

# Protocol: Calibration of Life Technologies Attune

## Setting up your experiment

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**Important!** The Attune must be properly calibrated before the first experimental run.

1. Start [Attune® Cytometric Software](#).
2. Log in under your user account **User: admin Password: password**.
3. In the **"Functions"** Menu, Select **"Start up"** - Press **"Next"** when ready.
4. Go to <http://fireflybio.com/productsupport> to download the **Template File & Run Protocol File**.
5. In the left-hand panel, right click on **"My Experiments"** and select **"New Plate Experiment Using Template"**.
6. Select the **Firefly Template** file (a .gpt file).
7. Name the plate according to your experiment, ensure not to change the plate type from **96 Well Flat Bottom**, click **"OK"**.
8. In the **Plate View**, highlight the wells to be run.
9. In the **Attune® Ribbon**, select the **"New Sample"** icon. Wells should appear in the plate setup grid.
10. Select a well and click on the **"Plate Setup"** menu. Click on the pull down arrow next to **"Save As"** and select **"Load..."**
11. Open the **Firefly Run** protocol (a .grp file). Click **"Apply to Experiment"**, and Select **"Yes"** in the user prompt that appears.
12. Select the **"Sample Information"** sub menu. Name your samples accordingly, making sure to **leave the well identifier intact (A01,A02,etc)**.
13. Double-click on any well to go to the Workspace.
14. Load 1mL of provided **Firefly Calibration** beads into a 5mL round bottom tube.
15. Place the cytometer into **Tube Mode** by lifting the manual valve to display a tube. Lower the pedestal and load the tube.
16. Locate your experiment in the **Experiment Explorer** panel. Double click on **"Calibration Beads"** sample under **"Calibration"** A red arrow should appear across from the specimen name.
17. Click on **"Collection Panel"** and select **"Run"**.
18. In the **"Instrument Configuration"** panel, adjust the corresponding gains until the right-most peak aligns with the green gate. Once all gains are correct, go back to **"Collection Panel"** menu and select **"Stop"** Select **"Save"**.
19. In **"Experiment Explorer"**, right click on **"Calibration Beads"** and select **"Save Instrument Settings as Global"** Select **"Yes"** in the user prompt.
20. In **"Experiment Explorer"**, right click on your experiment plate and select **"Save as Template"**.
21. Save the template and use this for future Firefly mirSelect Assays.

# Protocol: Operation of Life Technologies Attune

## Running Multiple Samples on the Attune

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**Important!** This protocol may only be run after your Attune has been properly calibrated.

1. Start [Attune® Cytometric Software](#).
2. Log in under your user account [User: admin Password: password](#).
3. In the ["Functions" Menu](#), Select ["Start up"](#) - Press ["Next"](#) when ready.
4. In the left-hand panel, right click on ["My Experiments"](#) and select ["New Plate Experiment Using Template"](#).
5. Select your saved template.
6. Name the plate according to your experiment, ensure not to change the plate type from [96 Well Flat Bottom](#), click ["OK"](#).
7. In the [Plate View](#), highlight the wells to be run.
8. In the [Attune® Ribbon](#), select the ["New Sample"](#) icon. Wells should appear in the plate setup grid.
9. Select a well and click on the ["Plate Setup"](#) menu. Click on the pull down arrow next to ["Save As"](#) and select ["Load..."](#)
10. Open the [Firefly Run](#) protocol (a .grp file). Click ["Apply to Experiment"](#), and Select ["Yes"](#) in the user prompt that appears.
11. Select the ["Sample Information"](#) sub menu. Name your samples accordingly, making sure to [leave the well identifier intact \(A01,A02,etc\)](#).
12. Double click on any well to go to the [Workspace](#).
13. [Load your plate](#) into the Attune® autosampler
14. Double click on your experiment in the ["Experiment Explorer"](#).
15. Ensure the manual valve is in ["Plate Collection Mode"](#).
16. Select ["Collect Plate"](#) under the ["Collection Panel"](#).
17. After running, in the ["Functions"](#) menu, select ["Rinse"](#).
18. Right click on your specimen and select ["Export FCS File\(s\)"](#).