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 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. Make sure the Gel-Dye mixture has an even blue color before transferring to the spin filter. Insufficient mixing of gel and 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. Prepare Gel-Dye by adding 520 µL Low MW Protein Express Gel Matrix to 20 µL Protein Express Dye Solution using a Reverse Pipetting Technique. Vortex and transfer to a spin filter.

2. Prepare Destain solution by adding 250 µL Low MW Protein Express Gel Matrix to a spin filter. Centrifuge the Gel-Dye and Destain solutions at 9300 rcf for 5 minutes at RT. Ensure that all of the gel has passed through the filters and then discard the filters.

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

4. Add Destain solution to chip wells 2 and 9 (as shown in Figure 1) using a Reverse Pipetting Technique.

5. Add prepared Gel-Dye to chip wells 3, 7, 8 and 10 (as shown in Figure 1) using a Reverse Pipetting Technique.

6. 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.

7. Clean both sides of the chip window with the supplied clean room cloth dampened with 70% isopropanol.

Note: Use High Resolution Chip for either Low MW Protein or Glycan Profiling. Only one assay type should be run on a chip.
Protein Sample, Ladder, and Buffer Preparation

Note: The Low MW Protein Ladder should be kept frozen. It is recommended that you aliquot the ladder into 12 µL lots for individual use to avoid freeze-thawing.

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.

2. Add 2 µL (or 5 µL for High Sensitivity) protein sample to 7 µL denaturing solution.

3. Transfer 12 µL Low MW Protein Express Ladder (note: stored at -20°C, not with rest of the kit box) to a microcentrifuge tube. 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 (or 32 µL for High Sensitivity) water to the samples and 120 µL water to the ladder and mix.

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.

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, 3, 4, 7, 8 and 10) twice with water.

3. Add 120 µL of molecular biology-grade water to active wells.

4. Make sure to cover all wells with Parafilm® and store at RT.
Assay Specifications

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<th>Specification</th>
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<tr>
<td>Sizing Range</td>
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| Sizing Resolution\(^1\)                | ± 10% 14 - 80 kDa,  
|                                        | ± 20% <14 kDa                                                          |
| Sizing Accuracy                        | ± 20% up to 80 kDa,  
|                                        | ± 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.     |
| Sample Capacity per Chip Prep          | 400 samples (four 96-well plates or one 384-well plate)               |

\(^1\) 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.

For complete Low MW Protein Assay User Guide, go to:

http://www.perkinelmer.com/labchipsystems