

Life Technologies Attune with plate handler protocol for multiplex miRNA assays



This protocol contains instruction for setup a Life Technologies Attune with plate handler. 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.

Requirements

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

Cytometer setup/verification (first use only)

1. Download the Attune settings files.
2. Cut/remove the plate seal from a covered well on the provided filter plate.
3. Invert and vortex the particle mix, add 35 μ L or 75 μ L or 1x capture particle mix to an uncovered well on the filter plate. Add 175 μ L of run buffer on top of the particle mix.
4. Start the Attune Software. If necessary run the start up under the functions menu.
5. In the left-hand panel, right click on my experiments and select new plate experiment using template. Load the Firefly™ template.
6. When prompted name the plate “Test run” and click OK.
7. Under plate view, select the well to be run. Click the new sample icon.
8. Select a well in the plate setup grid and click on the plate setup menu. Click the pull down arrow next to save as and select load.
9. Open the Firefly™ Run protocol file (.grp file). Click apply to experiment.

Failure to load run protocol will cause loss of data.

10. Load your plate into the autosampler and double click on any well to go to the workspace.
11. Select collect plate under the collection panel.
12. Right click your specimen and export the FCS file into the Firefly™ Analysis Workbench. 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 indicated 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 Life Technologies Attune for Firefly Particles. This protocol assumes a completed assay in 96-well plate format, with particles suspended in run buffer.

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7. Load your plate into the autosampler and double click on any well to go to the workspace.
8. Select collect plate under the collection panel.
9. Right click your specimen and export the FCS file into the Firefly™ Analysis Workbench. For detailed support, see the Firefly Analysis Workbench quick start guide.