

revvity

LabChip User Guide

Low pI Charge Variant Assay User Guide

For LabChip® GXII Touch

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Specifications

Assay Specifications

Table 1. Assay Specifications

Sample Type	Protein
pI Range	3.0 - 7.0
Amount of Sample Required	25 μ L with concentration between 0.5 - 10 mg/mL (12.5 μ g to 250 μ g of protein, total) Optimal concentration: 2 mg/mL
Resolution	Comparable to IEX and conventional CZE
Reproducibility ^a	CV < 5% for varying concentration from 1 - 3 mg/mL CV < 3% at constant concentration
Assay Run Time	1.8 - 3.5 hours for a 96-well plate. Three assay durations: 68s, 90s, and 110s
Chip Lifetime	500 samples
Samples per Chip Prep	Up to 96 samples
Samples per Reagent Kit	120 samples
For Research Use Only	

a. Based on protein main peak.

Storage Conditions

Chip Storage: Store chips at 2 - 8°C.

Storage: When not in use, store Running Buffer and Storage Buffer at 2 - 8°C. Store Labeling Buffer and Dye Concentrate at -20°C. Do not leave chips, buffers, or reagents at room temperature overnight.

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. Remove the Labeling Buffer and Dye Concentrate from the padded shipping pack and allow to warm from -20°C to room temperature for 45 minutes before use. Protect the Dye Concentrate from light.

Reagent Kit Contents

Low pI Charge Variant Reagent Kit, CLS154134

Table 2. Reagents







Items at -20°C	Vial	Quantity
Labeling Buffer	Orange 	1 vial, 1 mL
Dye Concentrate	Blue 	5 vials, 7 µL (24 samples/vial)
Items at 2 - 8°C	Vial	Quantity
Running Buffer pH 5.6	Red 	1 bottle, 25 mL
Running Buffer pH 7.2	Green 	1 bottle, 25 mL
Running Buffer pH 8.5	Yellow 	1 bottle, 25 mL
Chip Storage Buffer	Purple 	1 bottle, 25 mL

Table 3. Consumable Items

Item	Supplier and Catalog Number	Quantity
Detection Window Cleaning Cloth	VWR [®] , Cat. # 21912-046	1
Swab	ITW Texwipe [®] , Cat. # TX758B	3
Centrifuge Tubes, 2.0 mL	(Not sold separately)	5
Ladder Tubes, 0.2 mL	(Not sold separately)	10
Buffer Tubes, 0.75 mL	(Not sold separately)	16

Low pI Charge Variant LabChip

Table 4. LabChips

Item	Part Number
Low pI Charge Variant LabChip (for use with GXII Touch)	CLS153418

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 as 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 DMAC. Avoid inhalation and contact with skin and eyes.

Usage

The Low pI Charge Variant 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 refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.*
- *Remove the Labeling Buffer and Dye Concentrate from the padded shipping pack and allow to warm from -20°C to room temperature (20 - 25°C) for 45 minutes. Protect the Dye Concentrate 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

- 18 megohm, 0.22-µm filtered water (Milli-Q® or equivalent)
- Anhydrous (99.8%) N,N-dimethylformamide (Sigma Aldrich Cat. # 227056-100 mL)
- Commercially available desalting method, for example a Zeba Spin Desalting Plate or Column (Thermo- Pierce: Cat. # 89807 or 89882)
- Plastic, disposable syringe (> 0.2 mL) with needle
- Centrifuge with rotor that accepts a 96-well PCR plate
- Revvity Hard-Shell thin-wall 96-well skirted PCR plate (blue), Cat. # 6008870 (recommended)

Notes:

- *After a chip is used for the Low pl Charge Variant assay, it should be designated for this assay only. Do not run other LabChip GXII Touch assays with this chip.*
- *Thoroughly clean the electrodes of the instrument with water (Milli-Q® or equivalent) before placing the chip in the instrument.*
- *Keep the chip in the chip storage container during preparation and when carrying from one location to another.*

Preparing the Running Buffer

- 1 Determine the desired pH of the Running Buffer. A Running Buffer with a pH approximately 2 units higher than the protein isoelectric point is recommended.
- 2 Mix the appropriate Running Buffers pH 5.6 (red cap ●), pH 7.2 (green cap ●), or pH 8.5 (yellow cap ●) at the ratio corresponding to the desired pH (see [Table 5](#), [Table 6](#), [Table 7 on page 14](#), and [Optimizing Running Buffer pH on page 20](#)).

Table 5. Running Buffer pH Adjustment for pH 5.6 to 7.2

pH 5.6 (μL) (red cap ●)	pH 7.2 (μL) (green cap ●)	pH (± 0.1)
0	1200	7.2
60	1140	6.9
120	1080	6.6
150	1050	6.5
300	900	6.2
420	780	6.1
600	600	5.9
840	360	5.8
1200	0	5.6

Table 6. Running Buffer pH Adjustment for pH 7.3 to 8.5

pH 7.2 (μL) (green cap ●)	pH 8.5 (μL) (yellow cap ●)	pH (± 0.1)
0	1200	8.5
60	1140	8.3
120	1080	8.2
150	1050	8.2
300	900	8.0
420	780	7.9
600	600	7.7
840	360	7.3
1200	0	7.2

- 3 Vortex the Running Buffer solution for about 10 seconds and spin down.

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 1](#)). For more details on how to set up a vacuum line see [page 35](#).
- 3 Rinse and completely aspirate each active chip well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q[®] or equivalent). Do not allow active wells to remain dry.
- 4 If any water spilled 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.

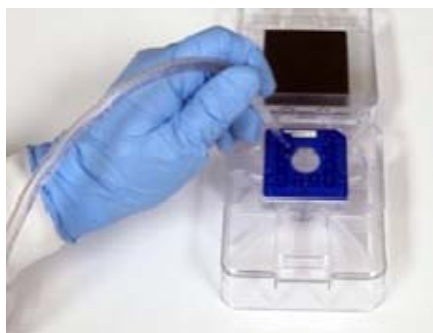
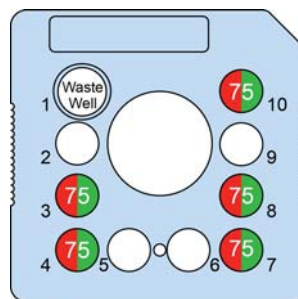


Figure 1. Using a vacuum to aspirate the chip wells is more effective than using a pipette. See [page 35](#) for details.

- 5 **When using pH 5.6 to 7.2 Running Buffer:** Using a reverse pipetting technique, add 75 μL of the **Running Buffer solution** to chip wells 3, 4, 7, 8, and 10 (as shown in [Figure 2](#).)



 Running Buffer

Figure 2. Chip Preparation for pH 5.6 to 7.2 Running Buffer

For pH 7.3 to 8.5 Running Buffer: Using a reverse pipetting technique, add **120 μL** of pH 7.2 ● Running Buffer to chip wells 3, 4, and 8, and add **120 μL** of pH 7.3 to 8.5 Running Buffer solution to wells 7 and 10 (as shown in [Figure 3.](#))

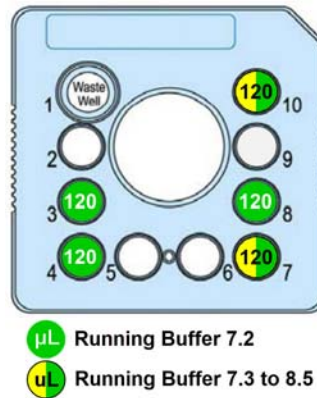


Figure 3. Chip Preparation for pH 7.3 to 8.5 Running Buffer

- 6 Make sure the rims of the chip wells are clean and dry.
- 7 **IMPORTANT:** Ensure chip well 1 (waste well) is empty before placing the chip in the LabChip GXII Touch.

Note: Each chip preparation is sufficient for running 96 samples.

