Protein Clear™ HR Assay Quick Guide
LabChip® GXII Touch

Preparing Gel-Dye and Protein Clear HR Chip

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light. If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

This assay requires consistent adherence to the user and quick guide protocols as written, or results may be compromised by increased variability.

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 2-8°C.*
- *The VeriMAb™ standard can be prepared and stored for up to 7 days; however, for best results, prepare fresh.*

HT (High-Throughput) workflow, up to 96 samples - LT (Low-Throughput) workflow, up to 48 samples

1. Allow the chip and all refrigerated reagents to equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light. If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.
2. Invert and 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 Protein Clear HR 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. Once the Dye Solution is added to the Gel Matrix, immediately cap and invert the spin filter to minimize dye concentrate interaction with filter material; then vortex for 10 seconds until the gel and dye are well-mixed.
6. For Destain Solution, transfer 250 µL (HT) or 180 µL (LT) of Protein Clear HR Gel Matrix (red cap) to a second spin filter.
7. Spin the Gel-Dye mix and the Destain Solution at 9300 rcf for 5-8 min at RT.
   Note: Make sure microcentrifuge is set to 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.
8. Use a pipette tip attached to a vacuum line to thoroughly aspirate all fluid from the chip wells.
9. 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.

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10. Using a reverse pipetting technique, add Destain Solution to chip wells 2 and 9 as shown in Figure 1 (HT) or Figure 2 (LT).
11. 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 three weeks.
12. 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).
13. Inspect the tops of the wells to ensure that they are clean. If any droplets of liquid are present, use the aspirator to remove them.
14. Use the provided Detection Window Cleaning Cloth dampened with 70% Isopropanol to clean the chip detection window.

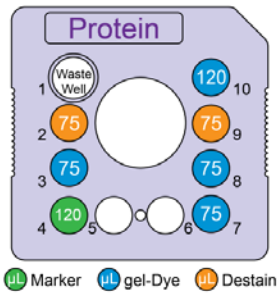

Figure 1. High-Throughput (HT)

Preparing VeriMAb™ Standard and Ladder for Priming & Calibration

Note: *The Prime & Calibrate step must complete successfully before starting a sample run with the Protein Clear HR Assay. Calibration step can take up to 60 minutes. Sample plate can be prepared while the calibration step is in progress. For best assay performance the time between calibration completion and starting the sample testing should be minimized.*

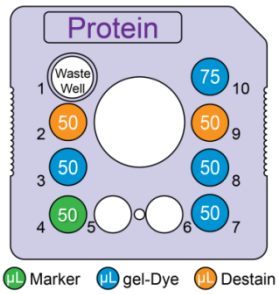

Figure 2. Low-Throughput (LT)

CRITICAL: The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use.
The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light.
If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

VeriMAb Standard

1. Prepare Non-Reducing Sample Buffer by transferring 90 µL of Protein Clear HR Sample Buffer (white cap) to a microfuge tube. Add 3 µL of 250 mM IAM solution.
2. Transfer 35 µL of Non-Reducing Sample Buffer to one well of a PCR plate, a PCR tube, or a 0.6 mL microfuge tube.
3. Add 5 µL VeriMAb Standard (orange cap) to the 35 µL Non-Reducing Sample Buffer.
4. Seal the plate or cap the tube and spin briefly to ensure that contents are at the bottom of the buffer.
5. Denature the VeriMAb Standard in Non-Reducing Sample Buffer at 70°C for 10 minutes, then cool to room temperature.
6. Add 70 µL water (Milli-Q® or equivalent) and mix thoroughly by pipetting up and down.
7. Transfer 70 µL of prepared VeriMAb Standard to a clean well of a PCR plate.
8. Ensure there are no bubbles in the VeriMAb standard.

Ladder

1. Pipette 15 µL of Protein Clear HR Ladder (yellow cap) into a supplied Ladder Tube.
2. Add 150 µL of water (Milli-Q® or equivalent) to the ladder and mix thoroughly by pipetting up and down.
3. Ensure there are no bubbles in the ladder tube.

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Sipper Wash Buffer

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

Prime and Calibrate

1. Place the PCR plate, Ladder Tube and Buffer tube in the appropriate positions on the instrument plate holder. Chip type and assay will automatically be selected from the RFID tag on the LabChip.
2. Touch the "Prime and Calibrate" button.
3. Select the plate type and indicate which well on the PCR plate contains the VeriMAb Standard.
4. Touch the "Prime and Calibrate" button to start the Prime and Calibrate step. **NOTE:** The maximum number of samples per calibrated chip prep is 96.

