Protein Express Assay Quick Guide
LabChip® GXII Touch and LabChip® GXII

Preparing Gel-Dye and Protein Express Chip

Notes: *The Dye Solution contains DMSO and* ***must be thawed completely*** *before use. The dye is light sensitive.* ***Do not expose the Dye Solution or Gel-Dye to light for any length of time.*** *Keep the prepared Gel-Dye Solution in the dark. Gel matrix is extremely viscous. Ensure that the correct volume of gel is transferred to the spin filter by using a reverse pipetting technique and pipetting slowly. Incorrect ratios of gel to dye will cause inconsistent assay results. Gel-Dye mixture can be stored in the dark for 3 weeks at 4°C.* ***Allow the chip and all reagents to equilibrate to RT before use (approximately 20 to 30 minutes).***

1. Vortex the thawed Dye Solution for 20 seconds and quickly spin down before use.
2. Using a reverse pipetting technique, transfer 520 µL (HT) or 280 µL (LT) of Protein Express Gel Matrix (red cap) to the top basket of a provided spin filter.
3. Add 20 µL (HT) or 10.7 µL (LT) of Protein Express Dye Solution (blue cap) to the Gel Matrix in the spin filter.
4. Cap the spin filter, invert, and vortex in the inverted orientation until the Gel-Dye appears a uniform blue color.
5. For Destain Solution, transfer 250 µL (HT) or 180 µL (LT) of Protein Express Gel Matrix (red cap) to a second spin filter.
6. Spin the Gel-Dye mix and the Destain Solution at 9300 rcf for 5 min at RT. Ensure that all the material has passed through the filter (spin longer if necessary), then discard the filter baskets and cap the tubes. Store in the dark until ready to use.
7. Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells.
8. Each active chip well (1, 2, 3, 4, 7, 8, 9, and 10) should be rinsed and completely aspirated twice with water (Milli-Q® or equivalent). Do not allow active wells to remain dry.
9. Using a reverse pipetting technique, add Destain Solution to chip wells 2 and 9 as shown in Figure 1 (HT) or Figure 2 (LT).
10. Using a reverse pipetting technique, add Gel-Dye Solution from spin filter tube to chip wells 3, 7, 8, and 10 as shown in Figure 1 (HT) or Figure 2 (LT). Store any unused Gel-Dye Solution in the dark at 4°C for up to three weeks.
11. Using a reverse pipetting technique, add 120 µL (HT) or 50 µL (LT) of Marker solution (green cap) to chip well 4 as shown in Figure 1 (HT) or Figure 2 (LT).
12. Use the provided Detection Window Cleaning Cloth dampened with 70% Isopropanol to clean the chip detection window.

Note: Up to 48 samples can be analyzed in LT mode.

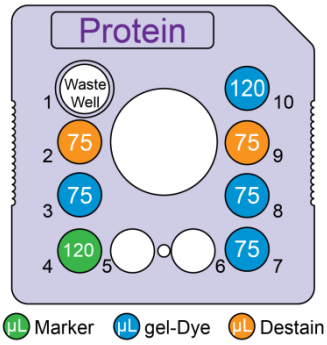

Figure 1. Reagent placement for High-throughput (up to 384 samples)

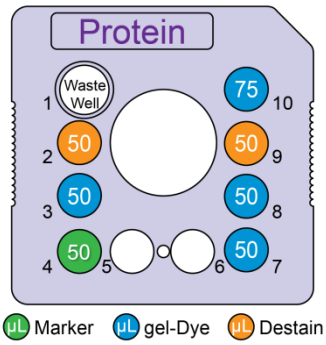

Figure 2. Reagent placement for Low-throughput (up to 48 samples)

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Preparing Protein Samples, Ladder and Buffer

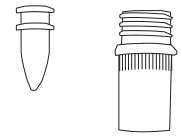
Note: *Samples can be prepared in either a 96-well or 384-well PCR plate or in 0.6 mL microcentrifuge tubes (and subsequently pipetted into a plate).*

1 If samples need to be reduced, prepare sample denaturing solution. Pipette 700 µL of Protein Express Sample Buffer (white cap) into a 2.0 mL centrifuge vial. Add 24.5 µL of BME or 1 M DTT or 3.75 µL of 100 mM TCEP.

2 For each sample to be analyzed, pipette 7 µL of denaturing solution into the wells of a plate. Add 2 µL (5 µL if running the High Sensitivity Assay) of each protein sample.

3 Ensure the Protein Express Ladder has been warmed to room temperature, then vortex gently for 10 seconds. Briefly spin the ladder vial. Ensure no precipitate is visible in the solution.

4 Pipette 12 µL of Protein Express Ladder into a microcentrifuge tube. **Do not add denaturing solution to the ladder.**



Ladder Tube | Buffer Tube

5 Denature samples and ladder at 100°C for 5 minutes. Optimum denaturing conditions may vary by sample type.

6 Add 35 µL of water (32 µL if running High Sensitivity Assay) and mix well by pipetting up and down.

7 Add 120 µL of water (Milli-Q® or equivalent) to the ladder and mix well.

8 Transfer 120 µL of prepared ladder to the provided 0.2 mL Ladder Tube.

9 Add 750 µL of Protein Express Wash Buffer (purple cap ●) to the provided 0.75 mL Buffer Tube.

10 Spin the sample plate at 1200 rcf for 2 minutes.

Chip Cleaning and Storage

Note: *After use, the chip must be cleaned and stored in the chip container. The cleaning procedure can be conducted the following day, when running overnight.*

1 Remove reagents from each well using a vacuum.

2 Rinse and thoroughly aspirate each active well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water.

3 Add 120 µL of water (Milli-Q® or equivalent) to the active wells.

4 Cover all wells with Parafilm® and store at RT.

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Assay Specifications

Sizing Range	P100 Assay: 14 kDa - 100 kDa P200 Assay: 14 kDa - 200 kDa
Sizing Resolution	± 10% difference in molecular weight
Sizing Accuracy	± 20%
Linear Concentration Range	5.0 - 2000 ng/µL
Maximum Total Protein Concentration	10 mg/mL
Quantitation Reproducibility	30% CV up to 120 kDa. Above 120 kDa, quantitation is not specified.
Sample Capacity per HT Chip Prep	384 samples (four 96-well plates or one 384-well plate)
Sample Capacity per LT Chip Prep	48 samples
For Research Use Only	

For the complete *Protein Express Assay User Guide*, go to:

http://www.perkinelmer.com/labchipsystems

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