LabChip User Guide

Protein Clear HR Assay User Guide

For LabChip® GXII Touch

 PN CLS146230 Rev
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CE

Contents

Specifications

Assay Specifications

Table 1. Assay Specifications

Sizing Range	14 - 250 kDa
Linear Concentration Range	10 - 1000 ng/µL (mAb, non-reduced main peak)
Maximum Sample Concentration	2000 ng/µL
Linearity (R^2)	> 0.995
Sizing Resolution[a]	Resolution >1.0 for VeriMAb reference standard
Sizing Precision RSD (CV)	< 2%
Relative Migration Time Precision RSD (CV)	< 2%
Separation Time per Sample	65 seconds
Percent Purity Reproducibility	< 0.5% main non-reduced IgG, < 5% all other peaks
Sensitivity (LOD)	5 ng/µL (mAb, non-reduced main peak)
Reagent Kit Primes	10
Chip Lifetime	400 samples
Maximum Number of Samples per Calibrated HT Chip Prep	96 samples
Maximum Number of Samples per Calibrated LT Chip Prep	48 samples
Maximum Sample Volume	2 µL

a. Resolution is defined as the difference in migration times divided by the sum of the full width half max for two closely migrating peaks.

Sample Conditions

Table 2. Sample Conditions

Buffers, Salts and Additives	Refer to “Compatible Buffers, Salts and Additives” on page 38 for compatibility with specific buffers, salts and additives. If your conditions are not listed, contact Revvity (see page 40) 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.

Kit Contents

Chip Storage: Prior to use, store chips refrigerated at 2°C - 8°C. After use, store chips at room temperature and use within 30 days.

Reagent Storage: Store Protein Clear HR Dye Solution (blue cap) at -20°C when not in use. Store all other reagents at 2°C - 8°C when not in use.

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light.
If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

Table 3. Protein Clear HR Reagent Kit Contents, P/N CLS960014

Reagent	Vial	Quantity
Protein Clear HR Dye Solution	Blue	1 vial, 0.13 mL
Protein Clear HR Sample Buffer	White	7 vials, 1.5 mL each
Protein Clear HR Gel Matrix	Red	3 vials, 1.7 mL each
Protein Clear HR Ladder	Yellow	1 vial, 0.11 mL
Protein Clear HR Lower Marker	Green	2 vials, 0.5 mL each
Protein Clear HR Wash Buffer	Purple	3 vials, 1.8 mL each
VeriMAb Standard	Orange	1 vial, 0.04 mL

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

Table 5. Protein Clear HR LabChips

Item	Part Number
Protein Clear HR LabChip for use with GXII Touch HT	CLS148695
Protein Clear HR LabChip for use with GXII Touch 24	CLS148696

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 Clear HR 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.

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light.
If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

Preparation Procedures

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light.
If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

Additional Items Required

- 0.6 mL centrifuge tubes and/or 96-well plates for denaturing protein samples.
- Means for heating samples to 70°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.
- Non-reducing agents: 250 mM IAM (iodoacetamide)

Preparing the Gel-Dye Solution

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light.
If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

Notes:

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.*

The Gel-Dye mixture can be stored in the dark for 3 weeks at 2-8°C.

The VeriMAb™ standard can be prepared and stored for up to 7 days at 2-8°C; however, for best results, prepare fresh.

For High Throughput Chip Preparation (up to 96 samples)

CRITICAL:

This assay requires exact and consistent adherence to the protocol as shown below, or results may be compromised by increased variability.

1. Invert and vortex the thawed Dye Solution at max speed for 20 seconds and quickly spin down before use.
2. Using a reverse pipetting technique, transfer 520 µL of Protein Clear HR 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 32, to accurately transfer the correct amount of gel to the spin filter. Incorrect ratios of gel to dye will cause inconsistent assay results.*

3. Add 20 µL of Protein Clear HR Dye Solution (blue cap 🔵) to the 520 µL Gel Matrix in the spin filter. For best results, make fresh and use immediately.
4. When transferring Gel-Dye mixture into the spin filter, invert as quickly as possible to minimize dye concentration interaction with the filter material.
5. Once the Dye Solution is added to the Gel Matrix, immediately cap and invert the spin filter to minimize dye concentrate interaction with filter material; then vortex for 10 seconds until the gel and dye are well-mixed.
6. For Destain Solution, transfer 250 µL of Protein Clear HR Gel Matrix (red cap 🔴) to a second spin filter.
7. Spin the Gel-Dye mix and the Destain Solution at 9300 rcf for 5 - 8 minutes at RT. Ensure that the microcentrifuge is set to RT and the material has passed through the filter (spin longer if necessary), then discard the filter baskets and cap the tubes. Store in the dark until ready to use.

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

For Low Throughput Chip Preparation (48 samples)

CRITICAL:

This assay requires exact and consistent adherence to the protocol as shown below, or results may be compromised by increased variability.

