Low MW Protein Express Assay Quick Guide
LabChip® GXII Touch

Preparing Gel-Dye and High Resolution Chip (Low MW Protein)

Notes: *The Dye solution contains DMSO and* ***must be thawed completely*** *before use.*

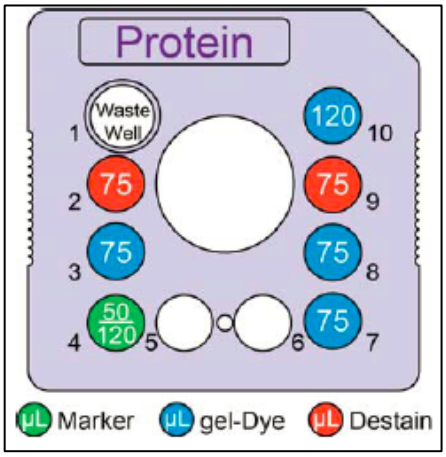
The dye is light sensitive. ***Do not expose the Dye or Gel-Dye solution to light for any length of time.*** *Keep the Dye and the prepared Gel-Dye solution in the dark.*

Gel matrix is extremely viscous. Make sure the Gel-Dye solution has an even blue color before transferring to the spin filter. Insufficient mixing of gel and dye will cause inconsistent assay results. Store Gel-Dye solution in the dark at 2 - 8°C for up to 3 weeks.

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

Remove the LMW Protein Express Ladder from the padded shipping pack and allow to warm from -20°C to room temperature (20 - 25°C) for 45 minutes.

1. Prepare Gel-Dye solution by adding **520 µL** LMW Protein Express Gel Matrix to **20 µL** Protein Express Dye Solution using a Reverse Pipetting Technique. Vortex until well mixed and then transfer to a spin filter.
2. Prepare Destain solution by adding **250 µL** LMW Protein Express Gel Matrix to a spin filter.
3. Centrifuge the Gel-Dye and Destain solutions at **9300 rcf for 8 minutes at RT**. Ensure that all of the gel has passed through the filters and then discard the filters.
4. Rinse and aspirate each active well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q® or equivalent) .
5. Add Destain solution to chip wells 2 and 9 (as shown in Figure 1) using a Reverse Pipetting Technique.
6. Add prepared Gel-Dye solution to chip wells 3, 7, 8, and 10 (as shown in Figure 1) using a Reverse Pipetting Technique.
7. Add Protein Express Lower Marker to chip well 4 (as shown in Figure 1). Add ***50 µL*** Protein Express Lower Marker for 96-well plate and **120 µL** Marker for 8 hour or multi-plate analysis.
8. Clean both sides of the chip window with the supplied clean room cloth dampened with 70% isopropanol.



Note: Use the High Resolution Chip for either Low MW Protein Express or Glycan Profiling assays. Only one assay type should be run on a chip.

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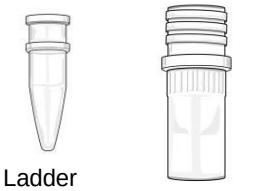
Protein Sample, Ladder, and Buffer Preparation

Note: The LMW Protein Ladder should be kept frozen. It is recommended that you aliquot the ladder into 12 µL lots for individual use after thawing for the first time. Store the aliquots at -20°C.

1. Prepare denaturing solution by adding **24.5 µL** BME, **24.5 µL** 1M DTT or **3.75 µL** 100 mM TCEP to **700 µL** Protein Express Sample Buffer. Vortex for 10 seconds.
2. Add **2 µL** protein sample to **7 µL** denaturing solution. Samples can be prepared in a microtiter plate or in microcentrifuge tubes. Cover the plate to minimize evaporation.
3. Pipettte **12 µL of** LMW Protein Express Ladder (stored at -20°C, not with rest of the kit box) to a microcentrifuge tube or into the well of a microtiter plate. *Do not add denaturing solution to the ladder.*
4. Denature samples and ladder at **100°C for 5 minutes**. *Optimum denaturing conditions may vary by sample type*.
5. Add **35 µL** water (Milli-Q® or equivalent) to the samples and **120 µL** water (Milli-Q® or equivalent) to the ladder and mix. Vortex and shake for a few seconds.
6. Transfer samples (**44 µL**) to a microtiter plate.
7. Transfer **120 µL** prepared ladder to the provided 0.2 mL Ladder Tube.
8. Add **750 µL** Protein Express Wash Buffer to the provided Buffer Tube.



Ladder Tube Buffer Tube

Cleaning and Storing the Chip

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

1. Place the chip into the chip storage container. The sipper should be submerged in the fluid reservoir.
2. Remove the reagents from each chip well using a vacuum.
3. Rinse and completely aspirate each active well (1, 2, 3, 4, 7, 8, 9, and 10) twice with water (Milli-Q® or equivalent).
4. Add **120 µL** of water (Milli-Q® or equivalent) to the active wells.
5. 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 within 30 days of analyzing the first sample. See Assay Specifications on page 3 for Chip Lifetime.

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Assay Specifications

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

Sizing Range	5 kDa – 80 kDa
Sizing Resolution[1]	± 10% 14 - 80 kDa, ± 20% <14 kDa
Sizing Accuracy	± 20% up to 80kDa ± 10% (CAII, BLG)
Sizing Reproducibility	3% CV (CAII, BLG)
Linear Concentration Range	30 - 2000 ng/µL (BLG, CAII in PBS)
Maximum Total Protein Concentration	10 mg/mL
Quantitation Reproducibility	30% CV up to 80 kDa. Above 80 kDa, quantitation is not specified.
Chip Lifetime	HT: 400 samples 24: 200 samples
Samples per Chip Prep	HT: up to 384 samples LT: up to 48 samples
Chip Preps per Reagent Kit	HT: 4 chip preps LT: 4 chip preps

[1] Resolution is defined as the height of the valley between two peaks to be no more than 50% of the maximum peak height. Actual separation performance can depend on the sample and application.

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For the complete Low MW Protein Express Assay User Guide, go to: http://www.perkinelmer.com/

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