Preparing the Buffer Tube

- 1 Add **750 μL** of Running Buffer to the 0.75 mL Buffer Tube.
For pH 5.6 to 7.2, use the same Running Buffer solution as used in the chip. **For pH 7.3 to 8.5,** use pH 7.2 Running Buffer in the buffer tube. Ensure there are no air bubbles in the Buffer Tube.
- 2 Click **Unload Plate** to access the Buffer Tube slot.
- 3 Insert the Buffer Tube into the buffer slot on the LabChip GXII Touch.

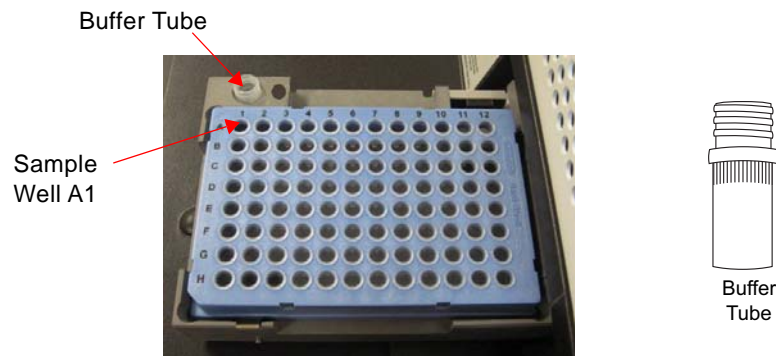


Figure 4. Location of the Buffer Tube in the GXII Touch

- 4 Click **Load Plate** to retract the plate carrier.

Preparing the Low pI Charge Variant Samples

For the first run on a chip using Running Buffer pH 7.3 to 8.5, prepare a labeled Bovine Serum Albumin (BSA) protein (not provided) according to steps 2-10 below. Perform a chip conditioning run in which the BSA sample is sipped 36 times with the HT Low pI Charge Variant 110s_RB 8 Chip Conditioning assay.

- 1 (Recommended) If the protein sample contains cell culture media, salt (>10 mM), surfactant, or excipients, then desalt the sample before labeling. Use a commercially available desalting method, for example a Zeba Spin Desalting Plate or Column (Thermo-Pierce: Cat. # 89807 or 89882). See [Using the Zeba Column to Clean Up Sample on page 11](#) for details.

Note: The Low pI Charge Variant dye reacts with the ϵ -amino group of lysine residues via an amide linkage; avoid using amine-containing buffers.

- 2 To each sample well of a 96-well plate, add **5 μ L** of **Labeling Buffer** (orange cap ●) and **25 μ L** of **sample** (2 mg/mL is optimal, 0.5 - 10 mg/mL is allowed).
- 3 When using running buffer solution with pH 7.3 to 8.5, add **3 μ L** of NaHCO₃ solution (1000 mM in Milli-Q water, not provided) to each sample.

Note: Dye solution should be used **immediately** (begin dispensing within **5 minutes** of mixing). Prepare the Dye solution after the Labeling Buffer and Samples have been dispensed into wells. If more than 24 samples are to be labeled, prepare and dispense Dye solution in batches of 24 samples when using a single-channel pipette; for a multi-channel pipette or liquid handler, multiple aliquots of Dye solution may be combined.

Important Note: Users should read the safety information in the MSDS for N,N-dimethylformamide before handling.

- 4 Add **5 μ L¹** of **Dye Concentrate** (blue cap ●) to **145 μ L anhydrous (99.8%) N,N-dimethylformamide** in a microcentrifuge tube and vortex for **10 seconds** (use a syringe to extract ~200 μ L of the N,N-dimethylformamide from the bottle and dispense into an intermediate tube). Each 150 μ L aliquot of Dye solution is sufficient for labeling 24 samples.
- 5 To each sample, add **5 μ L** of **Dye solution** and mix by pipetting up and down.

1. Optimization: Dye concentration up to 4X (in DMF) can be used to increase protein fluorescence.

- 6 Seal the sample plate and incubate at room temperature for 10 minutes, protected from light.
- 7 For Running Buffer solutions with pH 5.6 to 7.2: To each sample, add **60 μL of water** (Milli-Q[®] or equivalent)
For Running Buffer solutions with pH 7.3 to 8.5: To each sample, add **60 μL of Running Buffer pH 7.2.**
- 8 Mix by pipetting up and down a few times or with a plate shaker. Avoid creating air bubbles. Centrifuge sample plate for 1 minute at 1000 rpm.
- 9 (Optional) Remove excess dye using Zeba Spin Desalting Plate or Column (see [Using the Zeba Column to Clean Up Sample](#) below).
- 10 Click **Unload Plate**, place the sample plate in the instrument, and click **Load Plate** to retract the plate carrier.

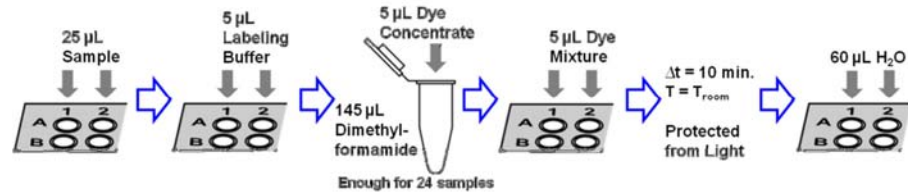


Figure 5. Preparing the Low pI Charge Variant Samples

Using the Zeba Column to Clean Up Sample

- 1 Snap off the bottom of the Zeba column and loosen the cap.
- 2 Put the column into an Eppendorf tube and centrifuge for 1 minute at 1,500 x G to remove the storage buffer. (Spin longer if necessary to completely remove the storage buffer, but do not over dry.)
- 3 Discard the Eppendorf tube with storage buffer, and wipe the bottom of the Zeba column to remove any liquid.
- 4 The resin in the Zeba column will compact during centrifugation, producing a slope. Mark the side of the column at the top of the compacted resin slope. Place the column in the microcentrifuge with the mark facing outward during the subsequent centrifugation.
- 5 Gently layer **50 - 75 μL** of sample onto the top of the sloped resin and then centrifuge for 2 min at 1,500 x G. (Spin longer if necessary to spin the sample completely through the column.)
- 6 Recover the filtered sample and process for Charge Variant analysis.

Inserting a Chip into the LabChip GXII Touch

Thoroughly clean the electrodes of the instrument with water (Milli-Q[®] or equivalent) before placing the chip in the instrument if changing the pH of the running buffer.

- 1 Verify the sample plate and Buffer Tube are placed in 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 provided Detection Window Cleaning Cloth dampened with a 70% isopropanol solution in DI water.
- 3 Touch the **Unload Chip** button on the **Home** screen (see [Figure 6](#)). The Chip door opens.

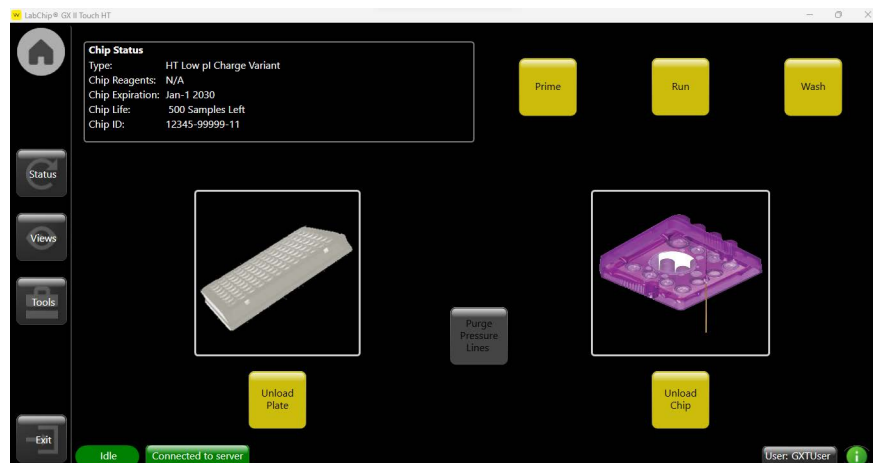


Figure 6. Home Screen

- 4 Insert the chip into the LabChip GXII Touch instrument (see [Figure 7](#)) and close the chip door securely.