1. Invert and vortex the thawed Dye Solution at max speed for 20 seconds and quickly spin down before use.
2. Using a reverse pipetting technique, transfer 280 µL of Protein Clear HR 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 32, to accurately transfer the correct amount of gel to the spin filter. Incorrect ratios of gel to dye will cause inconsistent assay results.*

3. Add 10.7 µL of Protein Clear HR Dye Solution (blue cap 🔵) to the 280 µL Gel Matrix in the spin filter. For best results, make fresh and use immediately.
4. When transferring Gel-Dye mixture into the spin filter, invert as quickly as possible to minimize dye concentration interaction with the filter material.
5. Once the Dye Solution is added to the Gel Matrix, immediately cap and invert the spin filter to minimize dye concentrate interaction with filter material; then vortex for 10 seconds until the gel and dye are well-mixed.
6. For Destain Solution, transfer 180 µL of Protein Clear HR Gel Matrix (red cap 🔴) to a second spin filter.
7. Spin the Gel-Dye mix and the Destain Solution at 9300 rcf for 5 - 8 minutes at RT. Ensure that the microcentrifuge is set to RT and the material has passed through the filter (spin longer if necessary), then discard the filter baskets and cap the tubes. Store in the dark until ready to use.

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

Preparing the VeriMAb, Ladder, and Buffer Tube

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light.
If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

1. Prepare Non-Reducing Sample Buffer by transferring 90 µL of Protein Clear HR Sample Buffer (white cap ◯) to a microcentrifuge tube. Add 3 µL of 250 mM IAM solution.
2. Transfer 35 µL of Non-Reducing Sample Buffer to one well of a PCR plate, a PCR tube, or a 0.6 mL microcentrifuge tube.
3. Add 5 µL VeriMAb Standard (orange cap ●) to the 35 µL Non-Reducing Sample Buffer. Seal the plate or cap the tube and spin briefly to ensure the contents are at the bottom of the buffer.
4. Denature the VeriMAb Standard in the Non-Reducing Sample Buffer at 70°C for 10 minutes, then cool to room temperature.
5. Add 70 µL water (Milli-Q® or equivalent) and mix thoroughly by pipetting up and down.
6. Transfer 70 µL of prepared VeriMAb Standard to a clean well of a PCR plate.
7. Ensure the Protein Clear HR Ladder (yellow cap ●) 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.
8. Pipette 15 µL of Protein Clear HR Ladder into a supplied Ladder Tube.
9. Add 150 µL of water (Milli-Q® or equivalent) to the Ladder Tube and mix thoroughly by pipetting up and down. Ensure there are no bubbles in the ladder tube.
10. Insert the Ladder Tube into ladder slot on the LabChip GXII Touch instrument.

11 Transfer 750 µL of Protein Clear HR 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.

12 Insert the Buffer Tube into the buffer slot on the LabChip GXII Touch instrument.

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

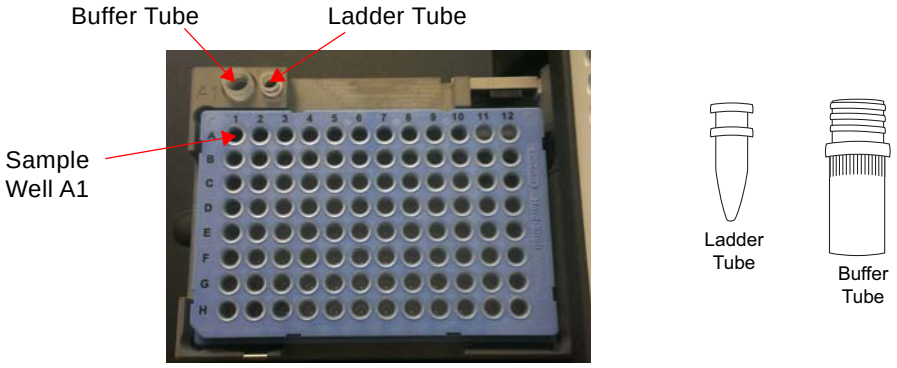

Figure 1. Locations of the Buffer Tube and Ladder Tube in the GXII Touch instrument.

Preparing the Chip

1 ***CRITICAL:*** *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 39.

3 Each active chip well (1, 2, 3, 4, 7, 8, 9, and 10) should be rinsed and completely aspirated 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.

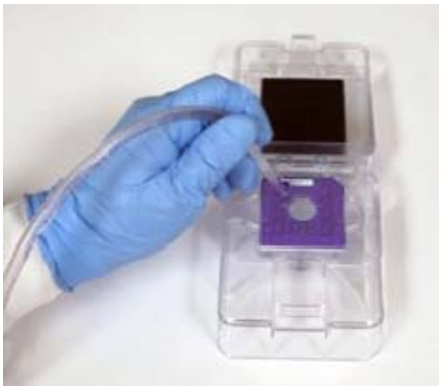

Figure 2. Using a vacuum to aspirate the chip wells is more effective than using a pipette. See page 39 for more details.

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

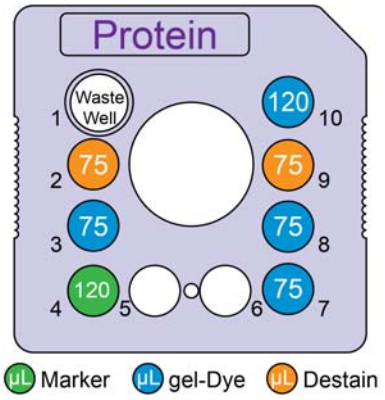

Figure 3. Reagent placement for High-throughput (up to 96 samples).

