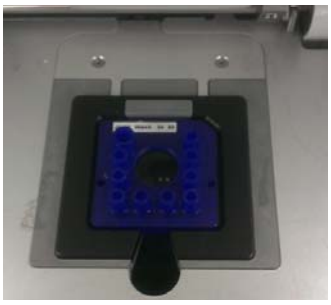

Figure 6. Chip in the LabChip GXII Touch Instrument

5 Touch the *Load Plate* button on the *Home* screen (Figure 5) to retract the sample plate and move the sipper to the Buffer Tube. (For GXII, press the *Eject* button.)

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

Running the Assay

Note: *Chips can be primed independently from running assays on the LabChip GXII Touch instrument. Touch the Prime button on the Home screen (Figure 5). Select the desired assay from the Assay drop-down list (see Figure 8).Touch the Prime button on the Chip Priming screen. Make sure the Buffer Tube is placed on the instrument.*

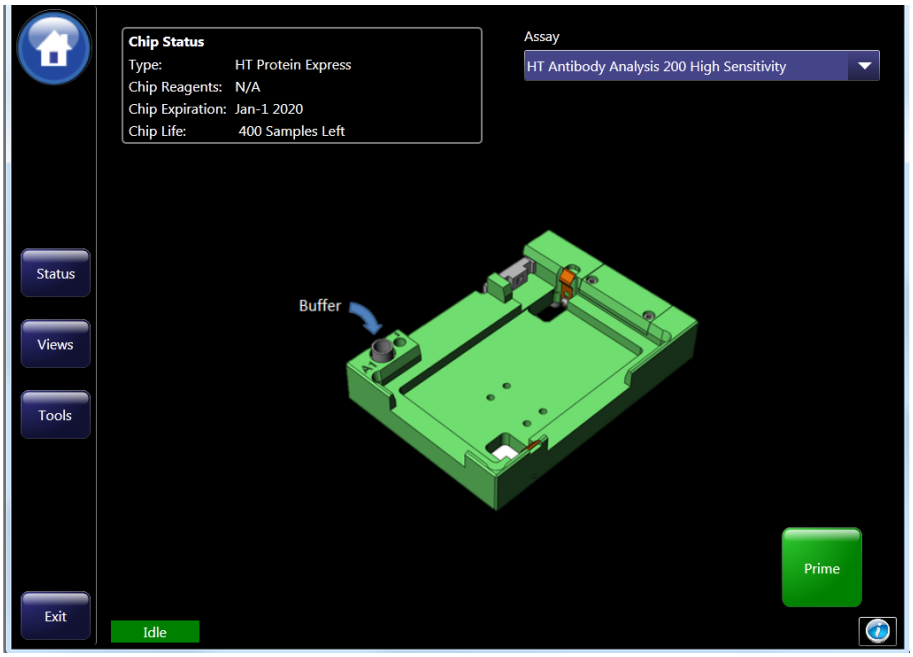

Figure 7. Chip Priming Screen

1 Touch/click the *Run* button (see Figure 5 on page 14).

2 Select the appropriate assay type (see Figure 8), 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 9). Touch the green arrow.