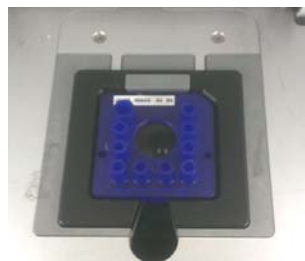


Figure 7. Chip in the LabChip GXII Touch

Running the Assay

For the first run on a chip using Running Buffer pH 7.3 to 8.5, prepare a labeled Bovine Serum Albumin (BSA) protein (not provided) according to steps 2-10 in [Preparing the Low pl Charge Variant Samples on page 10](#). Perform a chip conditioning run in which the BSA sample is sipped 36 times with the HT Low pl Charge Variant 110s_RB 8 Chip Conditioning 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 6](#)). Select the desired assay from the Assay drop-down list. Touch the Prime button on the Chip Priming screen (see [Figure 8](#)). Make sure the Buffer Tube is placed on the instrument.

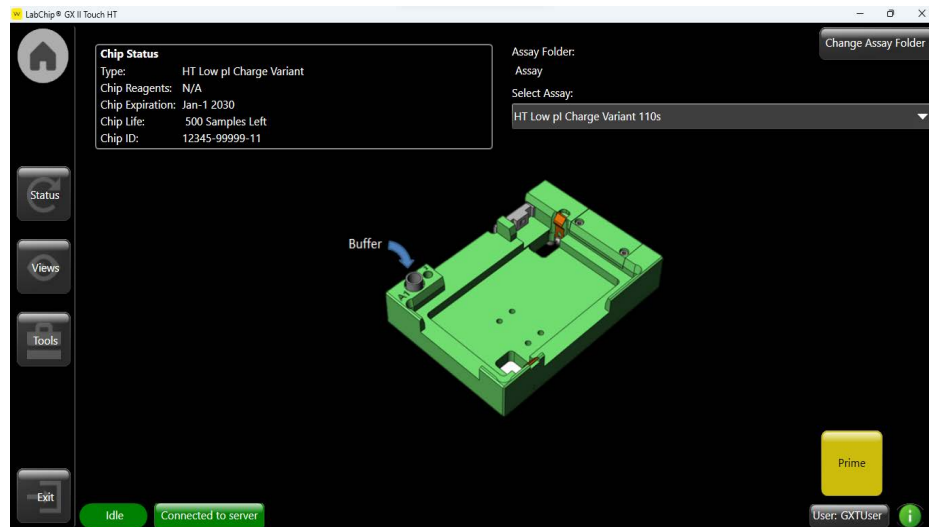


Figure 8. Chip Priming Screen

- 1 Touch the **Run** button on the Home screen (see [Figure 6 on page 12](#)).
- 2 In the Select Wells screen, select the appropriate **Assay Type**, **Plate Type**, **Well Pattern**, and whether to read wells in **Columns or Rows**. Refer to [Table 7](#) for Assay Type and Running Buffer pH. Select the number of times each well is sampled under *Adv. Settings* ([Figure 9](#)). Touch the green arrow.

Note: The pH values listed in [Table 7](#) are recommendations for achieving high resolution of charge variants within the time allowed by the indicated assay. If required, the resolution can be increased by increasing the pH. However, migration speeds decrease with increasing pH, so a longer assay may be required.

Table 7. Recommended Run Conditions

pl of Main Variant	Running Buffer pH	Assay
7.0 - 6.0	8.5	Low pl Charge Variant 68s
6.0 - 5.0	7.2	Low pl Charge Variant 90s
5.0 - 3.6	6.2	Low pl Charge Variant 110s
3.6 - 3.0	5.6	Low pl Charge Variant 110s

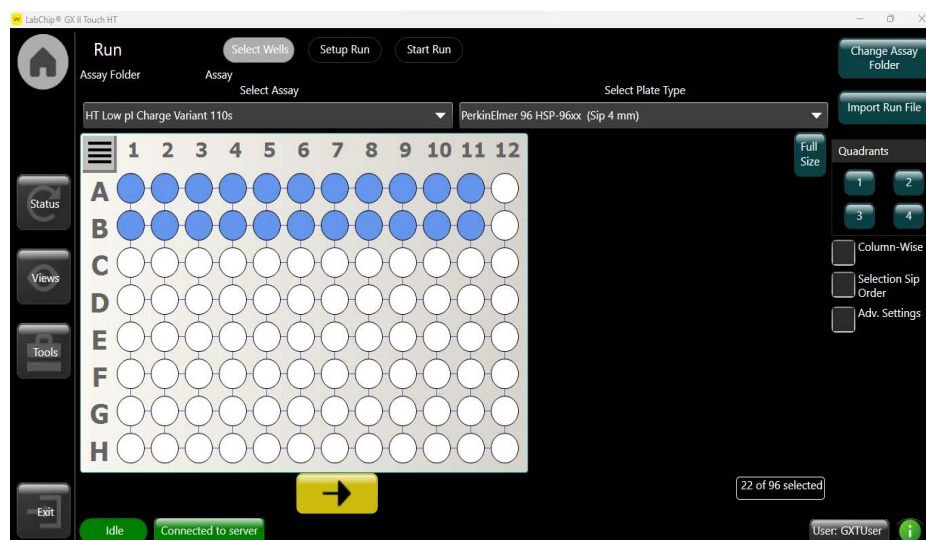


Figure 9. Select Wells Screen

- In the Setup Run screen, 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. The Start Run screen opens.

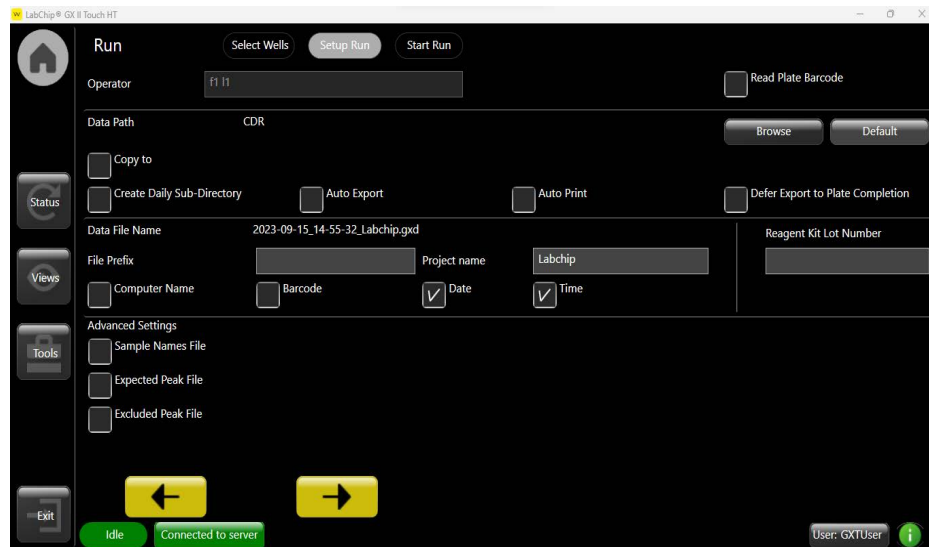


Figure 10. Setup Run Screen

- 4 Touch **Start** to begin the run.