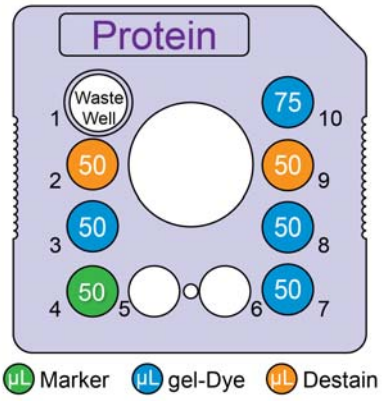

Figure 4. Reagent placement for Low-throughput (up to 48 samples).

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 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

1. Check that the sample plate, Buffer Tube, and Ladder Tube are placed on the instrument properly.
2. Touch the *Load Plate* button on the *Home* screen (Figure 5) to retract the sample plate and move the sipper to the Buffer Tube.
3. Remove the chip from the chip storage container and inspect the chip window. Clean BOTH sides of the chip window with the Revvity-supplied Detection Window Cleaning cloth dampened with a 70% isopropanol solution in DI water.
4. Touch the *Unload Chip* button on the *Home* screen.

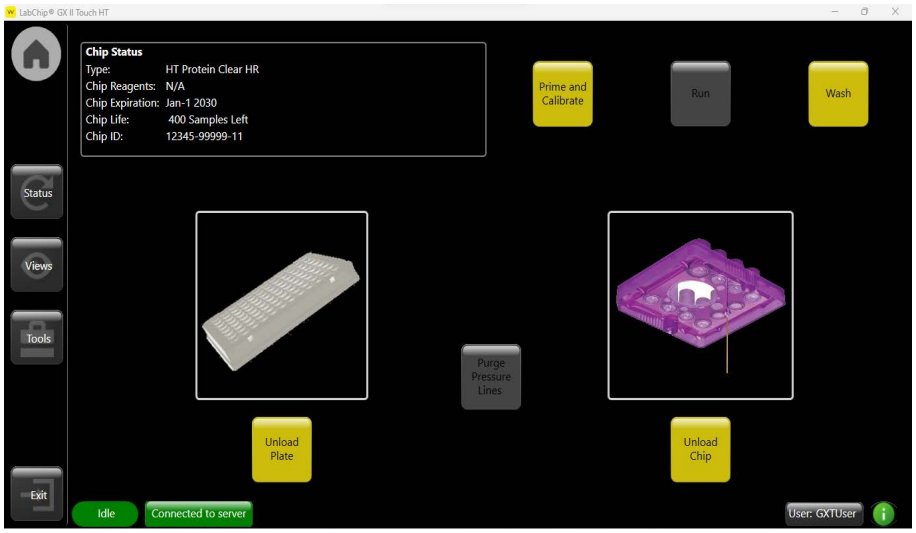

Figure 5. *Home* screen.

5. Insert the chip into the LabChip GXII Touch instrument (Figure 6) and close the chip door securely.

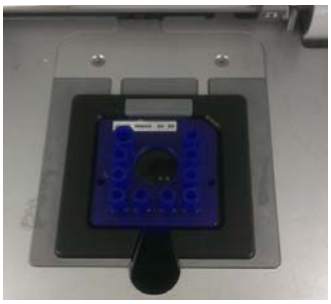

Figure 6. Chip in the LabChip GXII Touch instrument.

Priming and Calibrating

1 Touch the *Prime and Calibrate* button on the *Home* screen (Figure 7).

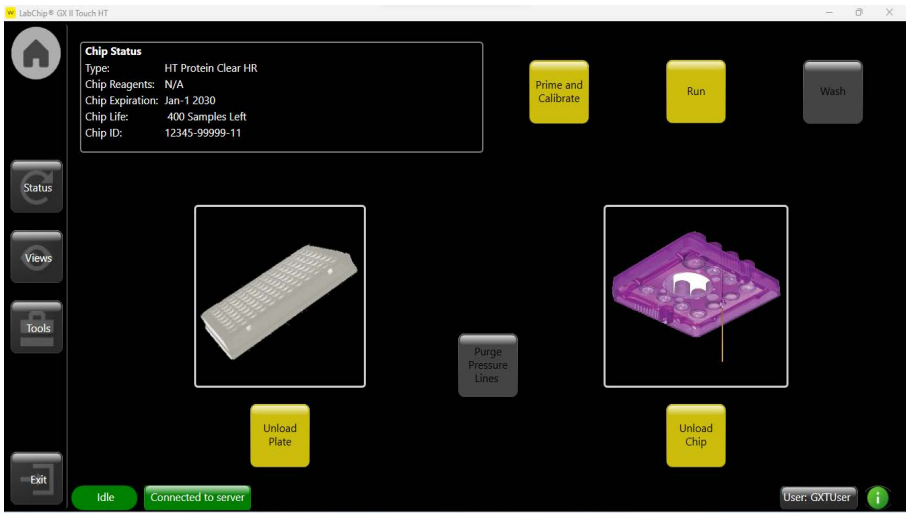

Figure 7. *Prime and Calibrate* button.