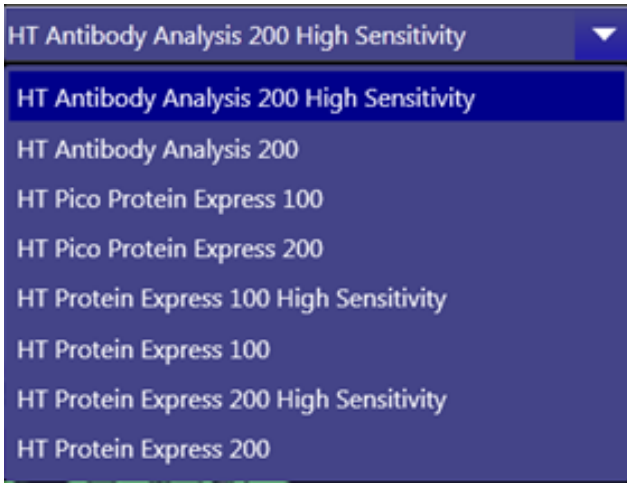

Figure 8. Assay List

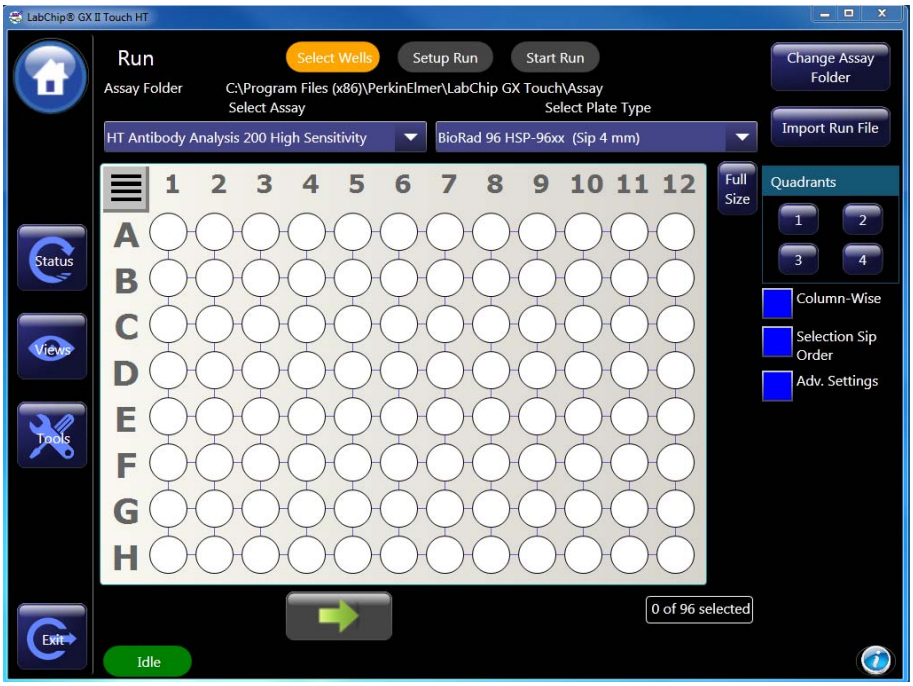

Figure 9. Selecting Wells

For Protein Express assays, appropriate assay types are:

- **Protein Express 100:** For sizing of proteins in the 14 kDa to 100 kDa range.
- **Protein Express 100 High Sensitivity:** For sizing of proteins in the 14 kDa to 100 kDa range. Greater sensitivity but requires a larger amount of sample. Slightly lower resolution may be observed.

- **Protein Express 200:** For sizing of proteins in the 14 kDa to 200 kDa range.
- **Protein Express 200 High Sensitivity:** For sizing of proteins in the 14 kDa to 200 kDa range. Greater sensitivity but requires a larger amount of sample. Slightly lower resolution may be observed.
- **Antibody Analysis:** For analyzing Ab samples. Same choices of assays as in Protein Express.

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 10). Touch the green arrow.

