

Millipore Guava HT protocol for multiplex miRNA assays

This protocol contains instruction for setting up a Millipore Guava HT System. If you will be using a different system, consult the appropriate protocol in our flow cytometry protocols for multiplex miRNA assays page, or contact our Technical Support team at technical@abcam.com.

For first time use of a system, please follow the cytometer setup/verification. After a system is properly validated, those settings can be saved and used for routine assay readout with the method for assay readout.

Please read this protocol carefully before cytometer operation. Failure to properly set up a system may result in unusable data, loss of product or system damage. For more detailed instructions on system operation details, please consult the Millipore Guava operation manual.

Requirements

- Multiplex miRNA assay
- Blade (scalpel or scissors)
- Pipette and tips

Cytometer setup/verification (first use only)

1. Go to abcam.com/FireflyCytometerInfo to download the appropriate Guava settings file.
2. Cut/remove the plate seal from a covered well on the provided filter plate.
3. Invert and vortex the particle mix, add 35 µL or 75 µL or 1x capture particle mix to the uncovered filter plate well. Add 175 µL of run buffer on top of the particle mix.
4. Open the Guava InCyte software.
5. Click worklist editor.
6. Select your wells to be run. Click acquire wells. Change time to acquire to 90 s, mix sample for to 5 s and events to acquire to 50,000. Leave all other settings as is.

Failure to increase event limits may cause low particle yield in analysis.

7. Click run worklist. Select a location and filename for your data.
8. 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.

9. Select analysis method as new and click OK.
10. When prompted, select an assay well to adjust settings and click retrieve settings.
11. Select the settings file downloaded in Step 1.
12. While the machine is adjusting settings, confirm that the event count is increasing and click next within 30 s or your sample will be lost.
13. Click resume to run your test well.
14. After scanning/cleaning is complete, export the FCS file.
15. Download the Firefly™ Analysis Workbench. For detailed support, see the Firefly Analysis Workbench quick start guide.

16. Open the Firefly Analysis Workbench and load the FCS file saved in Step 14. When prompted, load the PLX file for your particle mix. Once the data loads, hover your mouse over the well to see the quality control score. A score of 75 or higher indicates successful settings. ***If the quality control score is below 75, perform a thorough system clean, and repeat this protocol. If the score remains low, contact our Technical Support team at technical@abcam.com.***

Assay readout

Important - only run this protocol after you have calibrated your Guava EasyCyte for particles. This protocol assumes a complete assay in 96-well plate format, with particles suspended in run buffer.

1. Open the Guava InCyte software.
2. Click worklist editor.
3. Select your wells to be run. Click acquire wells. Change time to acquire to 90 s, mix sample for to 5 s and events to acquire to 50,000. Leave all other settings as is.

Failure to increase event limits may cause low particle yield in analysis.

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 the settings downloaded from the setup/verification protocol above.
9. While the machine is adjusting settings, confirm that the event count is increasing and click next within 30 s or your sample will be lost.
10. Click resume to run your assay wells.
11. After scanning/cleaning, save the FCS file and analyze using the Firefly™ Analysis Workbench. For detailed support, see the Firefly Analysis Workbench quick start guide.