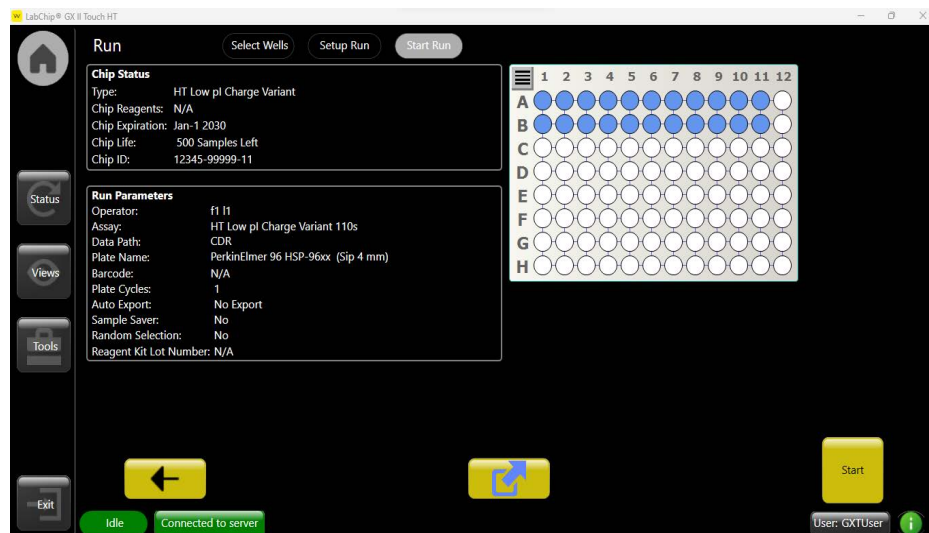


Figure 11. Start Run Screen

Washing and Storing the Chip

At the end of the day or when changing Running Buffer pH, wash the chip. At the end of the day, store the chip in the chip container. The procedure below can be conducted the following day when running overnight.

- 1 Place the chip into the chip storage container. Make sure the sipper is submerged in the fluid reservoir.
- 2 Remove the Running Buffer from each chip well using vacuum.
- 3 Rinse and completely aspirate each active chip well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q[®] or equivalent).
- 4 Add **120 μ L** of **Storage Buffer** (purple cap ●) to each active well and to the waste well.
- 5 Place the chip in the LabChip GXII Touch instrument and place a Buffer Tube containing Running Buffer in the Buffer Tube slot.
- 6 Touch the **Wash** button on the **Home** screen.
- 7 Touch the **Wash** button on the **Wash** screen (see [Figure 12](#)).

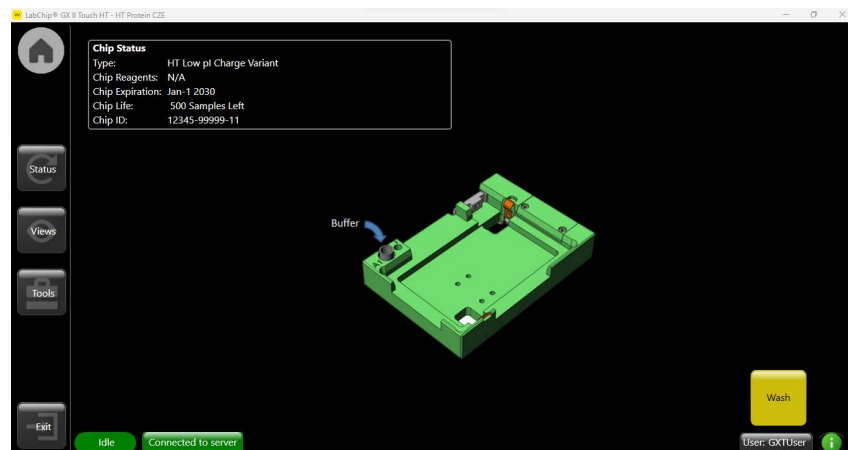


Figure 12. Wash Screen

- 8 After the wash is complete, remove the chip from the instrument and place the chip in the chip storage container.
- 9 Cover the wells with Parafilm[®] to prevent evaporation and store the chip at 2 - 8°C. Allowing chip wells to dry may lead to changes in chip performance.

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 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 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

Peaks in the Electropherogram

The profile of charge variants for each sample is shown in the Electropherogram tabs of the LabChip GX Touch software. The software analyzes these profiles, assigning a migration time (and other parameters) to each detected peak. The more basic variants migrate faster (and appear earlier) than the more acidic variants. Unless removed via desalting (see [Preparing the Low pI Charge Variant Samples on page 10](#)), the electropherogram will contain a free dye peak that typically elutes at 30 ± 3 sec, depending on the pH of the Running Buffer. The free dye peak should be excluded from the analysis, as described in [Eliminating Extraneous Peaks on page 19](#).

Figure 13 shows a charge variant profile of a negative charged protein in the LabChip Low pI Charge Variant Assay. Due to stronger electrophoretic force, acidic variants move toward the anode faster and arrive earlier at the detection window than main variants and basic variants. Acidic, main, and basic variants are separated, resolved, and labeled in the LabChip Low pI Charge Variant Assay.

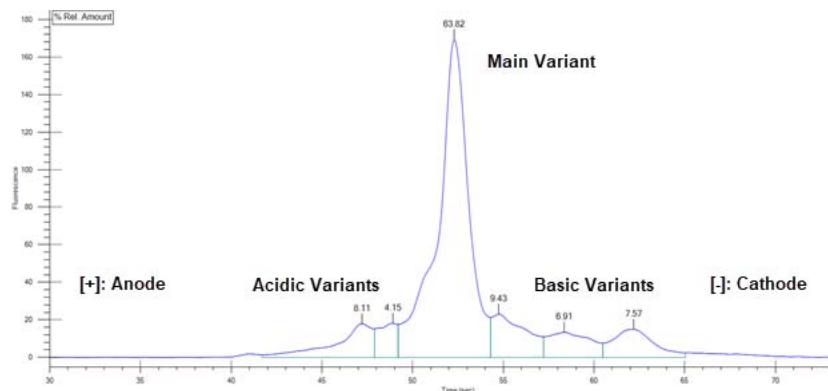


Figure 13. Electropherogram of a Negative Charged Protein

Eliminating Extraneous Peaks

To accurately quantify the % Relative Amount of each variant, extraneous peaks (such as the free dye peak and low-signal peaks that appear along the baseline as shown in [Figure 14](#)) must be excluded from the analysis. To exclude these peaks, several default settings of the Peak Find tab of the Analysis Settings can be adjusted. The default analysis setting of Minimum Peak Height is 0.2, to allow most peaks to be detected automatically. To exclude low-signal, extraneous peaks along the baseline, increase the Minimum Peak Height. Note that this may also exclude low-signal peaks associated with the sample profile. To exclude any remaining peaks in an electropherogram, right click them individually and select *Exclude Peak*. Alternatively, if all the sample profiles in a file span similar time ranges, adjusting the Start Time (sec) and End Time (sec) to a narrower range will also eliminate extraneous peaks. Finally, the *Excluded Peaks* function under *Analysis Settings* can be used to eliminate peaks that appear at approximately the same migration time in each profile of a plate, e.g., the free dye peak.