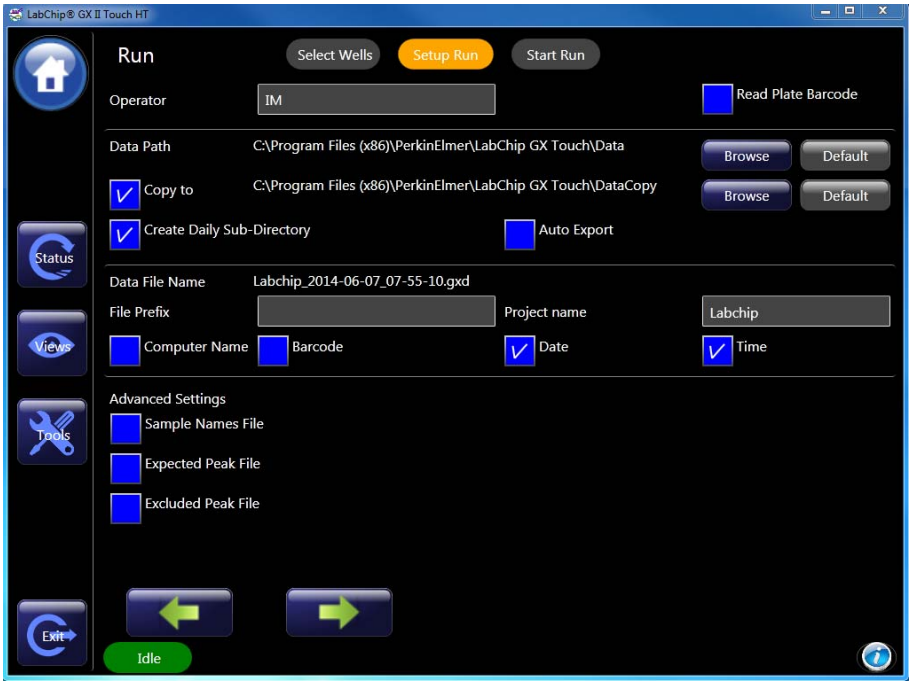

Figure 10. Run Setup Screen

4 Touch/click *Start* to begin the run.

Figure 11. Starting a Run

Cleaning and Storing the Chip

After use, the chip must be cleaned and stored in the chip storage container.

1 Place the chip into the chip storage container. The sipper should be 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, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q® or equivalent).

4 Add 120 µL of water (Milli-Q® or equivalent) to the active wells.

5 Cover the wells with Parafilm® to prevent evaporation and store the chip at room temperature until next use. Allowing chip wells to dry may lead to changes in chip performance. Use to the total lifetime within 30 days of analyzing the first sample. See Assay Specifications on page 3 for Chip Lifetime.

Chip Cartridge Cleaning

1 Daily

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

b Use the provided lint-free swab dampened with water (Milli-Q® or equivalent) to clean 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 GXII Touch or 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

Protein Express Ladder Result

The electropherogram of a typical Protein Express ladder is shown in Figure 12. Peaks to the right of the lower marker and system peaks in order of increasing migration time correspond to proteins of increasing size i.e., 15.9 kDa, 20.4 kDa, 28.9 kDa, 48.4 kDa, 68.4 kDa and 119.2 kDa respectively.

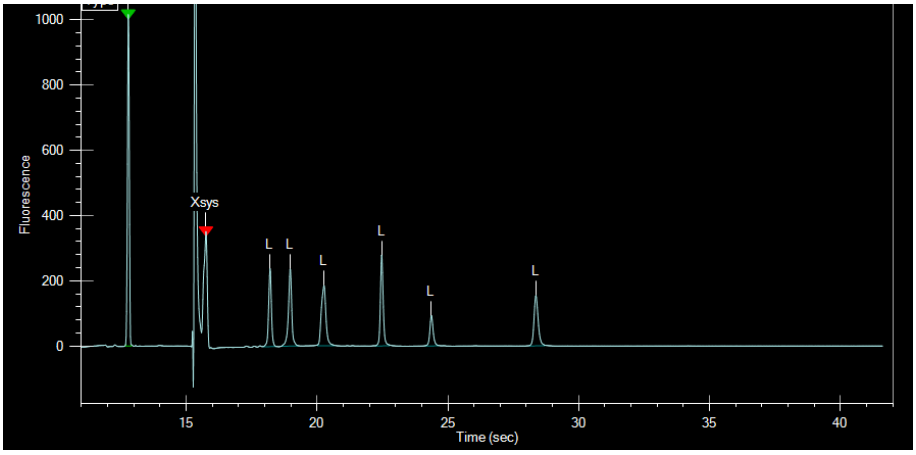

Figure 12. Protein Express Ladder Electropherogram

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 there may be debris in the samples, spin the sample plate down at 3000 rpm 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 the marker well or the chip remaining idle on the 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. (For GXII, press the *Chip* button on the front of the instrument.)
- 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.
- Rinse and completely aspirate chip well 4 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).

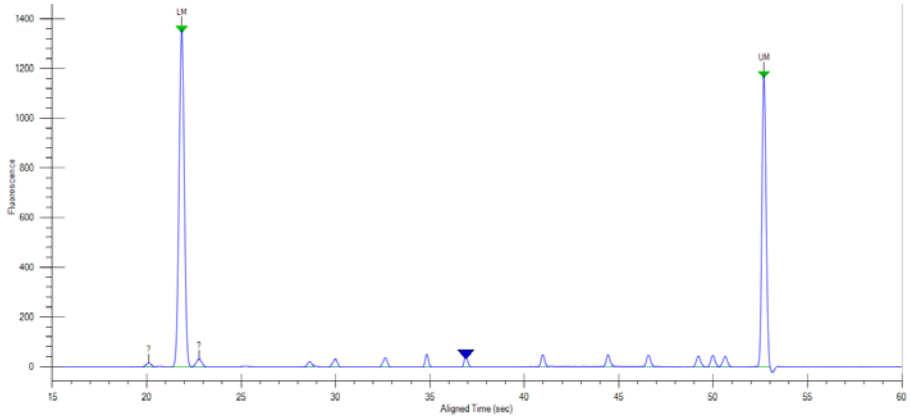

Figure 13. Small Ladder Peaks in Sample Well caused by Delayed Sip

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.

