Protein Charge Variant Assay Quick Guide
LabChip® GXII Touch

Note: ***We highly recommend that first-time users read the full User Guide for this assay, before proceeding.***

Allow the Chip and Reagents to equilibrate to room temperature for 30 minutes before use.
The Dye Concentrate is light sensitive. Do not expose the Dye to light for any length of time.

Chip Preparation

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

Once a chip has been used for the Protein Charge Variant assay, it should be designated for this assay only. Do not run other LabChip GXII Touch assays with this chip.

1. Rinse and aspirate each active well (1, 3, 4, 7, 8, & 10) of the chip once, with molecular biology-grade water.
2. 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).
3. Vortex the Running Buffer mixture for about 10 seconds and spin down.
4. Add **75 µL** of the Running Buffer mixture to wells 3, 4, 7, 8, & 10 of the chip (as shown in Figure 1).
5. Add **750 µL** of the Running Buffer mixture 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**

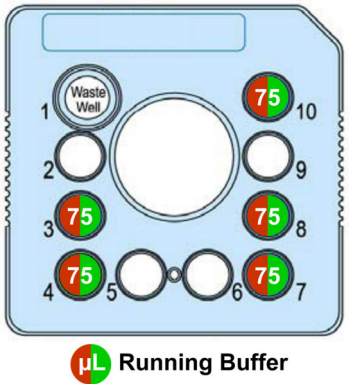

Figure 1: Chip Preparation

Thoroughly clean the electrodes of the instrument with molecular biology-grade water before placing the chip in the instrument if the Protein Express, the Low MW Protein, the Pico Protein, or the ProfilerPro Glycan Profiling assay was run previously.

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 Protein Charge Variant dye reacts with the ε-amino group of lysine residues via an amide linkage; avoid using amine-containing buffers.*
2. To a single 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).

PN CLS140172, Rev. B

3. Add **5 µL** of Dye Concentrate to **145 µL** anhydrous (99.8%) N,N-dimethylformamide in a microcentrifuge tube and vortex for 10 s (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. 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 may be combined.*
4. To each sample, add **5 µL** of Dye Mixture and mix by pipetting up and down.
5. Incubate sample plate at room temperature for **10 minutes**, protected from light.
6. To each sample, add **60 µL** of molecular biology-grade water 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.
8. Place sample plate in instrument.

Running the Assay

For selection of the appropriate **Assay** (**Protein Charge Variant 68s**, **Protein Charge Variant 90s**, or **Protein Charge Variant 110s**), refer to Table 2.

Table 2: Recommended Run Conditions

pI of Main Variant	Running Buffer pH	Assay
9.5 - 9.1	7.2	**Protein Charge Variant 68s**
9.0 - 8.7	6.2	**Protein Charge Variant 68s**
8.6 - 8.0	5.9	**Protein Charge Variant 90s**
7.9 - 7.0	5.6	**Protein 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 may be increased by increasing the pH. However, migration speeds decrease with increasing pH, and thus a longer assay may be required.

PN CLS140172, Rev. B

Protein Charge Variant Assay Quick Guide
LabChip® GXII Touch

Chip Cleaning and Storage

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

1. Remove the Running Buffer from each well using vacuum.
2. Rinse and thoroughly aspirate each active well (1, 3, 4, 7, 8, & 10) once, with molecular biology-grade water.
3. Add **75 µL** of Storage Buffer ● to each active well, and **120 µL** to the waste well, as shown in Figure 2.
4. Place the chip back on the LabChip GXII Touch and ensure that a Buffer Tube containing Running Buffer is in the Buffer Tube slot. Click the **Wash** button.
5. After the wash is complete, remove the chip from the LabChip GXII Touch and place in chip container.
6. Cover all wells with Parafilm®, close container, and store at 4 ºC.

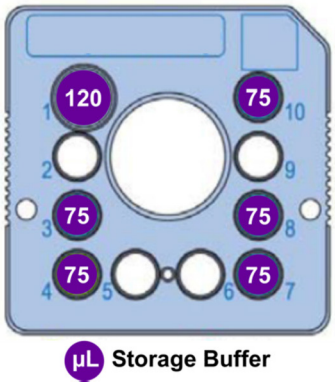

Figure 2: Chip Storage

Assay Specifications

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.
Number of Samples per Kit	120 samples
Number of Samples per Chip Prep	96 samples
For Research Use Only	

For complete Protein Charge Variant Assay User Guide, go to:

http://www.perkinelmer.com/labchipsystems

PN CLS140172, Rev. B