The *Prime and Calibrate* screen opens (Figure 8).

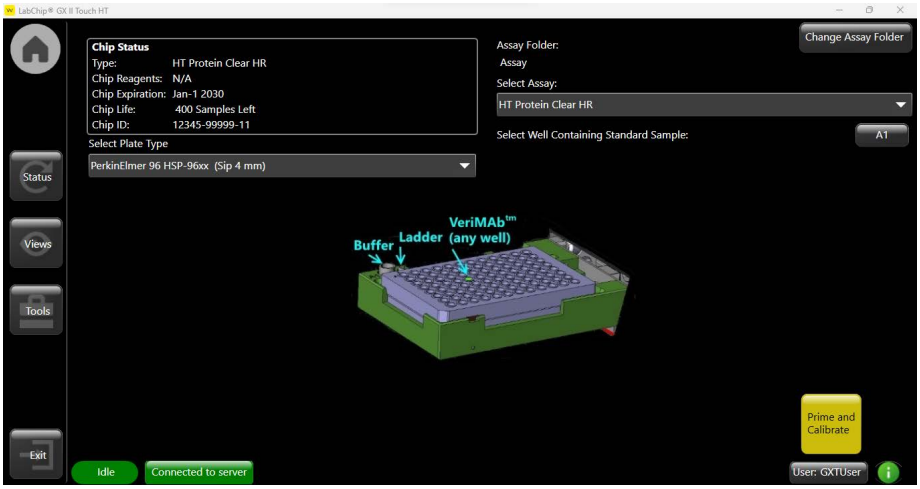

Figure 8. Prime and Calibrate button on the *Prime and Calibrate* screen.

2 Select the plate type from the *Select Plate Type* drop-down list.

3 Touch the *Select Well Containing Standard Sample* button to select the well that contains the VeriMAb sample.

4 Touch the *Prime and Calibrate* button to begin the prime and calibration process.

Workaround Procedure

IMPORTANT: If repeating the prime and calibration process after the completion of a run, the following workaround procedure is needed to avoid incomplete runs:

1 After the completed run, remove the chip from the instrument and place it in the jewel case.

2 Aspirate the liquid from the Waste Well (well 1) without touching the dent in the center of well. Rinsing the well is not necessary.

3 Aspirate the liquid from the protein Marker Well (well 4) and replenish with the recommended volume.

4 Remove the “completed run plate” and replace it with a new sample plate.

5 Follow standard procedures for assay plate setup.

6 Recalibrate, reprime, and run the new plate.

Notes:

- *Do not keep the chip door open for any length of time once the chip is installed.*
- *For best run performance, minimize time between calibration completion and run commencement.*

Preparing the Protein Samples

Note: *The following is a general protocol for antibody sample preparation. Optimization of the type or concentration of reducing agent and stabilizing agent and/or optimization of denaturing conditions may be necessary depending on the specific molecules to be analyzed. Use of a hardshell 96-well PCR plate and a thermal cycler is recommended for efficient sample preparation.*

1 Prepare Reducing and/or Non-Reducing Sample buffer.

2 For each sample to be analyzed, pipette 18 µL of Reducing or Non-Reducing Sample Buffer into a well in a 96-well PCR plate.

3 The sample buffer calculation is described as follows:
   - Transfer 700 µL of Protein Clear HR Sample Buffer (white cap ◯) into a microfuge tube.
   - For Reducing Sample Buffer, add 24.5 µL of BME or 1M DTT.
   - For Non-Reducing Sample Buffer, add 24.5 µL of 250 mM IAM.

4 Add 2.5 µL of sample to each prepared well. When finished, cover the plate with foil seal to minimize evaporation.

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

6 Denature samples at 70°C for 10 min, then cool to room temperature. Optimum denaturing conditions may vary by sample type.

7 Add 35 µL of water (Milli-Q® or equivalent) to each sample and mix thoroughly by pipetting up and down a few times.

8 Spin the sample plate at 1200 rcf for 2 minutes to eliminate bubbles and move the fluid to the bottom of the wells.

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

Running the Assay

1. Confirm that the software has completed the *Prime and Calibration* step (see page 17). Clear the window indicating that the chip has been successfully calibrated.
2. Touch the *Unload Plate* button on the *Home* screen (Figure 5 on page 16) and replace the plate containing the VeriMAb Standard with the plate containing samples. Leave the buffer tube and ladder tube in place and touch the *Load Plate* button.
3. Touch the *Run* button on the *Home* screen.
4. Select the appropriate plate type, well pattern, and whether to read wells in columns or rows (Figure 9). Select number of times each well is sampled under *Adv. Settings*. Touch the green arrow.