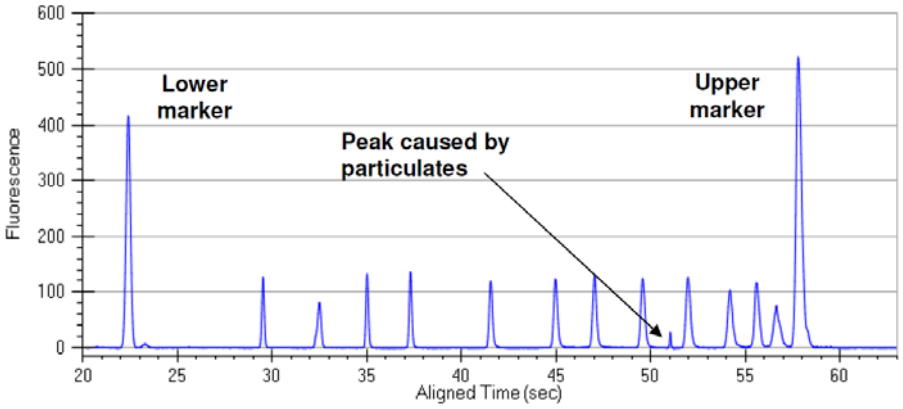

Figure 14. Unexpected Sharp Peak

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.

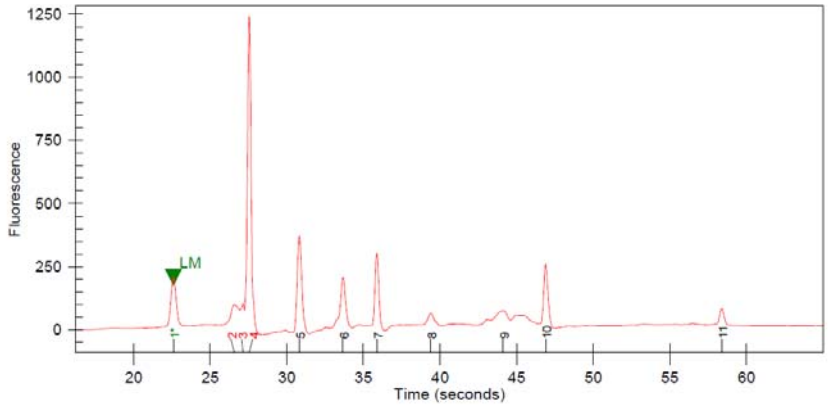

Figure 15. Humps in Several Electropherograms

Possible causes:

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

2 High concentrations of detergent in the sample buffer can sometimes cause humps in the electropherogram.

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.

2 Lower the detergent concentration in the sample (see Compatible Buffers, Salts and Additives on page 34).

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:

- *Protein Express Assay Lower Marker: 11-13.5 seconds*
- *Upper Ladder Protein on the first plate: 23-32 seconds*
- *Upper Ladder Protein on the third plate: 20-29 seconds*

Possible causes:

1 Incorrect Gel to 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 solution was not primed properly into the chip.

What to do:

1 Prepare fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye solution. 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 spinning the sample plate at 3000 rpm for 5 minutes and/or selecting the Sip 4 mm plate type in the Select Wells screen before starting a new run. Unclog 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.

Symptom: High baseline fluorescence (e.g., greater than 1000 counts).

Possible causes:

1 The destain wells (2 and 9) do not contain Destain solution (gel matrix with no dye).

2 The destain wells (2 and 9) may have been contaminated with dye either because the well was improperly flushed after priming or because dye was mistakenly pipetted into the well.

