ProteinEXact™ HR Assay Quick Guide
LabChip® GXII Touch

Preparing Gel-Dye and ProteinEXact HR Chip

CRITICAL:

- **The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. Protect the Protein Express Lower Marker from light.**
- **The Protein Clear HR Dye and the ProteinEXact HR Ladder must be removed from the padded shipping pack and allowed to warm from -20°C to room temperature for 45 minutes. Protect the Protein Clear HR Dye from light.**
- **After thawing, aliquot 15 µL of the ProteinEXact HR Ladder into each of the provided empty ladder tubes. Store these aliquots, including the original tube, at -20°C.**
- **This assay requires consistent adherence to the user and quick guide protocols as written, or results may be compromised by increased variability.**
- **Fresh Milli-Q® water should be obtained the day of the assay.**
- **Adherence to the full vortex time is important for assay performance.**

NOTES:

- *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 solution to light for any length of time****. Keep 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 solution can be stored in the dark for 3 weeks at 2 - 8°C.*
- *Up to 48 samples can be analyzed in LT mode and up to 96 samples can be analyzed in HT mode per calibrated chip prep.*

Gel-Dye and Destain Preparation

1. Allow all refrigerated reagents to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. From the freezer, take the Dye and one Ladder aliqoute and allow to warm from -20°C to room temperature for 45 minutes. Protect the Dye and Gel-Dye solution from light.
2. Vortex the thawed Protein Clear HR Dye solution (blue cap ) at max speed for 20 seconds and quickly spin down before use.
3. Using a reverse pipetting technique, transfer 520 µL (HT) or 280 µL (LT) of ProteinEXact Gel Matrix (red cap ) to the top basket of a provided spin filter.
4. Add 20 µL (HT) or 10.7 µL (LT) of Protein Clear HR Dye solution (blue cap ) to the Gel Matrix in the spin filter. For best results, make fresh and use immediately.
5. After the dye solution is added to the Gel Matrix, immediately cap and invert the spin filter 10 times to mix well and minimize dye concentrate interaction with filter material; then vortex tube, upside down for 20 seconds until the gel and dye solution are well mixed.
6. For Destain Solution, transfer 250 µL (HT) or 180 µL (LT) of ProteinEXact Gel Matrix (red cap ) to a second spin filter.
7. Spin the Gel-Dye solution and the Destain Solution at 9300 rcf for 8 min at RT.
   Note: *Ensure that the microcentrifuge is set to RT and all the material has passed through the filter (spin longer if necessary), and then discard the filter baskets and cap the tubes. Store in the dark until ready to use.*

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Chip Preparation

1. Allow the chip to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.
2. Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells.
3. Rinse and completely 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.
4. Using a reverse pipetting technique, add 75 µL (HT) or 50 µL (LT) of Destain Solution to chip wells 2 and 9 as shown in Figure 1 (HT) or Figure 2 (LT).
5. Using a reverse pipetting technique, add Gel-Dye solution from spin filter tube to chip wells 3, 7, 8, and 10 with volumes shown in Figure 1 (HT) or Figure 2 (LT).
6. Using a reverse pipetting technique, add 120 µL (HT) or 50 µL (LT) of Protein Express Lower Marker (green cap) to chip well 4 with volumes shown in Figure 1 (HT) or Figure 2 (LT).
7. Using a reverse pipetting technique, add 80 µL of Wash Buffer to waste well (for HT and LT).
8. Remove any liquid from the chip surface and the rims of the wells using the vacuum line.
9. Use the provided Detection Window Cleaning Cloth dampened with 70% Isopropanol to clean the chip detection window.

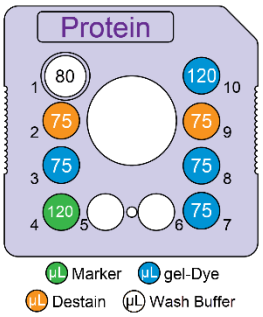

Figure 1. High-Throughput (HT)

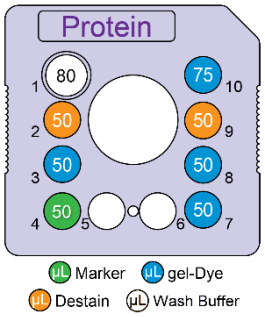

Figure 2. Low-Throughput (LT)

Ladder Preparation

Note: Store the ProteinEXact HR Ladder at -20°C. It is recommended that you aliquot the ladder into 15 µL lots for individual use to avoid multiple freeze/thaw cycles. Use the provided empty ladder tubes for making the aliqoutes.

1. Pipette 15 µL of ProteinEXact HR Ladder (yellow cap , stored at -20°C, not with refrigerated reagents) into a supplied Ladder Tube.
2. Add 150 µL of water (Milli-Q® or equivalent) to the ladder tube and mix thoroughly by pipetting up and down.
3. Ensure that no bubbles are in the ladder tube.