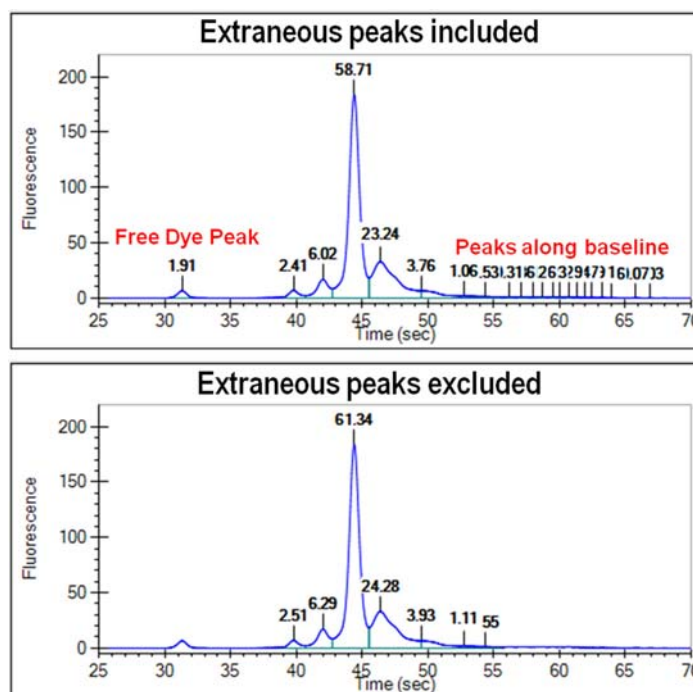


Figure 14. Electropherograms with Extraneous Peaks Included (top) and Excluded (bottom) from Analysis

Optimizing Running Buffer pH

As noted in [Preparing the Chip on page 8](#), the pH of the Running Buffer has a strong effect on the resolution of charge variants. Typically, as the pH increases the resolution improves. However, this improvement comes at the cost of slower migration. The optimum pH for a particular sample balances these two factors. To find this optimum, we recommend the following procedure:

Initial Test

- 1 Run samples using the recommendations in [Table 7](#) (see [Running the Assay on page 13](#)).
- 2 If the pIs of samples fall in multiple pI ranges in [Table 7](#), use the lowest pH and longest assay type.

pH Optimization

- 1 Rinse and completely aspirate each active chip well with water (Milli-Q[®] or equivalent) (see [Preparing the Chip on page 8](#)).
- 2 Prepare Running Buffer at 0.2 or 0.3 units higher than the pH used previously and then add to the wells of chip and to a new Buffer Tube.
- 3 Run samples using Running Buffer at the higher pH.
 - The samples from the initial test can be re-used throughout the optimization.
 - If the profile of the sample was near the end of the separation time in the previous run, use a longer assay type.
- 4 Visually compare the profiles from the run at the higher pH to those from the run at the previous pH to determine whether the resolution has increased. Indications of increased resolution:
 - Separation between peaks has increased.
 - Number of peaks has increased.
- 5 Repeat steps 1- 4 until the resolution no longer improves. Use the final pH in subsequent runs of sample.

Additionally, it is possible to determine the optimal pH by examining the reproducibility of the % Relative Amounts of the variants, as a function of pH. Higher pH should provide a higher reproducibility, as the resolution increases. For this method of optimization, we recommend labeling and running multiple samples of the same mAb (at least three), to increase the statistical significance of results.

Figure 15 shows the change in the profile of a mAb when run at various pH. As the pH of the Running Buffer increases, both the resolution and the migration time of the variants increase. Note also that the peak height decreases and that the peak width increases. Finally, the separation between the free dye peak and the mAb profile increases at higher pH, so samples with high pI that may co-elute with the free dye peak at low pH can be moved away by using a higher pH.

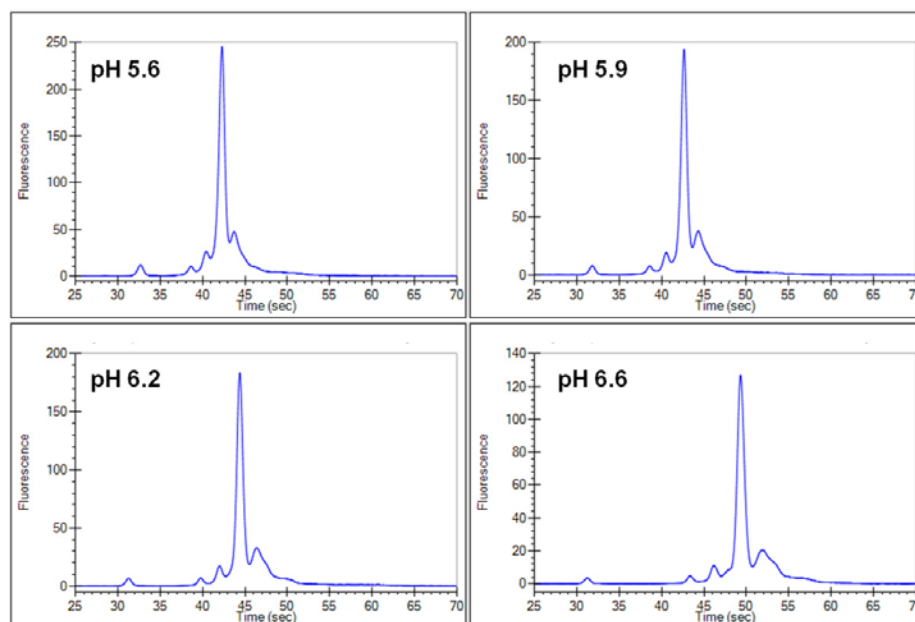


Figure 15. Charge variant profiles of a mAb sample (pI ~8.8) at various pH

Troubleshooting

Why don't I see a profile for my sample?

- 1 The running buffer pH may be too high or the selected assay type may be too short. Try a longer assay and/or a lower pH.
- 2 The plate well may contain air bubbles or not enough sample. The sipper may not be reaching the sample due to bubbles or low volume. Verify that there is sufficient sample volume to reach the sipper. To eliminate bubbles, centrifuge the sample plate for 1 minute at 1000 rpm before starting the run. Alternatively, manually dislodge the bubble using a pipette tip. Rerun these sample wells.
- 3 The chip sipper may be clogged. Remove the chip from the instrument and pipette 120 μL of Storage Buffer into well 1. Carefully insert the sipper into the pipette tip attached to the vacuum (that was used to aspirate the wells during chip preparation), until the pipette tip reaches the glue bead. With the vacuum running, hold the chip in place until several beads of liquid have emerged from the end of the sipper. Aspirate all fluid from well 1 and then place the chip in the instrument and restart the run.

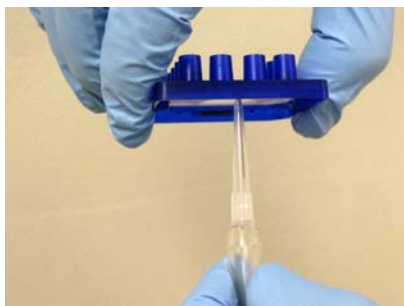


Figure 16. Removing a clog by suctioning the sipper with a vacuum line

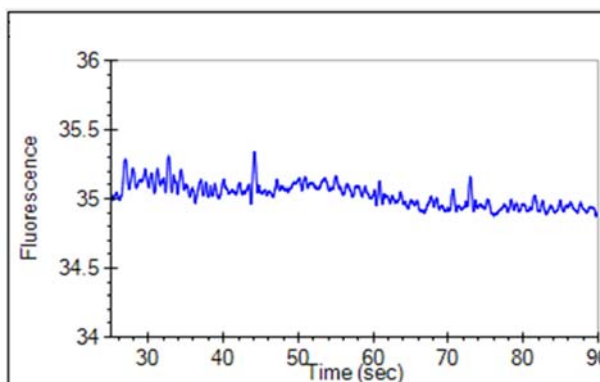


Figure 17. Example of a profile when the sipper is clogged

Why is my sample signal low?

- 1 The Dye solution may have been left at room temperature for too long. The Dye solution should be used immediately (begin dispensing within 5 minutes of mixing with N,N-dimethylformamide). Extended incubation of the Dye solution at room temperature will degrade the dye and decrease the amount of dye that can label the sample. [Figure 18](#) shows a sample where the dye solution was used immediately (blue trace) and a sample where the dye solution was older (red trace). When the Dye solution is used immediately, the dye peak is shorter and the mAb peaks are taller (blue trace).