What to do:

1 Prepare a fresh Destain solution. Wash and reprime the chip with the new Destain solution. See LabChip Kit Essential Practices on page 28 for instructions on how to wash and reprime the chip.

Symptom: Lower than expected signal for ladders and samples.

Possible causes:

1 Improper SDS concentration in Gel-Dye solution.

What to do:

1 Ensure that Dye Concentrate is completely thawed and mixed. Prepare fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye solution. See LabChip Kit Essential Practices on page 28 for instructions on how to wash and reprime the chip.

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 Software Help file or call PerkinElmer Technical Support (see page 36).

General

- Allow the chip, sample plate and all reagents to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.Protect the Dye, prepared Gel-Dye solution, and Lower Marker from light.
- 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 Detection Window Cleaning 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” (see page 29) will help avoid introducing bubbles into the chip when pipetting the gel.

1. PerkinElmer, 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 60 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

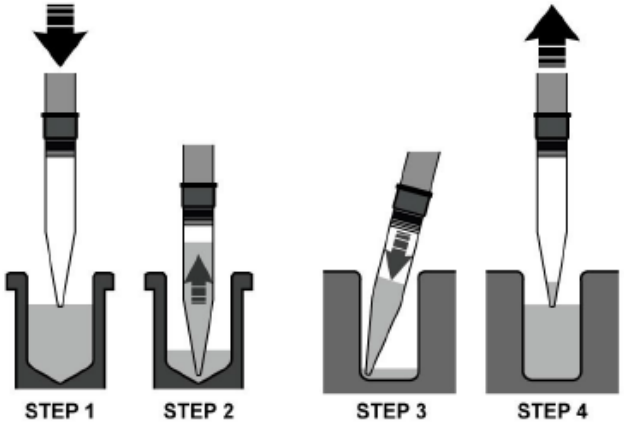

Figure 16. Reverse Pipetting

1. Depress the pipette plunger to the first stop, then continue to depress the plunger past the first stop, but not all the way to the second stop.
2. Aspirate the selected volume plus an excess amount from the tube. Leave the pipette tip in the solution for 5-10 seconds to ensure that the aspiration is complete.
3. Dispense the selected volume into the corner of the well by depressing plunger to the first stop. Hold the pipette for 10 seconds with the plunger at the first stop to ensure that the dispense is complete.
4. Withdraw the pipette from the well.
5. Retain the excess volume in the pipette tip if performing an additional Reverse Pipetting transfer, or return the excess volume to the source container for later use.

Reagents

- Store reagents as specified in Storage Conditions on page 4.
- Protect the Dye, Gel-Dye solution, and Lower Marker from light.
- The Gel-Dye solution expires 3 weeks after preparation.
- For optimal performance, use one reagent kit per chip.

Chips

Repriming Chips

1. Touch the *Unload Chip* button on the *Home* screen to open the instrument door. (For GXII, press the *Chip* button.) Place the chip into the instrument.
2. Close the chip door securely and choose the corresponding assay. (For GXII, latch the chip cartridge, push the chip cartridge in, and press the *Eject* button.)
3. Touch the *Prime* button on the *Home* screen, select the assay, and touch the *Prime* button to reprime the chip. (For GXII, run the desired assay. The chip is primed at the start of the run.)

Washing Chips

Important Note: *Wash chips only with water (Milli-Q® or equivalent). Use of any other reagents (including Wash Buffer) is likely to cause even more artifacts in subsequent data.*

Notes: *Some protein samples may have components which produce data with extra peaks, spikes or other artifacts. When these artifacts are present, washing chips on the LabChip GXII Touch or GXII immediately before the next use can often restore data quality.*

Chips should only be washed on the LabChip GXII Touch or GXII immediately before they are prepared with fresh reagents and primed on the instrument. Chips should not be washed and left with water in the chip channels for any extended period of time.

For most protein samples, the only chip cleaning protocol that is required is to rinse and aspirate the active wells twice with water (Milli-Q® or equivalent), and store the chip with 120 µL of water in each active well.

1. Thoroughly aspirate all fluid from the chip wells using a vacuum line.
2. Rinse and completely aspirate each active well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
3. Add 120 µL of water (Milli-Q® or equivalent) to each active well (1, 2, 3, 4, 7, 8, 9, and 10).

4 Touch the *Unload Chip* button on the *Home* screen and place the chip into the instrument. (For GXII, press the *Chip* button and load the chip into the instrument.)