Wash Buffer Preparation

1. Transfer 750 µL of Protein Express Wash Buffer (purple cap) to the supplied 0.75 mL Buffer Tube.
2. Ensure that no bubbles are in the buffer tube.

Sample Preparation

Note: *The following is a general protocol for antibody sample preparation. Optimization of the type or concentration of reducing agent and stabilizing agent and/or optimization of denaturing conditions may be necessary depending on the specific molecules to be analyzed. Use of a hardshell 96-well PCR plate and a thermal cycler is recommended for efficient sample preparation.*

1. Prepare Reducing and/or Non-Reducing Sample buffer. To prepare the sample buffer:
   - Transfer 700 µL of Protein Express Sample Buffer (white cap) into a microfuge tube.
   - For Reducing Sample Buffer, add 24.5 µL of BME or 1M DTT.
   - For Non-Reducing Sample Buffer, add 24.5 µL of 250 mM IAM.
2. For each sample to be analyzed, pipette 18 µL of Reducing or Non-Reducing Sample Buffer into a well in a 96-well PCR plate.
3. Add 2.5 µL of sample to each well prepared in step 2.
4. Seal the sample plate and denature samples at 70°C for 10 minutes, then cool to room temperature.
5. Add 35 µL water (Milli-Q® or equivalent) to each sample and mix thoroughly by pipetting up and down.
6. Spin the sample plate at 3000 rpm for 2-3 minutes.

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Starting the Sample Run

Option 1 – Load chip and samples at onset of experiment

1. Touch the **Run** button.
2. Enter the parameters for the run:
   - On the **Select Wells** tab, select the plate type and the sample wells.
   - On the **Setup Run** tab, select the file name and data storage location.
   - On the **Start Run** tab, type the total ladder concentration (specified on the reagent vial) in the **Total Ladder Concentration** text box.
3. Touch the **Start** button.

Option 2 – Prime chip while preparing samples

If desired, the chip can be primed while preparing the samples.

1. Insert the chip, ladder tube, and buffer tube into the instrument.
2. Touch the **Prime** button on the Home screen.
3. Touch the **Prime** button on the Prime screen.
4. Prepare the samples while the chip is being primed and warmed.
5. When the prime is complete, touch the **Unload Plate** button and place the sample plate in the instrument.
6. Touch the **Run** button.
7. Enter the parameters for the run:
   - On the **Select Wells** tab, select the plate type and the sample wells.
   - On the **Setup Run** tab, select the file name and data storage location. Select the **Skip Prime** and **Skip Warm** check boxes.
   - On the **Start Run** tab, type the total ladder concentration (specified on the reagent vial) in the **Total Ladder Concentration** text box.
8. Touch the **Start** button.

Cleaning and Storing the Chip

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

1. Place the chip into the chip storage container. The sipper must be submerged in the fluid reservoir.
2. Remove the reagents from each well of the chip using vacuum.
3. Rinse and 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 each active well.
5. Cover the wells with Parafilm® to prevent evaporation and store the chip at room temperature. Allowing chip wells to dry may lead to changes in chip performance. The chip must be used to its lifetime (to the total number of 400 samples) within 30 days of analyzing the first plate of samples.

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Instrument Maintenance

After removing the chip at the completion of the run, wipe the electrodes with a lint-free swab dampened with water (Milli-Q® or equivalent) to remove any gel.

Before running the first chip of the day:

1. Remove the chip from the instrument and touch the **Purge Pressure Lines** button to purge the internal pressure lines.
2. Clean the electrodes and O-rings with a lint-free swab dampened with water (Milli-Q® or equivalent).

ProteinEXact HR Assay Specifications

The ProteinEXact HR 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	6.5 - 250 kDa
Linear Concentration Range (R^2)	0.99 (10 – 1000 ng/µL) and 0.98 (10 – 2000 ng/µL)
Maximum Sample Concentration	2000 ng/µL
Concentration (CV)[1]	< 10%
Sizing Resolution[2]	≤10% difference in size
Sizing Precision RSD (CV)	< 2%
Separation Time per Sample	65 seconds
Sensitivity (LOD)[3]	0.2 ng/µL
Reagent Kit Primes	10
Chip Lifetime	HT: 400 samples 24: 400 samples
Samples per calibrated Chip Prep	HT: up to 96 samples LT: up to 48 samples
HT Chip Preps per Chip (96 samples)	4
LT Chip Preps per Chip (48 samples)	8
Minimum Sample Volume	2 µL

[1] Typical results. Concentration CV may vary ±5% based on individual proteins.
[2] Resolution is defined as the difference in migration times divided by the sum of the full width half max for two closely migrating peaks.
[3] Based on internal standards.

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For the complete ProteinEXact HR Assay User Guide, go to: http://www.revvity.com/

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