

Protein Express Assay Quick Guide

LabChip® GXII Touch and LabChip® GX II

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, Gel-Dye solution, or Lower Marker to light for any length of time. Keep the Dye, prepared Gel-Dye solution, and Lower Marker in the dark.

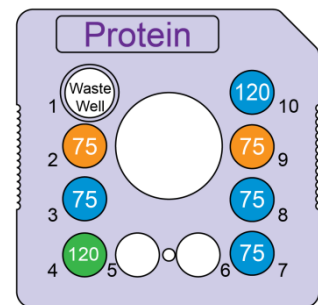
Gel matrix is extremely viscous. Ensure that the correct volume of gel is transferred to the spin filter by using a reverse pipetting technique and pipetting slowly. Incorrect ratios of gel to dye will cause inconsistent assay results. Gel-Dye solution can be stored in the dark for 3 weeks at 2 - 8°C.

Critical: Allow the chip and all reagents to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. Protect the Dye Solution and the Lower Marker from light.

Allow the chip and all reagents to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.

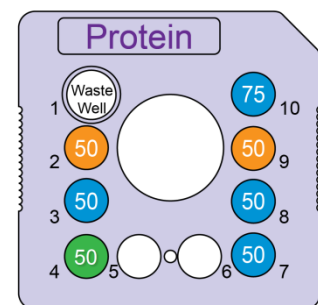
- 1 Vortex the thawed Dye Solution for 20 seconds and quickly spin down before use.
- 2 Using a reverse pipetting technique, transfer 520 μL (HT) or 280 μL (LT) of Protein Express Gel Matrix (red cap ●) to the top basket of a provided spin filter.
- 3 Add 20 μL (HT) or 10.7 μL (LT) of Protein Express 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 is 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 8 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 Rinse and thoroughly aspirate each active chip well (1, 2, 3, 4, 7, 8, 9, and 10) 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 2 - 8°C for up to 3 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.



● 120 μL Marker ● 75 μL gel-Dye ● 75 μL Destain

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



● 50 μL Marker ● 50 μL gel-Dye ● 50 μL Destain

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

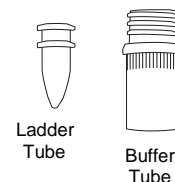
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Preparing Protein Samples, Ladder and Buffer

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.
- 3 Add 2 μL (5 μL if running the High Sensitivity Assay) of protein sample to each well.
- 4 Seal plate to minimize evaporation.
- 5 Ensure the Protein Express Ladder has been warmed to room temperature, and then vortex gently for 10 seconds. Briefly spin the ladder vial. Ensure no precipitate is visible in the solution.
- 6 Pipette 12 μL of Protein Express Ladder into a microcentrifuge tube.
Do not add denaturing solution to the ladder.
- 7 Denature samples and ladder at 100°C for 5 minutes. Optimum denaturing conditions may vary by sample type.
- 8 Add 35 μL (32 μL if running High Sensitivity Assay) of water (Milli-Q® or equivalent) to each sample well and mix by pipetting up and down a few times.
- 9 Add 120 μL of water (Milli-Q® or equivalent) to the ladder and mix well.
- 10 Transfer 120 μL of prepared ladder to the provided 0.2 mL Ladder Tube.
- 11 Add 750 μL of Protein Express Wash Buffer (purple cap ●) to the provided 0.75 mL Buffer Tube.
- 12 Spin the sample plate at 3000 rpm (1250 rcf) for 5 minutes.



Cleaning and Storing the Chip

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 chip well using a vacuum.
- 2 Rinse and thoroughly aspirate each active well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q® or equivalent).
- 3 Add 120 μL of water (Milli-Q® or equivalent) to the active wells.
- 4 Cover the wells with Parafilm® to prevent evaporation and store the chip at room temperature (20 - 25°C) until next use. Allowing chip wells to dry may lead to changes in chip performance. Use to the total lifetime within 30 days of analyzing the first sample.

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Assay Specifications

The Protein Express Assay is for use with LabChip GXII Touch or LabChip GX II instruments. LabChip GXII Touch and LabChip GX II instruments are for research use only and not for use in diagnostic procedures.

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|-------------------------------------|---|
| 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. |
| Chip Lifetime | HT: 400 samples 24: 200 samples |
| Samples per Chip Prep | HT: up to 384 samples 24: up to 48 samples |
| Chip Preps per Reagent Kit | 4 HT chip preps, or 8 LT chip preps |

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For the complete *Protein Express Assay User Guide*, go to: <http://www.revvity.com/>

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