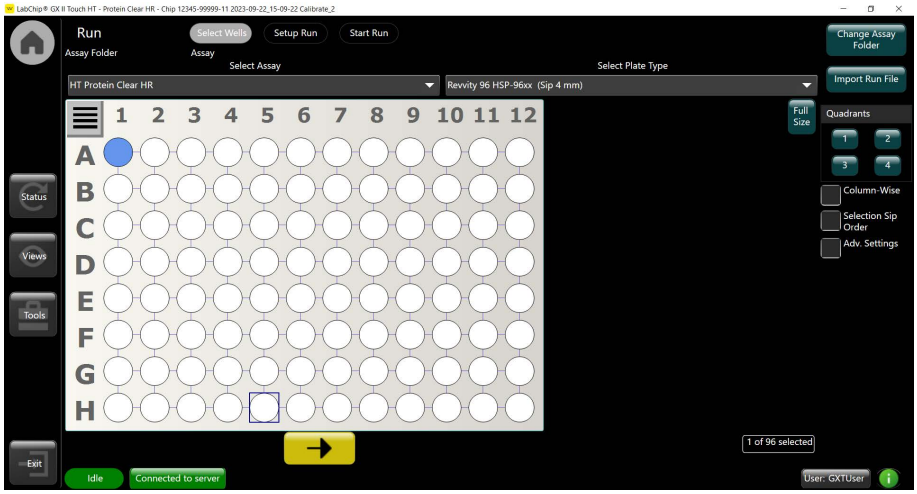

Figure 9. Selecting wells.

5. 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, *Auto Print* to print results table into a PDF, and *Defer Export to Plate Completion* to export results at the end of the run instead of as each well is completed (Figure 10). Touch the green arrow.

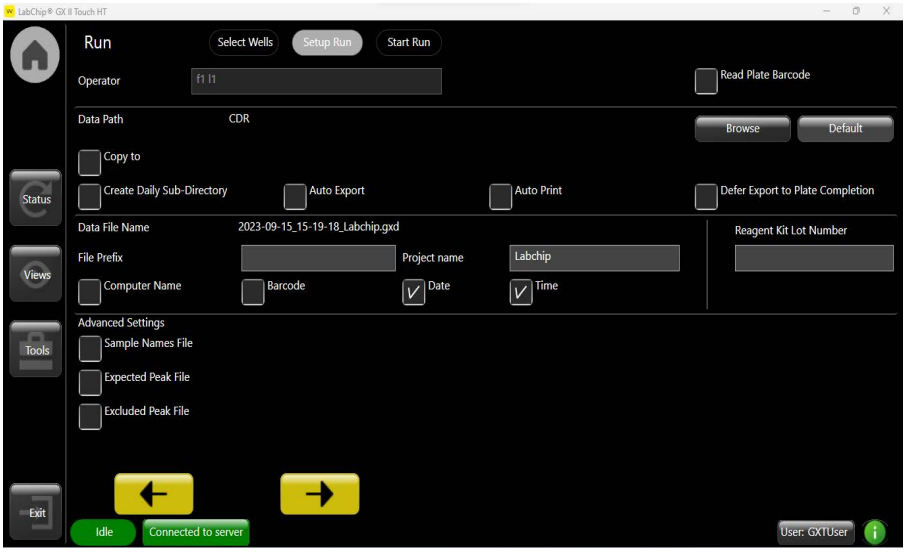

Figure 10. Run setup screen.

6 Touch the *Start* button to begin the run.

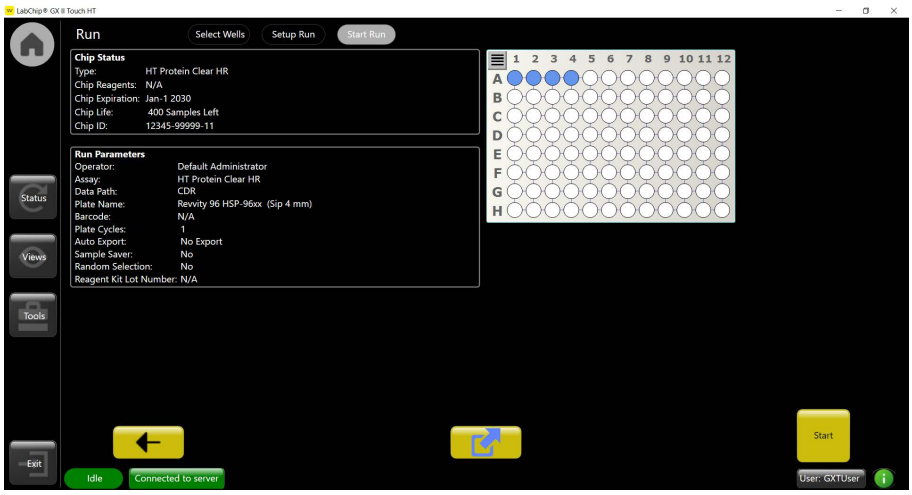

Figure 11. Starting a run.

Storing the Chip

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

1. Place the chip into the plastic storage container. The sipper should be submerged in the fluid reservoir.
2. Remove the reagents from each well of the chip using vacuum.
3. Each active well (1, 2, 3, 4, 7, 8, 9, and 10) should be rinsed and aspirated twice with water (Milli-Q® or equivalent).
4. Add 100 µL 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. The chip must be used to its lifetime (to the total number of 400 samples) within 30 days of analyzing the first plate of samples.

