Attune® NxT Acoustic Focusing Cytometer

Catalog Numbers A24858, A24859, A24860, A24861, A24862, A24863, A24864

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The functions of the Attune® NxT Acoustic Focusing Cytometer are controlled by the Attune® NxT Software. Basic instructions for using the Attune® NxT Acoustic Focusing Cytometer are described below.

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Attune® NxT Acoustic Focusing Cytometer User Guide*. For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Workflow

Startup → Run Performance Test → Create Experiment → Calculate Compensation → Collect Data

For detailed instructions on using the Attune® NxT Acoustic Focusing Cytometer, refer to the *Attune® NxT Acoustic Focusing Cytometer User Guide* (Pub. no. 100024235). For a detailed description of the Attune® NxT Software, refer to the *Attune® NxT Software User Guide* (Pub. no. 100024236). For detailed maintenance and troubleshooting information, refer to the *Attune® NxT Maintenance and Troubleshooting Guide* (Pub. no. 100024234). These user guides are supplied with the product; you can also download them from **www.lifetechnologies.com**.

Startup

Before You Begin

1. Check the levels in the fluid containers of the Attune® NxT Acoustic Focusing Cytometer.

   If empty, fill the focusing fluid, wash solution, and shutdown solution containers.

   Empty the waste container and fill it with full strength bleach solution up to the bleach fill mark on the bottle.
2. Power ON the Attune® NxT Acoustic Focusing Cytometer and the computer.
3. Log in to Windows® and launch the Attune® NxT Software.

IMPORTANT! A license control mechanism in the form of a DESkey device is required for the operation of the Attune® NxT Software. If a valid DESkey device is not present, the software will display a warning message.

Run Startup Function

1. Click **Startup** on the *Main Menu*, on the *Instrument ribbon tab,* or the *Collection panel*.
2. The Startup dialog opens and provides instructions to perform the Startup operation. Follow the instructions provided by the software.
3. After the Startup function is completed and no system errors are encountered, the Status bar displays the *Ready* icon.

   If any system errors are encountered during the Startup, the status bar displays the *Alarm* icon.

Run Performance Test

1. Click **Performance Test** on the *Main Menu* to view the *Performance test setup screen*.
2. Click **Check now** under the filter block image to verify that the filters are appropriate.

   Note: If you need to select a different optical configuration, refer to "Instrument Configuration" in the *Attune® NxT Software User Guide*.

Check instrument configuration

Check now

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3. Verify that the bead lot number you have selected matches the bead lot number of the Attune® Performance Tracking Beads you are using. The bead lot number is the **first six digits** printed on the label.

Lot #: 756080D
Expiration: 2/25/2015

4. Shake the Attune® Performance Tracking Bead bottle to resuspend the beads, and then add 3 drops of the bead suspension to 2 mL of focusing fluid in a 12 × 75-mm tube. Mix by gentle inversion or vortexing.

5. Load the tube by placing it in the sample tube lifter.

6. Click **Run Performance Test** to initiate the automated Performance test. During the procedure, the software provides progress information for the Performance test, which takes about 5 minutes to complete.

Run performance test

7. The result of the daily performance test is displayed at the end of the run. You can also review the most recent report by clicking **Current PT Result** on the Performance Test ribbon tab.

Note: For more information on viewing and interpreting the Performance Test reports, see "Performance Test Reports" in the *Attune® NxT Software User Guide.*

Create an Experiment

1. On the *Main Menu* or the *Home tab*, click **New Experiment** to open the *New Experiment dialog*.
   Alternatively, right-click on the **User Folder heading**, and then select **New Experiment** from the context menu.

