

Protein Charge Variant Assay Quick Guide

LabChip® GXII Touch

Note: We highly recommend that first-time users read the full Protein 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.

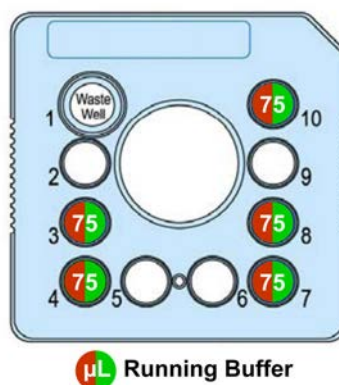
After a chip has been used for the Protein Charge Variant assay, it should be designated for this assay only. Do not run other LabChip GXII Touch assays with this chip.

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 mixture 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 mixture to wells 3, 4, 7, 8, & 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 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



Buffer Tube

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 Protein 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).

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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 *Protein Charge Variant Assay User Guide* for details.)
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.

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 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

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

1. Place the chip into the chip storage container. The sipper should be 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 ensure that a Buffer Tube containing Running Buffer is 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 Protein 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	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	HT: 500 samples 24: 500 samples
Samples per Chip Prep	Up to 96 samples per batch with LabChip GXII Touch HT Up to 48 samples per batch with LabChip GXII Touch 24
Samples per Reagent Kit	Up to 120 samples

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For the complete Protein Charge Variant Assay User Guide, go to: <http://www.perkinelmer.com/>



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