High pI Charge Variant Assay Quick Guide
LabChip® GXII Touch

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

This guide is also used for the Protein Charge Variant Assay in LabChip GX Touch software V1.8 or lower.

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 High pI 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 if the Protein Express, Low MW Protein, Pico Protein, or ProfilerPro Glycan Profiling assay was run previously.

1. Mix the pH 5.6 🔴 and pH 7.2 🟢 Running Buffers at the ratio corresponding to the desired pH (see Table 1 and Table 2).
2. Vortex the Running Buffer solution for about 10 seconds and spin down.
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 **75 µL** of the Running Buffer solution to wells 3, 4, 7, 8, and 10 of the chip (as shown in Figure 1).
5. Ensure chip well 1 (waste well) is empty before placing the chip in the LabChip GXII Touch.
6. Add **750 µL** of the Running Buffer solution to the provided buffer tube; place chip and buffer tube in instrument. Each chip preparation is sufficient for running 96 samples.

Table 1: Running Buffer pH Adjustment

pH 5.6 (µL) 🔴	pH 7.2 (µL) 🟢	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**

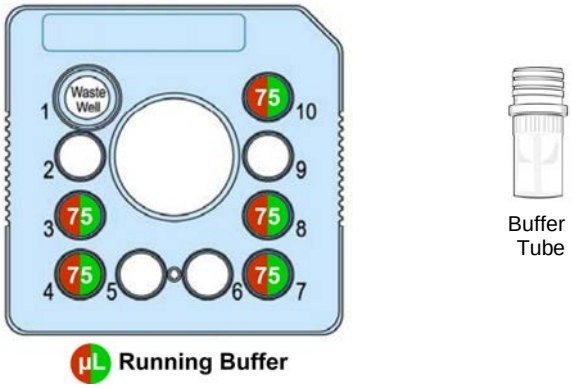

Figure 1: Chip Preparation

Sample Preparation

1. (Recommended) If the mAb 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 High 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 allowed).

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

3. Add **5 µL** 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 Mixture is sufficient for labeling 24 samples.
4. To each sample, add **5 µL** of Dye Mixture and mix by pipetting up and down.
5. Seal the sample plate and incubate at room temperature for **10 minutes**, protected from light.
6. To each sample, add **60 µL** of water (Milli-Q® or equivalent) and mix by pipetting up and down or with a plate shaker. Centrifuge sample plate for 1 minute at 1000 rpm.
7. (Optional) Remove excess dye using Zeba Spin Desalting Plate or Column. (See the *High pI Charge Variant Assay User Guide* for details.)
8. Place sample plate in instrument.

Running the Assay

For selection of the appropriate **Assay** (**High pI Charge Variant 68s**, **High pI Charge Variant 90s**, or **High pI Charge Variant 110s**), refer to Table 2. (The assays are named Protein Charge Variant 68s, 90s, and 110s when using the DNA 5K/RNA/CZE LabChip.)

Table 2: Recommended Run Conditions

pI of Main Variant	Running Buffer pH	Assay
9.5 - 9.1	7.2	**High pI Charge Variant 68s**
9.0 - 8.7	6.2	**High pI Charge Variant 68s**
8.6 - 8.0	5.9	**High pI Charge Variant 90s**
7.9 - 7.0	5.6	**High pI Charge Variant 110s**

The pH values listed in Table 2 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, and thus a longer assay may be required.

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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 µL** 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 High 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.

Reagent Kit	High pI Charge Variant (P/N CLS760670)
LabChip	High pI Charge Variant LabChip (P/N CLS153419) Prior to V1.9 software: • HT DNA 5K/RNA/CZE LabChip: P/N 760435 • 24 DNA 5K/RNA/CZE LabChip: P/N CLS138949
Sample Type	Monoclonal antibody (mAb)
pI Range	7.0 - 9.5
Amount of Sample Required	25 µL with concentration between 0.5 - 10 mg/mL (12.5 µg to 250 µg of mAb, total) Optimal concentration: 2 mg/mL
Resolution	Comparable to IEX and conventional CZE
Reproducibility	CV < 5% for varying concentration from 1 - 3 mg/mL CV < 3% at constant concentration
Assay Run Time	1.8 - 3 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	Up to 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 *High pI Charge Variant Assay User Guide* (P/N CLS140162), go to: http://www.revvity.com/

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