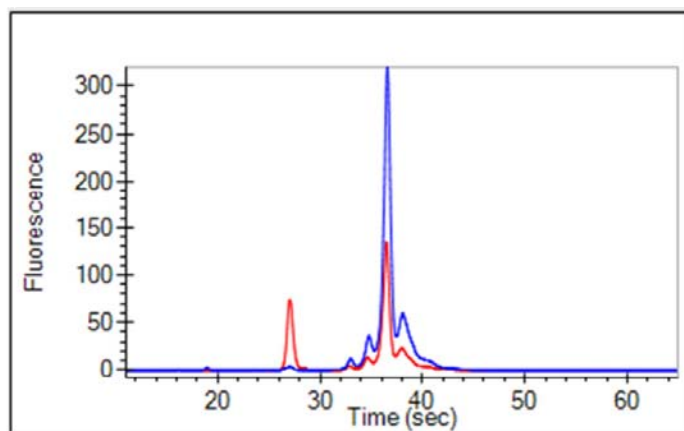


Figure 18. Dye Solution Comparison

- 2 The salt and/or excipient content of the sample may be too high. Higher conductivity of the sample typically results in less field amplified stacking of the sample during on-chip injection. Try desalting the sample before or after the labeling reaction. Alternatively, if there are high concentrations of amine-containing buffer or excipient present, then the signal of the mAb may decrease through competition for dye molecules during labeling.
- 3 The mAb sample concentration may be too low. The preferred range of input concentration is 0.5 - 10 mg/mL, and the optimal concentration is 2 mg/mL. Some samples, depending on the number of charge variants, give lower signals than others. Make sure that the sample concentration is ≥ 2 mg/mL.
- 4 The protein has a pI of 6-7 and is a difficult-to-label protein. Maximize the dye volume and use the pH 8.5 Running Buffer protocol to increase sample loading into the separation channel.

Why are there extra peaks in the profile of my sample?

- 1 Excipients with amines, such as histidine, can be labeled by the Low pI Charge Variant dye and appear in the profile as extra peaks. To remove these peaks, desalt the sample before or after the labeling reaction.

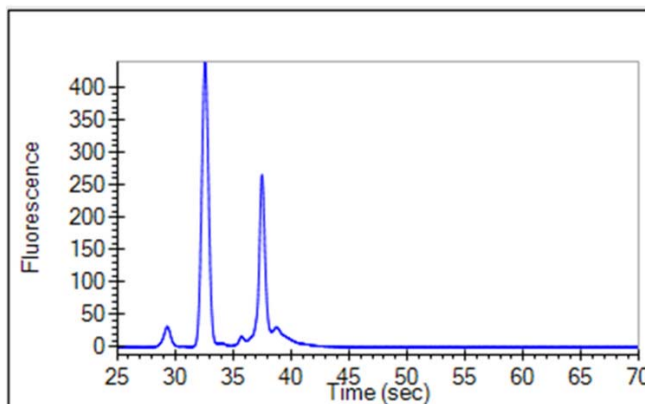


Figure 19. Example of an Excipient Peak (Tall Peak at ~33 s)

- 2 When using pH 8.5 Running Buffer, sample mixtures with pH \geq 8.0 may generate a broad peak that occurs late in the electropherogram. The peak is related to the dye molecule entering the separation channel and may grow in intensity over subsequent sample sips. To mitigate this, adjust the pH of the sample to 7.8 - 8.0 for analysis with pH 8.5 Running Buffer.

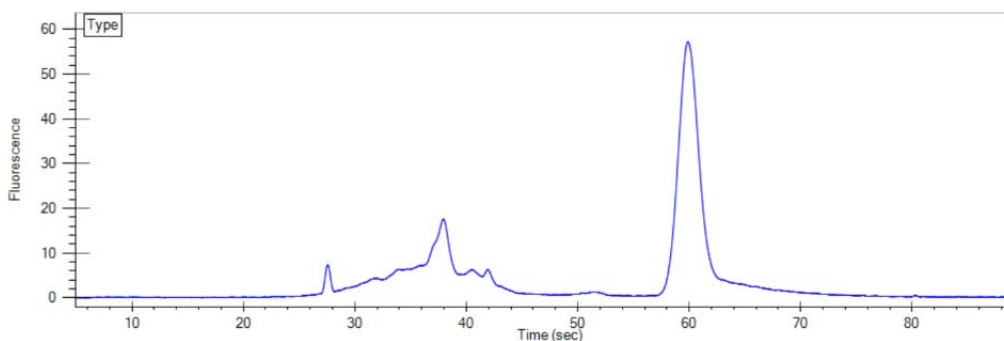


Figure 20. Example of a pH-Induced Dye Peak using pH 8.5 Running Buffer

Why is the resolution of charge variants in my sample so low?

- 1 The pH of the Running Buffer may be too low. Increase the pH to improve resolution. Higher pH running buffer increases the migration time and allows better separation. The higher pH may require the use of a longer assay time. See the Optimization of Running Buffer pH section for more detail. If the resolution is not sufficient at the highest pH of the Running Buffer system, then the pI of the sample is most likely outside the allowed range.

- Excipients or contaminants may be co-migrating with or affecting the migration of charge variants. Desalt the sample to remove any excipients that may interfere with or alter the profile. If the Protein Express, Pico Protein, or Glycan Profiling assay was run before the Low pI Charge Variant assay, clean the electrodes with water (Milli-Q® or equivalent).

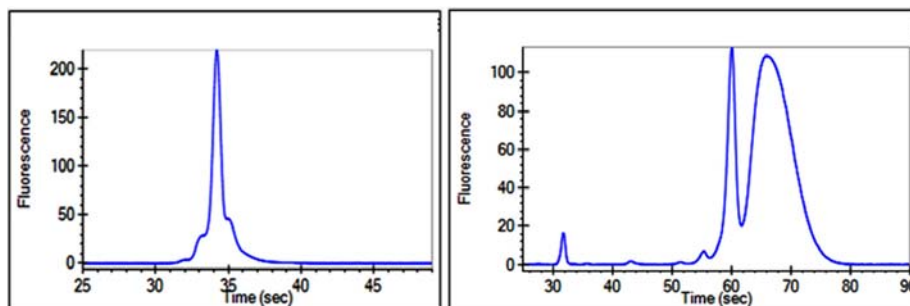


Figure 21. Examples of profiles with poor resolution caused by low pH (left) and poor resolution caused by presence of surfactant or excipient (right)

How can I reduce or eliminate carryover?

- Desalt the sample. Samples that contain cell culture media or high concentrations of salt, excipients or amine-containing buffer can potentially increase carryover. Desalting the sample prior to labeling may reduce the carryover.
- Use a longer assay type. Longer assays are better at suppressing carryover.

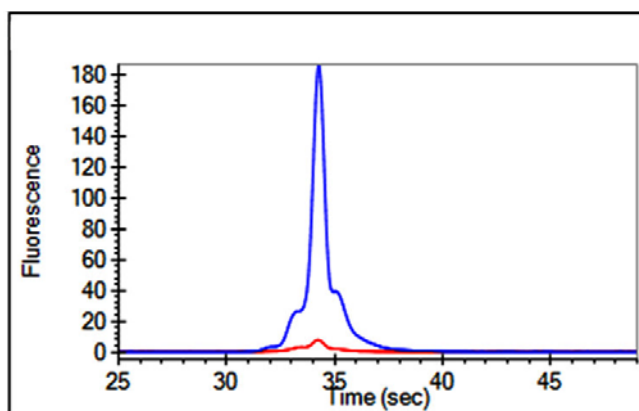


Figure 22. High Carryover in a Water Blank (red trace), Following a mAb Sample (blue trace)

Why does the migration time of the same sample vary?