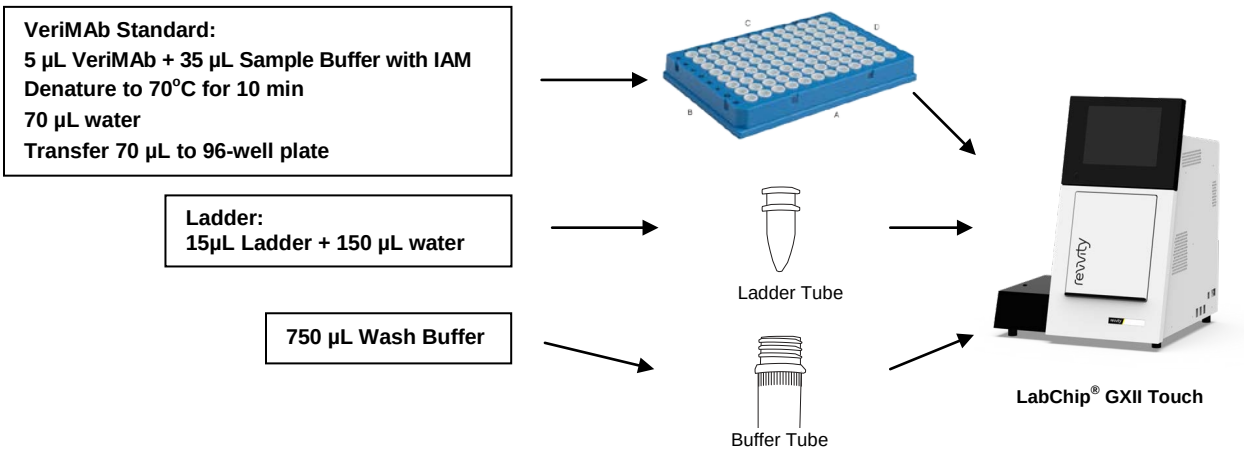
IMPORTANT NOTE:

After the completion of a run using the Protein Clear HR assay, the "Prime and Calibrate" button will become active in some versions of the LabChip GX Touch Controller software. However, the chip cannot be re-primed and calibrated unless the following steps are taken:

1. Remove the chip from the instrument.
2. Aspirate the contents from Well 1.
3. Aspirate and replace the contents of Well 4.

Failure to refresh the chip will cause the instrument to block the start of the second run.

Prime and Calibrate Workflow



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Preparing Protein Samples

CRITICAL:

The chip and all refrigerated reagents must equilibrate to room temperature (20 - 25°C) for at least 30 minutes before use. The dye must be removed from its padded shipping pack and allowed to warm from -20°C storage to room temperature for 45 minutes, protected from light. If the temperature in your lab is significantly lower than 20°C, please allow longer equilibration time.

Notes: *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.
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.

   The sample buffer calculation is described as follows:
   - Transfer 700 µL of Protein Clear HR 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.
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 min, 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 1200 rcf for 2 minutes.

Starting the Sample Run

1. Confirm that the software has completed the Prime & Calibration step. Clear the window indicating that the chip has been successfully calibrated.
2. Touch the “Unload Plate” button and replace the plate containing the VeriMAb Standard with the plate containing samples. Leave the buffer tube and ladder tube in place and touch the “Load Plate” button.
3. Touch the “Run” button and enter the parameters for Run Setup: Select plate type and sample wells; set file name and data storage location; confirm run setup; and touch the “Start” button.

Chip Cleaning and Storage

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

1. Place the chip into the plastic storage container. The sipper should be submerged in the fluid reservoir.
2. Remove the reagents from each well of the chip using vacuum.
3. Each active well (1, 2, 3, 4, 7, 8, 9, and 10) should be rinsed and aspirated twice with water (Milli-Q® or equivalent).
4. Add 100 µL of water (Milli-Q® or equivalent) to the active wells.
5. Cover the wells with Parafilm® to prevent evaporation and store at room temperature. Allowing chip wells to dry may cause may lead to changes in chip performance. The chip must be used to its lifetime (to the total number of samples) within 30 days of analyzing the first plate of samples.

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Instrument Maintenance

1. After removing the chip at the completion of the run, wipe the electrodes with a lint-free swab to remove any gel.
2. Touch the “Purge Pressure Lines” button to purge the internal pressure lines.
3. Clean the electrodes and O-rings with a lint-free swab moistened with water.

Protein Clear HR Assay Specifications

The Protein Clear 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	14 - 250 kDa
Linear Concentration Range	10 - 1000 ng/µL (mAb, non-reduced main peak)
Maximum Sample Concentration	2000 ng/µL
Linearity (R^2)	> 0.995
Sizing Resolution[1]	Resolution >1.0 for VeriMAb reference standard
Sizing Precision RSD (CV)	< 2%
Relative Migration Time Precision RSD (CV)	< 2%
Separation Time per Sample	65 seconds
Percent Purity Reproducibility	< 0.5% main non-reduced IgG, < 5% all other peaks
Sensitivity (LOD)	5 ng/µL (mAb, non-reduced main peak)
Reagent Kit Primes	10
Chip Lifetime	400 samples
Maximum number of samples per calibrated HT Chip Prep	96 samples
Maximum number of samples per calibrated LT Chip Prep	48 samples
Maximum Sample Volume	2 µL

[1] Resolution is defined as the difference in migration times divided by the sum of the full width half max for two closely migrating peaks.

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LabChip Reagent CoA: https://www.revvity.com/tools/COASearch

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

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