5 Close the chip door securely. (For GXII, close the chip cartridge, push the chip cartridge in, and press the *Eject* button.

6 Transfer 750 µL of water (Milli-Q® or equivalent) into the Buffer Tube. Install into the instrument.

7 Touch the *Wash* button on the Home screen. Then touch the *Wash* button on the Wash screen.(Figure 17).

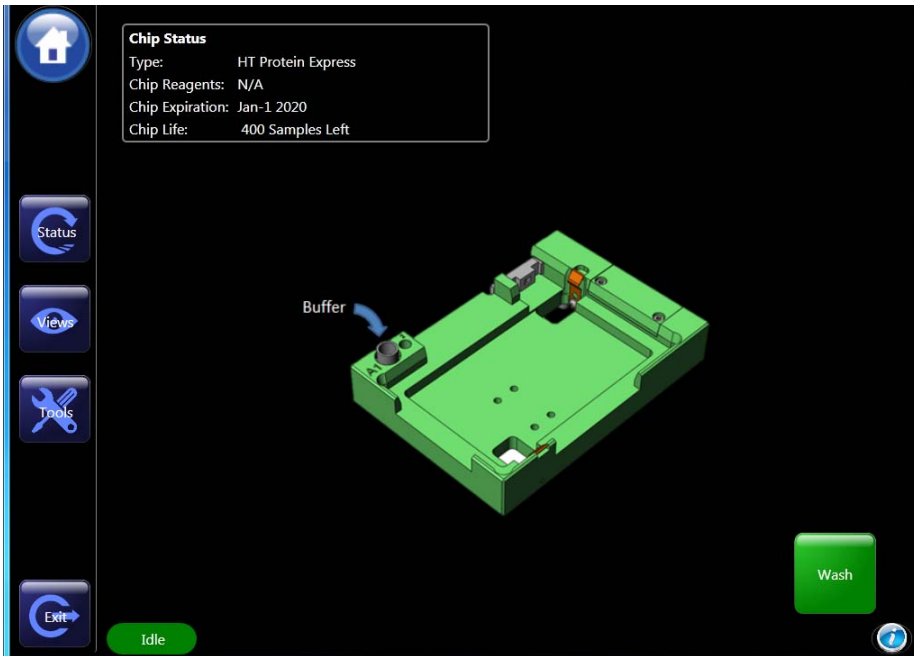

Figure 17. Wash Screen

8 After completion of the wash cycle, open the chip cartridge and return the chip to the chip container ensuring the sipper is immersed in fluid.

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

10 Replace fluid in the wells with freshly made reagents as described in Preparing the Chip on page 12. Do not let wells remain dry.

11 Transfer 750 µL of Wash Buffer (purple cap ●) into a clean Buffer Tube. Install into the instrument.

12 Install the Ladder Tube, sample plate and chip into the instrument and run the assay.

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 water (Milli-Q® or equivalent). Then suction the sipper with a vacuum line, as shown in Figure 18, 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.

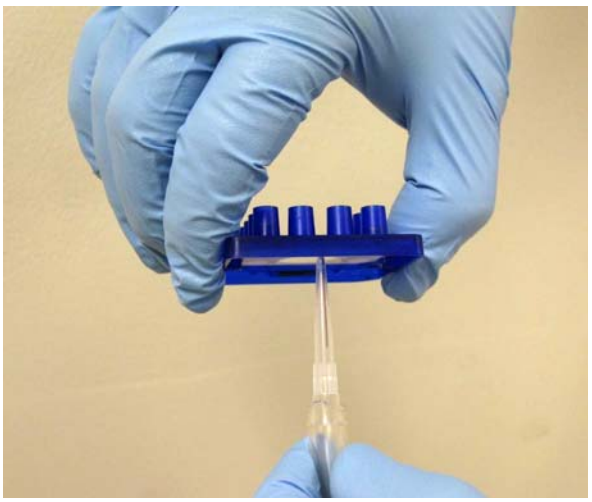

Figure 18. Removing an air bubble or clog by suctioning the sipper with a vacuum line

Other Considerations:

- Store chips as specified in Storage Conditions on page 4.
- 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 in the instrument for extended periods of time so that samples can be run as needed throughout the day. PerkinElmer recommends the chip be re-prepared after it has been idle for 8 hours, but the chip can be used continually over an 8-hour work day as long as the maximum recommended idle time of 8 hours and total chip lifetime number of samples are not exceeded.