Chip Cartridge Cleaning

1 **Daily**

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

b Touch the *Purge Pressure Lines* button on the Home screen (see Figure 5 on page 16).

c 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 instrument. Soak O-rings in water (Milli-Q® or equivalent) for several 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 Clear HR Ladder Result

The electropherogram of a typical Protein Clear HR 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, 119.2 kDa, and 250 kDa, respectively.

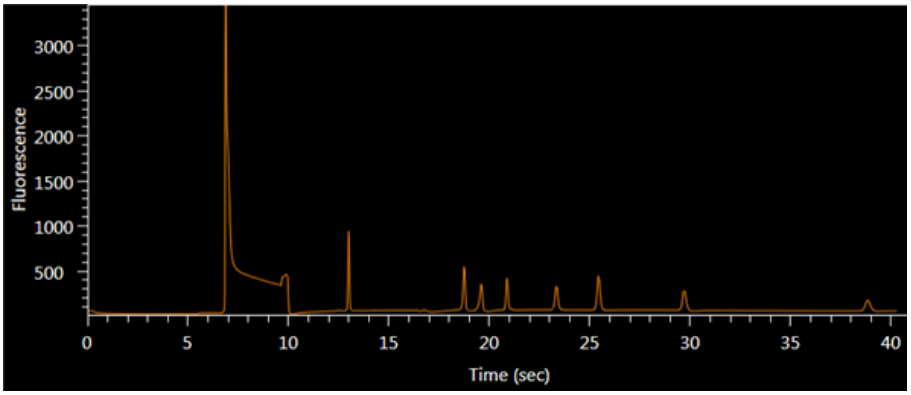

Figure 12. Protein Clear HR ladder electropherogram.

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 31 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 31 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 31 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.
- 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 31 for instructions on how to reprime the chip.

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

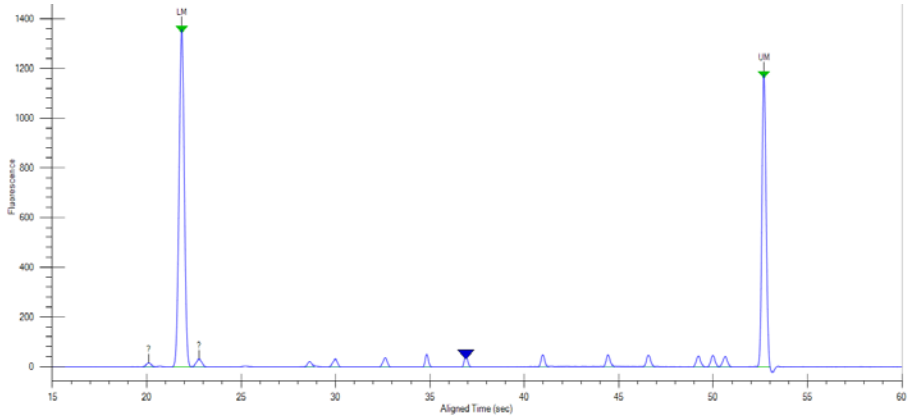

Figure 13. Small ladder peaks in sample well caused by delayed sip.

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 31 for instructions on how to reprime the chip.

Symptom: Unexpected sharp peaks.

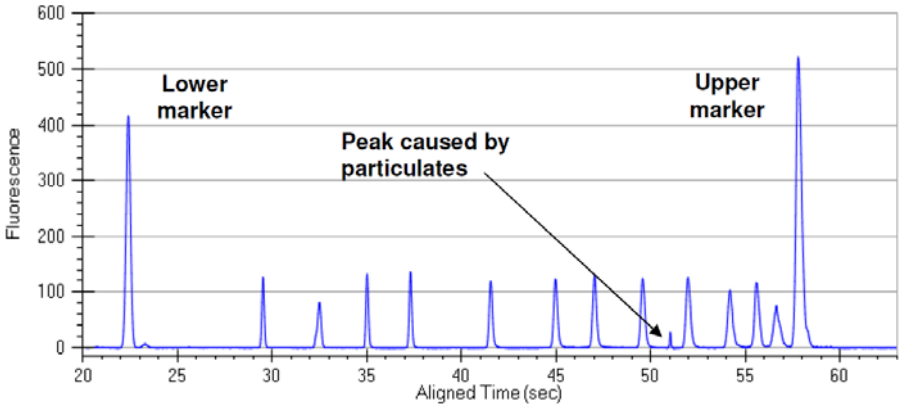

Figure 14. Unexpected sharp peak.

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.

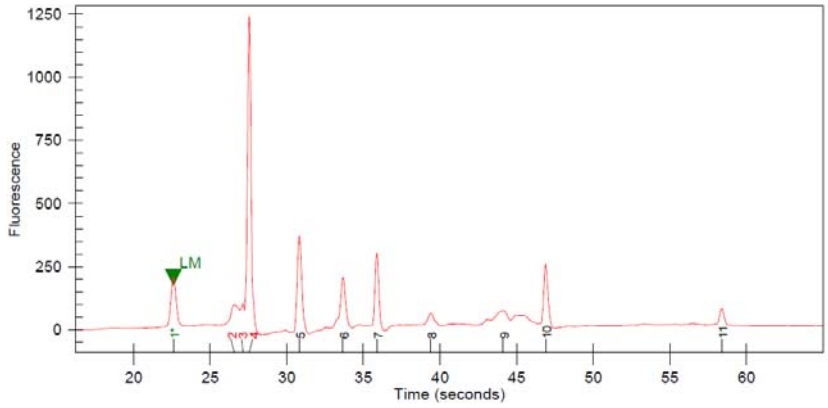

Figure 15. Humps in several electropherograms.