The microchannels of the chip have a dynamic coating that suppresses electroosmotic flow during the separation and variation in the degree of coating causes variation in migration time. Typically, the migration time of a sample will vary by 0.5 - 2 seconds, with low pI samples having higher variability than high pI samples. Different chips, GXII Touch instruments, and Running Buffer lots can contribute to the variability. Furthermore, the variability in migration time is typically lower for chips that have run more samples or have been stored with Low pI Charge Variant Storage Buffer for a longer time. Variation in migration time should not impact resolution or % Relative Amount reproducibility. When significant variation in migration time or abnormally slow migration (i.e., when the free dye peak elutes after ~33 seconds) is observed, we recommend washing the chip two or three times with storage buffer and then storing it overnight at room temperature to regenerate the dynamic coating.

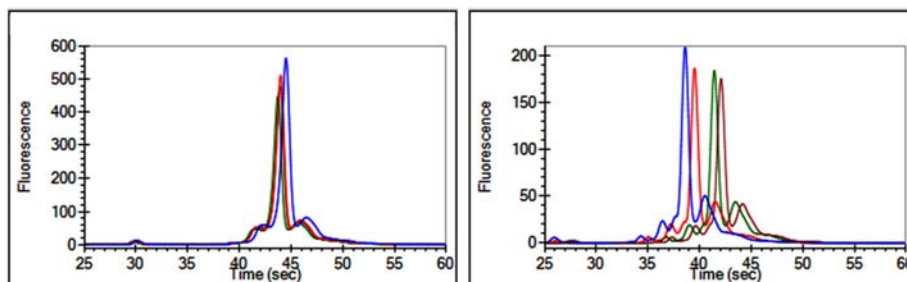


Figure 23. Examples of Typical (left) and Abnormal (right) Variation in Migration Time

Why is the CV of the % Relative Amount greater than the specified value?

Adjust the pH of the Running Buffer to confirm that the resolution is optimal. When running multiple samples of the same molecule, dilute the samples so the concentration of each differs by ± 0.5 mg/mL or less to eliminate this source of variability. Desalt samples before or after labeling to (1) remove excipients that may interfere with the profile, and (2) increase the signal.

In existing data, check the Show Peak Baselines option of the Properties tab to make sure that the software analyzes each profile of the same sample in the same way (same number of peaks, with peak baselines defined at the same position within the profile). Adjust the *Peak Find* parameters in the *Analysis Settings* interface, such as the Filter Width (sec) or Slope Threshold (/sec), to improve the reproducibility of the peak find algorithm. Make sure all extraneous peaks are excluded (see Typical Results section). Also make sure that all relevant peaks are included if summing basic and acidic variants.

What are good samples to use as positive controls?

Bovine serum albumin (BSA) can be used as positive control samples to make sure that the electrophoretic separation and the labeling reaction are working properly. The BSA is labeled at a concentration of 0.5 - 10 mg/mL.

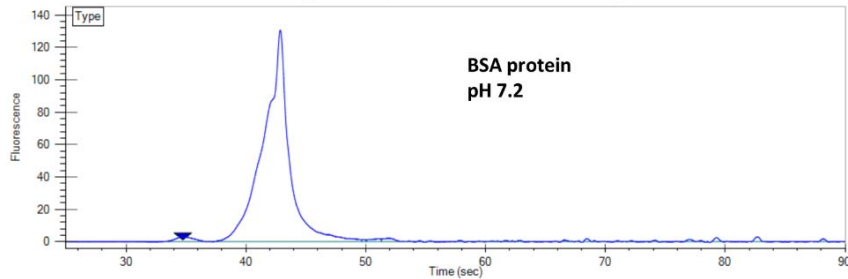


Figure 24. Assay with BSA as a Control

Why do electropherograms change when wells from different assays are selected at the same time?

If a well from the longer assay is selected first, then all data will appear in the electropherogram (including the irrelevant data of the shorter assay, collected during the sip - see Assay Performance in the FAQ section). However, if a well from the shorter assay is selected first, then all data will be truncated at the total assay time of the shorter assay (68 or 90 seconds). The additional 20 seconds of irrelevant data in the shorter run may include artifact peaks, particularly if the profile of the sample is near the end of the separation time (48 or 70 seconds). Set the end time to 48 or 70 seconds for the 68 and 90 second assay, respectively, to exclude these artifact peaks from the analysis. This issue will be addressed in a future software update.

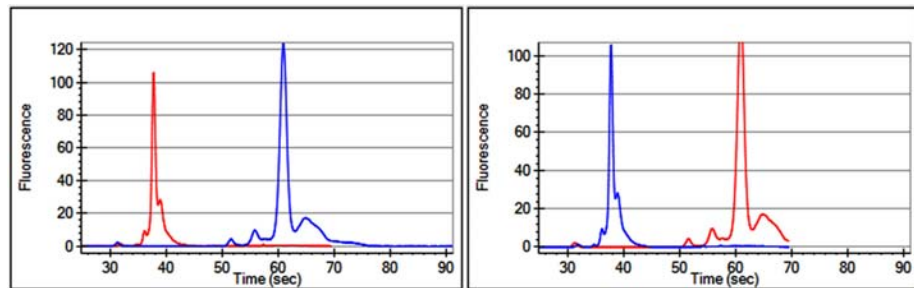


Figure 25. Examples of assays in which wells from the longer (left) or shorter (right) assays are chosen

Why is the baseline noisy, the migration of my sample slow, and the resolution of the variants poor?

If the Protein Express, the Low MW Protein, the Pico Protein, or the ProfilerPro Glycan Profiling assay was run on the instrument, then the electrodes must be cleaned before running the Low pI Charge Variant assay. If the electrodes are not cleaned, residual gel will contaminate the chip and adversely affect the performance. To fix this problem, remove the chip from the instrument, aspirate and rinse the wells of the chip as described in [Preparing the Chip on page 8](#), and add fresh Running Buffer. Next, replace the Buffer Tube with a new tube containing fresh Running Buffer. Then thoroughly clean the electrodes of the instrument using water (Milli-Q[®] or equivalent) and a swab. Place the chip back in the instrument and restart the run.

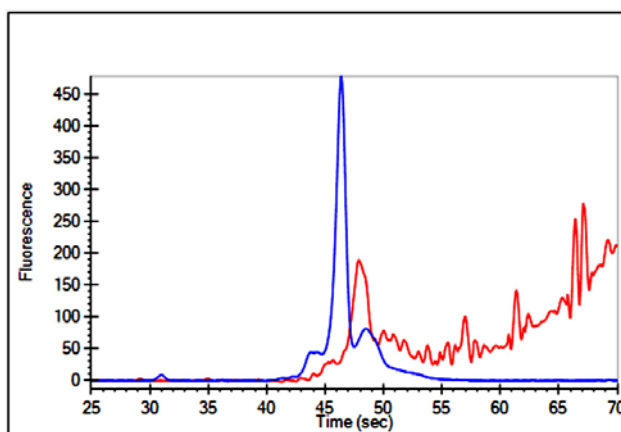


Figure 26. Example profiles showing the effect of residual Protein Express gel (red = contaminated; blue = cleaned)

Frequently Asked Questions

Kit Components

- 1 Is there a lower marker for this assay?

No. The assay has been optimized to minimize variation in migration time (see Troubleshooting section for more details). Alignment to a lower marker should not be necessary.

- 2 Is there a charge or pI ladder for this assay?

No. Similar to conventional CZE assays, this assay does not provide pI information.

Sample Preparation

- 1 May other desalting methods be used, besides Zeba Desalting plates and columns?

Yes, however Zeba Desalting plates and columns were used in the validation of the assay and are therefore preferred.

- 2 What happens to the dye when it is not used immediately after dilution with DMF?

When left at room temperature for extended periods of time (hours) the dye solution will degrade, causing inefficient labeling and resulting in low sample signal.

- 3 Can labeled samples be used on another day?

We recommend that samples are analyzed immediately after labeling. However, labeled samples can be frozen and analyzed later. A loss in signal may result from this storage.

- 4 Can labeled samples be reconstituted in less or more than 60 μ L of water (Milli-Q[®] or equivalent)?