2. Select the **Experiment type** you wish to create (Tube is the default selection).

3. Enter the **Experiment name** or use the default Experiment name.

4. Accept the default **Workspace** and default **Instrument Settings**.

5. Enter the number of **Tube Groups** for the Experiment and the number of **Tube Samples** for each Group.

6. Click **OK** to create the new Experiment.
   The New Experiment dialog closes and the software opens the first Sample in the Experiment and the Experiment Workspace.
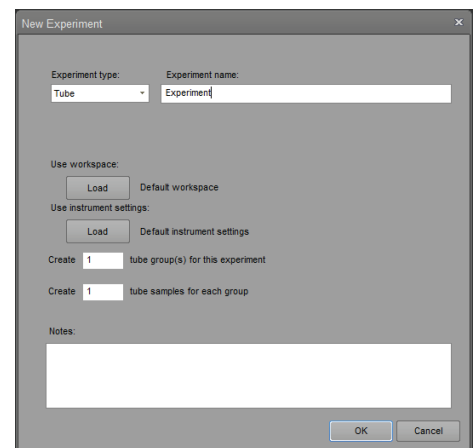
Calculate Compensation

Before You Begin

- Prepare the necessary compensation controls. You will need single-stained controls for each fluorophore you are using. If you select **Use Unstained Control**, you will also need an unstained or isotype-labeled control.
- We recommend that you optimize the instrument settings for compensation controls in the Compensation Workspace.
- If no compensation is necessary, you can optimize the instrument settings within the workspace of the sample. The procedure for optimizing samples is similar to that described for compensation control samples.
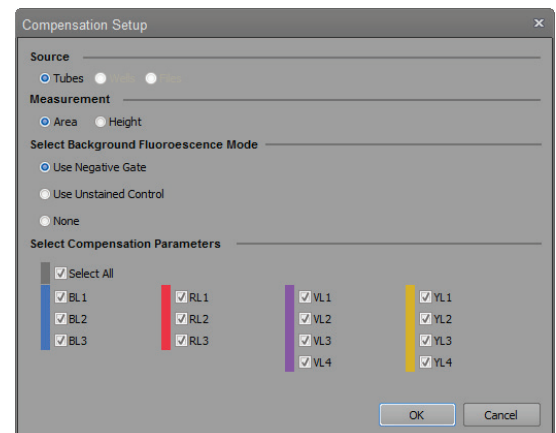
Compensation Setup

1. Click **Compensation Setup** on the *Compensation ribbon tab* to open the *Compensation Setup dialog*.
   Alternatively, double-click the **Compensation node** within your Experiment in Experiment Explorer.

2. On the Compensation Setup dialog, select the source of compensation controls, the measurement parameter, background fluorescence mode, and the compensation parameters.

   Note: For more information on each option, refer to "Compensation" in the *Attune® NxT Software User Guide*.

3. Click **OK**. The software creates or updates the compensation control for each selected parameter in the Experiment Explorer.
   The Compensation Workspace for the first control opens automatically.

   Note: You must select a minimum of 2 fluorescent channels or the OK button will be disabled.

Optimize Instrument Settings for Unstained Control

1. Double-click **UC** under the Compensation node in the Experiment Explorer to open the *Compensation Workspace* for the unstained control.
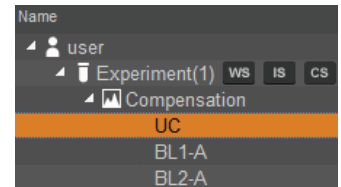
   The Compensation Workspace for the unstained control contains one SSC vs. FSC plot with a polygon gate, and histogram plots for each of the fluorescent parameters selected during the compensation setup.

2. Install the unstained control on the sample injection port.

3. Define the **Collection Criteria** using the *Collection Panel* and click **Run**.
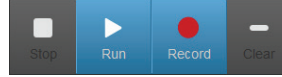
   IMPORTANT! Do not click **Record** at this point. It is critical that you optimize voltages prior to recording any sample or any compensation controls.

4. Resize the **R1** gate over the population of interest. This gate shape and location can be applied to all compensation controls by right-clicking and selecting **Apply gate shape to all controls**.

5. Adjust the **FSC and SSC voltages**, **Threshold**, and **Fluorescence Channels** using the *Instrument Configuration* tab to place the population on scale and to remove unwanted events and background.

