Low pI Charge Variant Assay Quick Guide
LabChip® GXII Touch

Note: ***We highly recommend that first-time users read the full Low pI Charge Variant Assay User Guide before proceeding.***

Critical: *Allow the chip 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.

Chip Preparation

Keep the chip in its container during preparation and when carrying from one location to another.

After a chip has been used for the Low pI Charge Variant assay, it should be designated for this assay only. Do not run other assays with this chip.

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 🔴, pH 7.2 🟢, or pH 8.5 🟡 at the ratio corresponding to the desired pH (see Table 1).

Table 1: Running Buffer pH Adjustment

pH 5.6 (µL) 🔴	pH 7.2 (µL) 🟢	Solution 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

pH 7.2 (µL) 🟢	pH 8.5 (µL) 🟡	Solution 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.
4. Rinse and completely aspirate each active chip well (1, 3, 4, 7, 8, and 10) twice with water (Milli-Q® or equivalent).
5. When using pH 5.6 to 7.2 Running Buffer solution, add **75 µL** of the Running Buffer solution to chip wells 3, 4, 7, 8, and 10 (see Figure 1).

   When using pH 7.3 to 8.5 Running Buffer solution, add **120 µL** of pH 7.2 Running Buffer 🟢 to wells 3, 4, and 8, and add **120 µL** of pH 7.3 to 8.5 Running Buffer solution to wells 7 and 10 (See Figure 2).

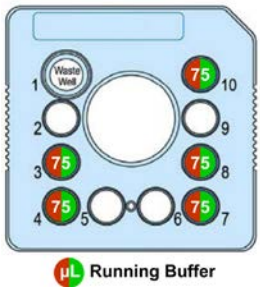

Figure 1: Chip Preparation for pH 5.6 to 7.2 Running Buffer

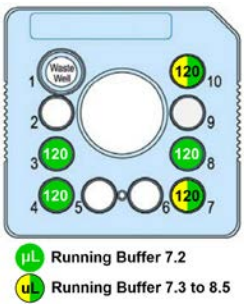

Figure 2: Chip Preparation for pH 7.3 to 8.5 Running Buffer

Running Buffer in Buffer Tube

6. Ensure chip well 1 (waste well) is empty.
7. Thoroughly clean the electrodes of the instrument with water (Milli-Q® or equivalent) before placing the chip in the instrument.
8. Add **750 µL** of Running Buffer to the 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.
9. Place the chip and buffer tube in the instrument. Each chip preparation is sufficient for running 96 samples.

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Sample Preparation

1. (Recommended) If the protein sample contains cell culture media, salt (>10mM), 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). *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 µL** of Labeling Buffer ● and **25 µL** of sample (2 mg/mL is optimal, 0.5 - 10 mg/mL is acceptable).
3. When using Running Buffer solution with pH 7.3 to 8.5, add **3 µL** of NaHCO3 solution (1000 mM in Milli-Q water, not provided).

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 labeling more than 24 samples, 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 can be combined.*

4. Add **5 µL**[1] of Dye Concentrate ● to **145 µL** anhydrous (99.8%) N,N-dimethylformamide in a microcentrifuge tube and vortex for 10 seconds (use a syringe to extract ~200 µL of the N,N-dimethylformamide from the bottle and dispense into an intermediate tube). Each 150 µL aliquot of Dye solution is sufficient for labeling 24 samples.
5. To each sample, add **5 µL** of Dye solution and mix by pipetting up and down.
6. Seal the sample plate and incubate at room temperature for **10 minutes**, protected from light.
7. For Running Buffers pH 5.6 to 7.2: To each sample, add **60 µL** of water (Milli-Q® or equivalent).

   For Running Buffers 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 or with a plate shaker. Centrifuge sample plate for 1 minute at 1000 rpm.
9. (Optional) Remove excess dye using Zeba Spin Desalting Plate or Column. (See the *Low pI Charge Variant Assay User Guide* for details.)
10. Place sample plate in instrument.

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 2-9 of the sample preparation instructions. 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. Click the **Run** button in the LabChip GX Touch software. The ***Run*** window opens.
2. In the ***Select Wells*** tab, select the appropriate **Assay**, **Plate Type**, and **Well Pattern**. For selection of the appropriate assay and Running Buffer **pH** refer to Table 2. In the ***Advanced Settings***, select the number of times each well is to be sampled. For an initial run of protein with unknown pI, use the **Protein Charge Variant 110s Assay**. If protein peaks appear in the time frame of 30-60 seconds, the experiment can be performed using a shorter version, either **Protein Charge Variant 68s** or **Protein Charge Variant 90s.**

Table 2: Recommended Initial Run Conditions

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

The pH values listed in Table 2 are recommendations for achieving high resolution of charge variants in 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.

3. In the ***Setup Run*** tab, select the destination of the file and the filename convention. We recommend including the Running Buffer pH in the File prefix.
4. Click **Start** on the ***Start Run*** tab to begin the run.

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

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Washing and Storing the Chip

At the end of each day, wash the chip and store in the chip container. You should also wash the chip when changing the pH of the running buffer.

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 ● to each active well.
5. Place the chip back on the LabChip GXII Touch 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.
8. After the wash is complete, remove the chip from the LabChip GXII Touch and place the chip in the chip storage container.
9. Cover all wells with Parafilm®, close the chip storage container, and store the chip at 2 - 8°C.

Assay Specifications

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.

Sample Type	Protein
pI Range	3.0 - 7.0
Amount of Sample Required	25 µL with concentration between 0.5 - 10 mg/mL (12.5 µg to 250 µg of protein, total) Optimal concentration: 2 mg/mL
Resolution	Comparable to IEX and conventional CZE
Reproducibility[2]	CV < 5% for varying concentration from 1 - 3 mg/mL CV < 3% at constant concentration
Assay Run Time	1.8 – 3.5 hr for a 96-well plate. Three assay durations: 68 s, 90 s, and 110 s.
Chip Lifetime	500 samples
Samples per Chip Prep	Up to 96 samples
Samples per Reagent Kit	120 samples
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LabChip Chip QC test data portal: https://www.revvity.com/tools/LabChipQCSearch

LabChip Reagent CoA: https://www.revvity.com/tools/COASearch

For the complete *Low pI Charge Variant Assay User Guide* (P/N CLS154074), go to: http://www.revvitycom.

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[2] Based on protein main peak.