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 four 96-well plates (400 samples) can be run with a single chip preparation when running the LabChip GXII Touch HT or LabChip GXII instrument. Up to 48 samples can be run with a single chip preparation when running the LabChip GXII Touch 24 instrument.

Compatible Buffers, Salts and Additives

Table 6. Compatible Buffers, Salts and Additives

Buffer & Salts	Concentration Limit	Additives	Concentration Limit
Tris Chloride	250 mM	Octyl Glucoside	2.5%
Tris Glycine	250 mM	Pluronic F68	0.1%
HEPES	500 mM	Sarcosyl	10%
PBS	8 X	CHAPS	0.5%
Sodium Citrate	150 mM	Tween 20	0.8%
Sodium Phosphate	250 mM	Triton X-100	0.6%
Sodium Acetate	600 mM	SDS	2%
Sodium Chloride	1000 mM	Zwittergent 3-14	0.4%
Sodium Azide	6%	PEG 3350	1%
Sodium Hydroxide	500 mM	Glycerol	30%
Potassium Chloride	900 mM	Urea	8 M
Ammonium Bicarbonate	1000 mM	Sucrose	1 M
Magnesium Chloride	300 mM	DMSO	25%
Imidazole	900 mM	EDTA	100 mM
PhosphoSafe		Ethanol	50%
BugBuster	2.5 X		
BPER			
POP Culture			
Insect POP Culture			

Table 7. Incompatible Buffers, Salts and Additives

Buffer & Salts	Concentration Limit	Additives	Concentration Limit
RIPA	All		

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 19). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 20).

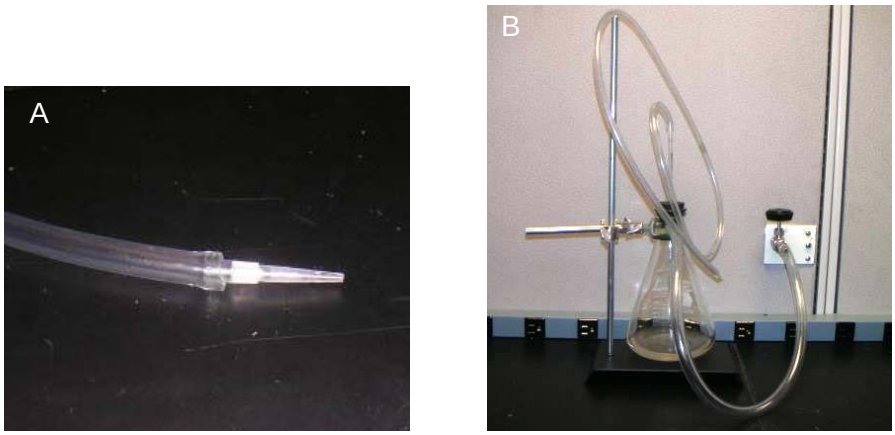

Figure 19. A: Permanent pipette tip attached to a house vacuum line; B: Vacuum line with trap

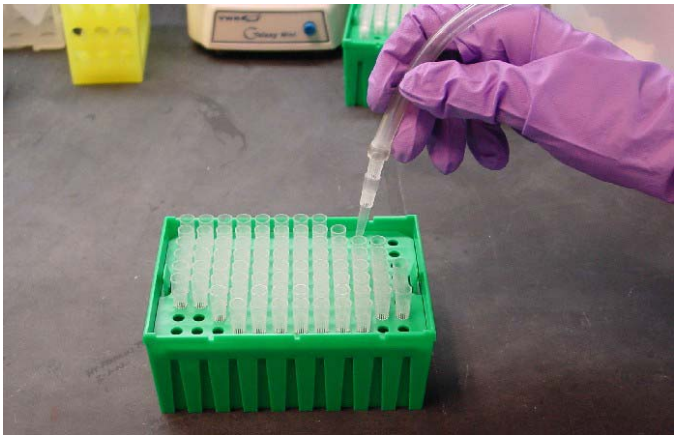

Figure 20. Replacing the Disposable Pipette Tip

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

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