Possible causes:

1 Electrode 7 is dirty and has contaminated the Gel-Dye mixture 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 38).

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:

- *Protein Clear HR Assay Lower Marker: 18-19 seconds*
- *120 kD Ladder Protein on the first plate: 41-45 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 31 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 Revvity. 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) and/or ensuring the Sip 4 mm plate type is selected in the Select Wells screen 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 31 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 31 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 matrix.

What to do:

1 Ensure that Dye Concentrate is completely thawed and mixed. Prepare a fresh Gel-Dye solution. Wash and reprime the chip with the new Gel-Dye mixture. See “LabChip Kit Essential Practices” on page 31 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 Revvity Technical Support at 1-800-762-4000.

General

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light.
If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

- Allow the sample plate (and the chip and all reagents) to equilibrate to room temperature (RT) for at least 30 minutes before use.
- 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 Detection Window Cleaning cloth can be used on the chip to clean the detection window.

1. Revvity, Inc. warrants that the LabChip Kit meets specification at the time of shipment, and is free from defects in material and workmanship. LabChip Kits are warranted for 60 days from the date of shipment. All claims under this warranty must be made within thirty days of the discovery of the defect.

- 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 32) will help avoid introducing bubbles into the chip when pipetting the gel.

Reverse Pipetting Technique

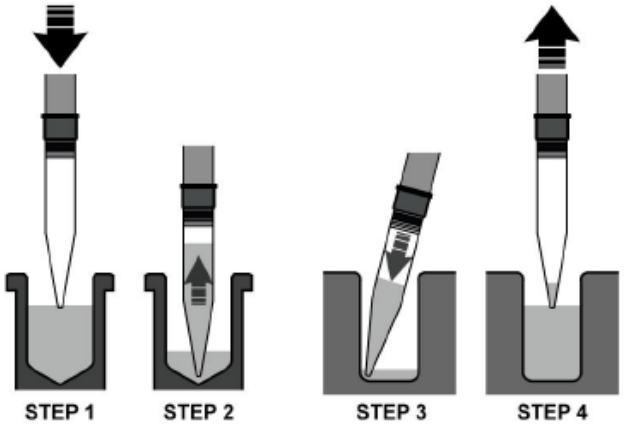

Figure 16. Reverse pipetting.

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

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light.
If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

- Store all reagents and Gel-Dye mixture (except dye alone) at 2-8°C when not in use. Store dye vial (blue cap) at -20°C when not in use.
- Protect the dye, Gel-Dye mixture, and marker from light. Store in dark when not in use.
- The Gel-Dye mixture expires 3 weeks after preparation.
- For optimal performance, use one reagent kit per chip. The Protein Clear HR Reagent Kit contains the reagents to run 384 samples, equivalent to four chip preparations in the high-throughput mode or eight chip preparations in low-throughput mode, whichever comes first.

Chips

General Guidelines

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light.
If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

- New chips should be stored refrigerated at 2°-8°C prior to first use.
- After running the first set of samples, chips must be stored at room temperature and used within 30 days
- 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. Revvity 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 of 400 samples are not exceeded.

Repriming 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 and Calibrate* button on the *Home* screen.

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 immediately before the next use can often restore data quality.*

Chips should only be washed on the LabChip GXII Touch 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.

- Thoroughly aspirate all fluid from the chip wells using a vacuum line.
- Each active well (1, 2, 3, 4, 7, 8, 9, and 10) should be rinsed and completely aspirated twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
- Add 120 µL of water (Milli-Q® or equivalent) to each active well (1, 2, 3, 4, 7, 8, 9, and 10).
- Touch the *Unload Chip* button on the *Home* screen and place the chip into the instrument.
- Close the chip door securely.
- Transfer 750 µL of water (Milli-Q® or equivalent) into the Buffer Tube. Install into the instrument.
- Touch the *Wash* button on the Home screen (Figure 5 on page 16). The wash cycle begins (Figure 17).

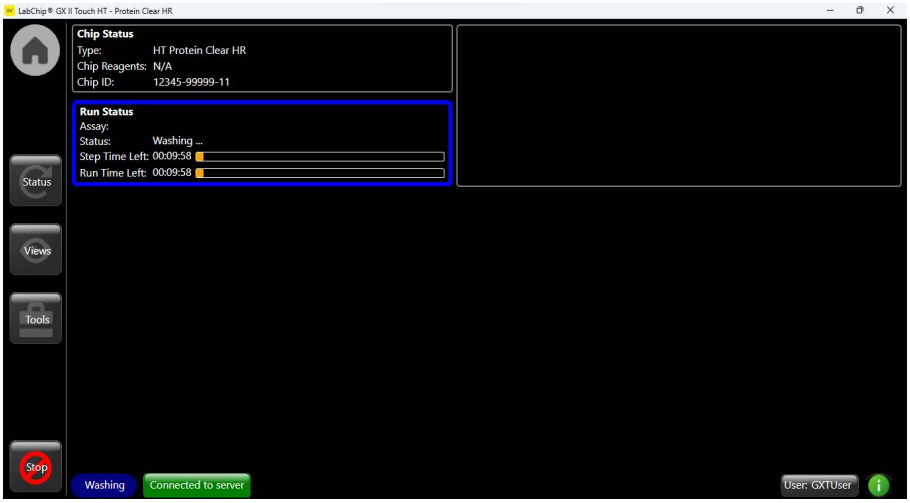

Figure 17. Wash screen.