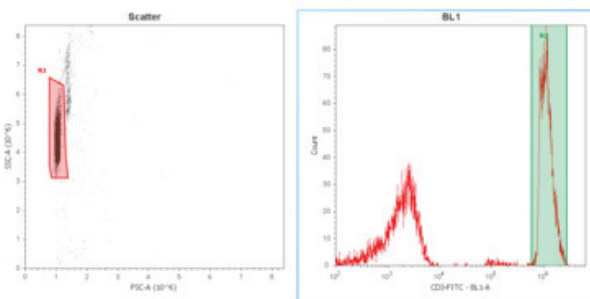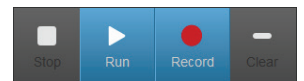6. Remove the unstained control from the sample injection port.
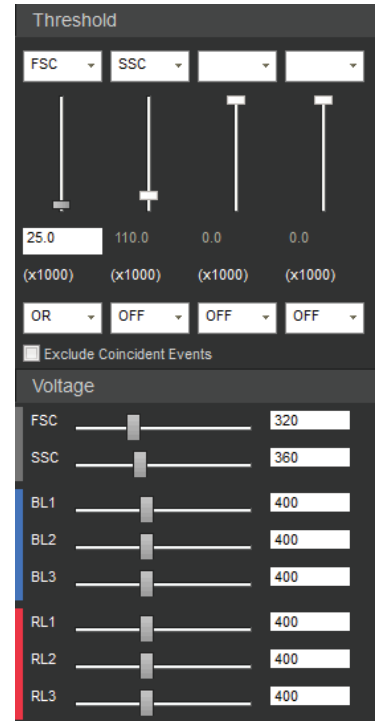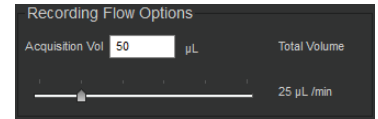
Optimize Instrument Settings for Single-Stained Controls

1. Continue with the single-stained controls using the Unstained control workspace to optimize each control.

2. Install the first single-stained control on the sample injection port.

3. On the *Collection Panel*, click **Next Sample**.

4. Using the same collection criteria and optimization procedures, adjust the instrument settings and gate for the population of interest.

Note: The Attune® NxT Software automatically executes the **Rinse** function each time the tube lifter is pushed down to remove the sample from the sample injection port.

Record Compensation

1. After compensation setup is complete, click **Next** on the Collection Panel tab. The workspace for the first defined control compensation control (e.g., UC) opens.

2. Install the tube containing the unstained control beads/cells on the sample injection port as prompted by the software.

3. Push up the tube loader to the active position in the sample injection port and click **Run** on the Collection Panel.

4. Wait until the sample equilibrates, and click **Record**.
   The Compensation matrix is calculated as new Compensation controls are recorded. The calculation will only include the controls that have been recorded.

5. Repeat the process for each of the single-stained controls, ensuring the **R1** (scatter) and **R2** (histogram) gates are set on the appropriate populations.

6. When the last compensation control is recorded, *Compensation matrix* automatically opens for your review. Click **OK** to accept the compensation values.

Collect Data

Run Sample

1. Double-click the **Sample** of interest in the Experiment Explorer panel to activate it.

   Workspace displays the default sample plots set up for the Experiment.
2. Enter the collection criteria in the Collection Panel.
3. Install the tube containing your sample on the sample injection port and lift up the tube loader to the active position.
4. Click **Run**. The events are displayed on the plots as the graphs are being populated.
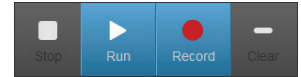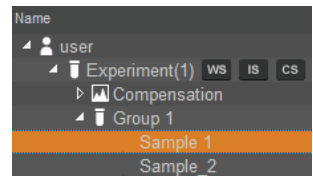
Record Data

1. While still in the **Run** mode, adjust the **PMT voltages** and **Threshold** values for the appropriate channels using the Instrument Configuration tab. Make sure the events are on scale.

   Note: While collecting samples, you can create plots, daughter plots, and statistics, and insert and adjust gates. **However, once compensation has been recorded and sample collection has started, you cannot adjust the PMT voltages and Threshold values.**
2. Select the desired **Gate** in the Workspace tab and set it on the population of interest.
3. Click **Record** to start data collection.

   Data is recorded until any of the conditions set in Collection Criteria is satisfied or you click **Stop**. You can also click **Pause** to temporarily halt the data collection.
4. Click **Stop** to stop the data collection. The Attune® NxT Software automatically saves the data in a unique FCS file.
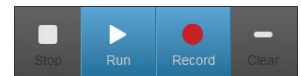
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