

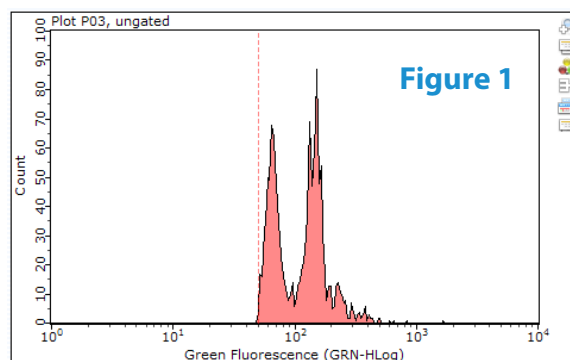
Protocol: Calibration of Millipore Guava 6HT/8HT

Calibrating your Experiment for use with Firefly Particles

IMPORTANT! The Guava EasyCyte has to be calibrated before the first experimental run!

1. Go to <https://fireflybio.com/productsupport> to download the Guava 8HT or Guava 6HT [settings file](#).
2. Turn on the Guava EasyCyte instrument. Open the Guava Soft icon on your desktop. Select the InCyte software and run a "Quick Clean" cycle within the "Capillary Tools" menu. When prompted to select wells, do not change anything and click "OK".
3. Vortex [Firefly Calibration beads](#) for 5 seconds on high and add 200ul to a Guava-compatible tube (VWR #89004-316).
4. Select "Worklist Editor" in the EasyCyte menu. Select tube [position T1](#) to be run. Check the "Acquire This Sample" box.
5. Select "Run Worklist." Select a location and filename for your data. This file can be deleted after calibration.
6. Select Analysis Method as "New" and click "OK".
7. Place the Calibration Beads tube from Step 1 into [position T1](#) in the Guava sample tray and click "OK".
8. When prompted, click "Retrieve Settings" and select the downloaded settings file. Select position T1 (containing your Calibration beads). Click "OK" to continue.
9. Open "Gain Controls".
10. Run Calibration beads for ~30 seconds. Drag the red bar on the [GRN-HLog](#) histogram (bottom right) until two peaks are clearly visible. Refer to Figure 1 as an example of a correct threshold. [The default threshold in the settings file may be correct.](#)
11. Click "Next". Click the "Save Settings" button to save this file for future Firefly assay runs. Use the "Capillary Cleaning Tools" button to run a "Quick Clean" cycle. Click the "Abort" button to finish the calibration procedure.

THRESHOLD: _____ on Green



Verification

1. To verify the calibration, load 35µL of [Firefly Multimix](#) to a new well, followed by 175µL of Run Buffer.
2. Follow "[Protocol: Operation of Millipore Guava 6HT/8HT: Running Multiple Samples on the Guava](#)".
3. Load the file into FireCode and ensure that all groups are properly appearing and that a quality control score of 70/100 is achieved.

Protocol: Operation of Millipore Guava 6HT/8HT

Running Multiple Samples on the Guava

IMPORTANT! Only run this protocol after you have calibrated your Guava EasyCyte for Firefly Particles. Refer to [Protocol: Calibration of Millipore Guava 6HT/8HT](#) for more information.

1. Open InCyte software.
2. Click ["Worklist Editor"](#).
3. Select your wells to be run. Click ["Acquire Wells"](#). Change ["Time to Acquire"](#) to 90 seconds and ["Mix Sample For"](#) to 5 seconds. Leave all other settings as is.
4. Click ["Run Worklist"](#). Select a location and filename for your data.
5. When prompted, remove the plate lid and load your assay plate with correct orientation, matching well A1 to the indicator on the tray. Reload any cleaning tubes as needed.
[Failure to correctly align and uncover assay wells may result in flow cell damage!](#)
6. Select Analysis Method as ["New"](#) and click ["OK"](#).
7. When prompted, select an assay well to adjust settings and click ["Retrieve Settings"](#).
8. Select your settings file, saved to your PC from the calibration. Refer to [Protocol: Calibration of Millipore Guava 6HT/8HT](#) to locate the file.
9. Confirm that the event count is increasing. Click ["Next"](#).
[Take care to hit "Next" within 30 seconds or your sample will be lost.](#)
10. Click ["Resume"](#) to run your assay wells.
11. .FCS files will be automatically exported to the directory selected in Step 4.