- 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.
- Thoroughly aspirate all fluid from the chip wells using a vacuum line.
- Replace fluid in the wells with freshly made reagents as described in “Preparing the Chip” on page 14. Do not let wells remain dry.
- Transfer 750 µL of Wash Buffer (purple cap ●) into the supplied 0.75 mL Buffer Tube. Install into the instrument.
- Install the Ladder Tube, sample plate and chip into the instrument and run the assay.

Removing Sipper Clogs

If air bubbles are not dislodged after a reprime:

- Remove the chip from the instrument
- Rinse and aspirate well 1 (the waste well) twice with water (Milli-Q® or equivalent).
- Use reverse pipetting (described on page 32) to add 100 µL water (Milli-Q® or equivalent) to well 1.
- 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.
- Aspirate the water from well 1, reinstall the chip into the instrument, and restart the run.

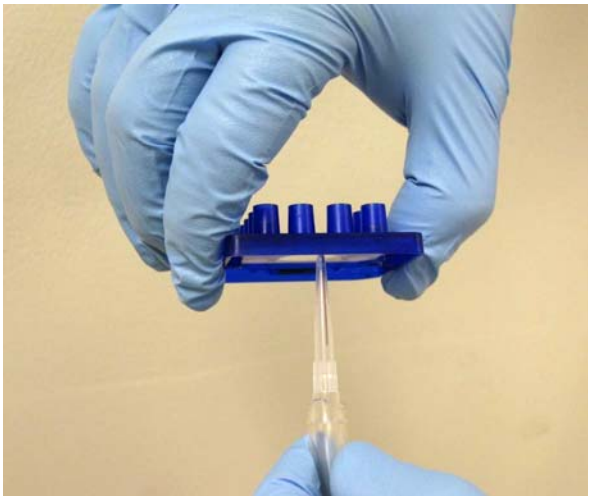

Figure 18. Removing an air bubble or clog by suctioning the sipper with a vacuum line.

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 one 96-well plate (96 samples) can be run with a single chip preparation when running the Protein Clear assay on the GXII Touch HT 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, Revvity 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).

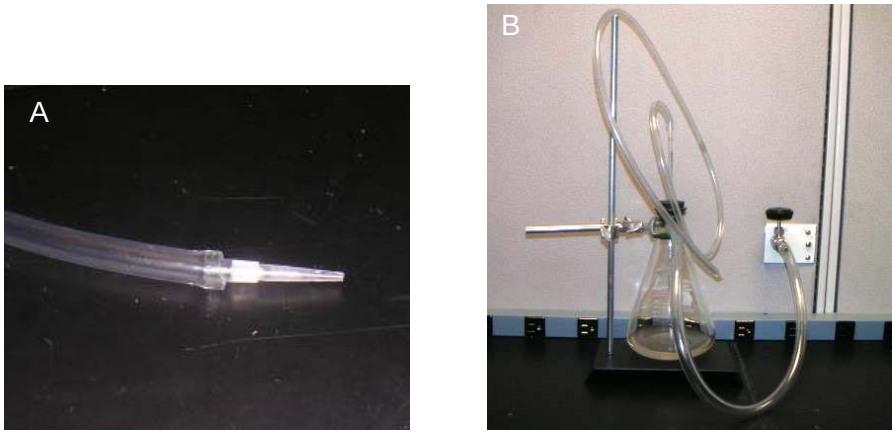

Figure 19. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap.

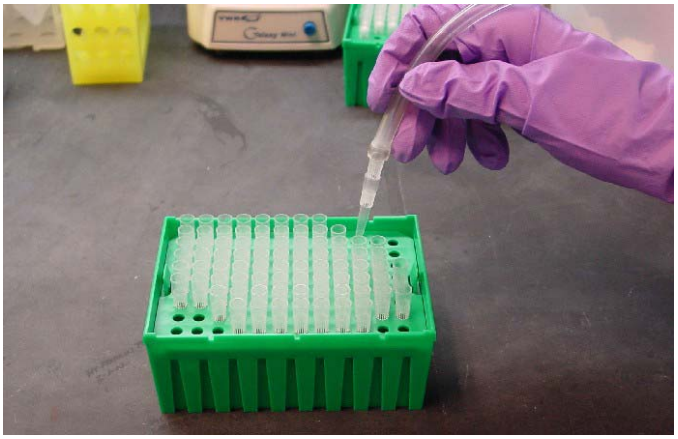

Figure 20. Replacing the disposable pipette tip.

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

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