The 60 μ L dilution gives the optimal signal and profile reproducibility. Other dilutions can be used, however, signal and reproducibility may be compromised.

- 5 Can the samples be labeled for more than 10 minutes, or less than 10 minutes?

The recommended 10 minutes is the shortest labeling time that will give the specified reproducibility. Shorter labeling times will compromise reproducibility and longer labeling times are not likely to affect reproducibility.

- 6 What is the lowest sample concentration that I can use?

The recommended range is 0.5 - 10 mg/mL. The reproducibility of % Relative Amount measurements may decrease for concentrations < 0.5 mg/mL, but detection below this limit is possible.

- 7 Can I label less than 24 samples?

Yes. To label less than 24 samples, reduce the volume of Dye Concentrate and N,N- dimethylformamide proportionately. For example, to label 12 samples, add 2.5 μ L Dye Concentrate to 72.5 μ L N,N-dimethylformamide.

- 8 Can I analyze a sample with a pI outside of the specified range?

Probably not. Lower pI mAbs may migrate too slowly and not elute before the end of the separation time, even for the 110-second assay. Higher pI mAbs may not be sufficiently resolved, even at pH 7.2.

- 9 Can high concentration proteins be run to chip lifetime?

Chip lifetime experiments were confirmed using protein at 2 mg/mL concentration. Higher concentration proteins can be run to lifetime, but the risk of protein aggregation and chip clogging is higher.

Assay Performance

- 1 How does this assay compare to conventional IEC, CZE and cIEF?

Typically, the resolution of the Low pI Charge Variant assay is the same or higher than that of IEC. The resolution of the Low pI Charge Variant assay is comparable to conventional CZE, and in most cases, the profile is similar to cIEF. The % Relative Amounts of variants reported by the Low pI Charge Variant assay are similar to those given by conventional assays. The analysis time of the Low pI Charge Variant assay is typically 10- to 50-fold faster than that of conventional assays.

- 2 Why does the electropherogram end 20 seconds before the time stated in the assay name?

The next sample is sipped after the end of the separation of the current sample, for 20 seconds. During this time, no relevant data are collected so these 20 seconds are excluded from the electropherogram. In the assay names, we have given the sum of the times required for the sip and the separation steps to provide the total time required to analyze each sample.

3 Can I run multiple samples that have different pIs at the same pH?

It is possible to run multiple samples with varying pIs at the same pH and achieve adequate resolution of charge variants for each sample. We recommend limiting the range of pI to no more than the ranges given in [Table 7](#). However, even with this limit, the resolution for all samples may not be optimal.

4 What should I do if I don't know the pI of my sample?

We suggest running the longest assay, using the running buffer at pH 5.6. Depending on the resolution, proceed with the optimization of the pH of the running buffer, as described in the [Optimization of Running Buffer pH](#) section.

5 Can I use my own running buffer or modify the current running buffer?

The Running Buffer provided in the kit has been optimized for resolution and reproducibility. We do not support the use of customer-developed running buffers.

6 Can this assay be run on the LabChip 90?

No. The LabChip 90 software does not support this assay.

7 If I am encountering difficulties, how do I confirm that the kit is working properly?

BSA can be used as positive control samples to check the labeling reaction and the chip. See the [Troubleshooting](#) section.

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 Revvity Technical Support at 1-800-762-4000.

General

- The assay requires exact and consistent adherence to the protocol as shown in this guide, or results may be compromised by increased variability.
- Allow the chip, sample plate, and all refrigerated reagents to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.
- Remove the Labeling Buffer and Dye Concentrate from the padded shipping pack and allow to warm from -20°C to room temperature (20 - 25°C) for 45 minutes. Protect the Dye Concentrate 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.
- Use only the supplied Detection Window Cleaning Cloth to clean the chip 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 33](#)) will help avoid introducing bubbles into the chip when pipetting the gel.

1. Revvity, Inc. warrants that the LabChip Kit meets specification at the time of shipment, and is free from defects in material and workmanship. LabChip Kits are warranted for 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

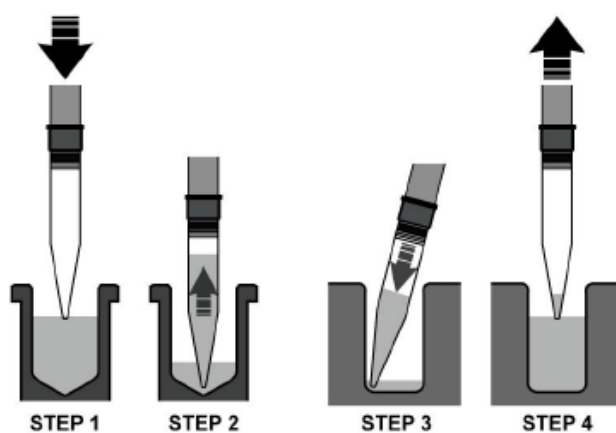


Figure 27. Reverse pipetting

- 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 as specified in [Storage Conditions on page 3](#).
- Protect the Dye Concentrate and Dye solution from light.

Chips

General Guidelines

- Store chips at 2 - 8°C.
- Do not allow the liquid in the chip container to freeze, as this may lead to poor chip performance. Do not submerge the chip in any solution.
- The entire chip surface must be thoroughly dry before use.
- 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 storage 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 number of samples are not exceeded.

Repriming Chips

Note: Place a Buffer tube filled with running buffer or water (Milli-Q® or equivalent) into the instrument while priming or washing chips.

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

Samples

- Prepared sample plates should be free of gas bubbles and particulate debris, both of which may inhibit sipper flow.
- Spin down sample plates containing gas bubbles and/or particulate debris at 3000 rpm (1250 rcf) for 5 minutes prior to analysis.
- Up to 96 samples can be run with a single chip preparation.

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 28). To avoid contamination, use a new disposable pipette tip over the permanent tip for each chip aspirated (Figure 29).

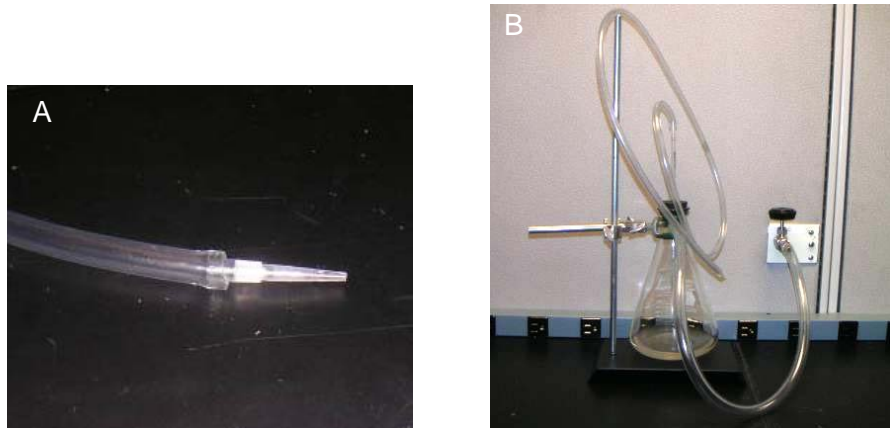


Figure 28. A: Permanent pipette tip attached to a house vacuum line; B: vacuum line with trap



Figure 29. Replacing the disposable pipette tip

Reordering Information

Table 8. Reordering information

Product	Part Number
Low pl Charge Variant Reagent Kit	CLS154134
Low pl Charge Variant LabChip	CLS153418

Customer Technical Support

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Hopkinton, MA 01748-1668 USA

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Fax: +1 203-944-4904

Email: L3LabChip@Revvity.com

Internet: www.Revvity.com

LabChip Chip QC test data portal:

<https://www.Revvity.com/tools/LabChipQCSearch>

LabChip Reagent CoA: <https://www.Revvity.com/tools/COASearch>

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

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