USER GUIDE

applied biosystems®
by life technologies™

Attune® Auto Sampler

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Contents

About this Guide

Audience

This user guide is for laboratory staff operating, maintaining, and analyzing data using the Applied Biosystems® Attune® Acoustic Focusing Cytometer equipped with the Attune® Auto Sampler sample loading device.

User Attention Words

Two user attention words appear in Life Technologies user documentation. Each word implies a particular level of observation or action as described below.

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

How to Obtain Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

When contacting customer support for instrument troubleshooting, provide the instrument model and the instrument serial number. Convey to the technical support any error messages that were displayed on your instrument and any troubleshooting that you have already performed
(see "Appendix D: Troubleshooting", page 55).

User Documentation

The guides listed below are shipped with the Applied Biosystems® Attune® Auto Sampler.

- *Attune® Auto Sampler Quick Reference Guide*
- *Attune® Auto Sampler User Guide*
- *Attune® Cytometric Software V2.1 Release Notes*

Safety Information

Note: See "Appendix F: Safety" on page 60 for the complete the chemical or instrument safety information.

Safety Alert Words

Four safety alert words appear in Life Technologies user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for IMPORTANT! safety alerts, each safety alert word in an Applied Biosystems® document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to Life Technologies instruments (see "Safety Symbols" on page 63).

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Life Technologies are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 72.

IMPORTANT! For the SDSs of chemicals not distributed by Life Technologies contact the chemical manufacturer.

1. Running Samples

Workflow

IMPORTANT! Although the daily Startup and Shutdown procedures are automated and require minimal user input, we recommend that you familiarize yourself with the Attune® Acoustic Focusing Cytometer, its operating principles, and the software user interface by reading the Attune® Acoustic Focusing Cytometer User Guide before starting your experiments. The Attune® Acoustic Focusing Cytometer User Guide is available for downloading at **www.lifetechnologies.com**.

Before You Begin

Required Solutions

- **Attune® Focusing Fluid** – is a buffered, azide-free support/carrier reagent for transporting particles through the capillary assembly. It contains a preservative and detergent designed to minimize bubble formation.
- **Attune® Wash Solution** – is a ready-to-use solution for removing cellular debris and dyes from the fluidics system of the instrument.
- **Attune® Shutdown Solution** – is a 10X salt-free solution that prevents the formation of bubbles and the accumulation of salt in the fluidics system of the instrument when the instrument is powered off. Prepare a 1:10 dilution of the shutdown solution in deionized water and add it to the shutdown tank.
- **10% bleach solution in deionized water** – decontaminates the fluidics lines. Prepare this solution fresh daily and use during the shutdown procedure.
- **Deionized water** – used for diluting Attune® Shutdown Solution and bleach, as well as for long-term storage of the instrument.

IMPORTANT! 10% Bleach is defined as a 1 in 10 dilution (1 part bleach to 9 parts deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

IMPORTANT! Reagents may be stored at colder temperatures, but running the Attune® Acoustic Focusing Cytometer with cold reagents (<15°C) will affect the data quality. Before you run the instrument, ensure that all fluids have equilibrated to room temperature.

Sample Requirements

- The Attune® Acoustic Focusing Cytometer with the Attune® Auto Sampler is designed to handle samples in 96-well or 384-well standard or deep well plates with round (U), flat, or conical (V) bottoms.

  Note: We strongly recommend using round bottom plates for any assay in which homogeneous sampling and consistency of concentration is essential.

- The method used to prepare a specimen depends on the sample type and the assay desired.
- In general, the maximum recommended sample concentration for analysis is 1×10^6 cells/mL. If the concentration of your sample is $>1 \times 10^6$ cells/mL, dilute it down prior to running it on the Attune® cytometer.
- The maximum recommended sample concentration for the Standard 500 µL/minute and 1,000 µL/minute collection rates is 5×10^5 cells/mL.

IMPORTANT! Although running a full 384-well plate in the standard mode requires only 950 mL Attune® Focusing Fluid, it is necessary to have at least 1.2 liters of focusing fluid in the focusing fluid tank, because the sensor in the tank is located at the 150 mL level and cannot detect if the focusing fluid levels drop below that.

Startup

During Startup, the Attune® Acoustic Focusing Cytometer:

- Warms the lasers to operating temperature
- Initializes the pumps
- Primes the instrument fluidics
- Informs the user of System Status (Ready, Attention, Clog, etc.)
- Initializes the motors and pumps in the Attune® Auto Sampler when it is connected and powered on

The Startup function ensures that all fluidic lines are clean, the fluidic lines and the system's two pumps are filled with fresh focusing fluid, and the lasers are warmed to operating temperature. We recommend performing the Startup function on a daily basis when the instrument is in use.

Before You Begin

1. Check the levels in the fluid containers located in the fluidics compartments of both the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler. If empty, fill the focusing fluid, wash solution, and shutdown solution containers. If full, empty the waste container. For more information, refer to the Attune® Acoustic Focusing Cytometer User Guide.

   Note: If you are running a full 384-well plate in the standard mode, make sure that the focusing fluid tank contains at least 1.2 liters of Attune® Focusing Fluid.

2. Power on the Attune® Acoustic Focusing Cytometer, the Attune® Auto Sampler, and the computer.
3. Verify the optical filters are appropriate for your experiment on the Attune® Acoustic Focusing Cytometer.
4. Start up the computer and log in to Windows.
5. Launch the Attune® Cytometric Software.

Note: To power up the system when it is in the *Standby* mode (i.e., the Auto Sampler open tray icon is blinking every 1–2 seconds), first launch the Attune® Cytometric Software, and then then power on the Attune® Acoustic Focusing Cytometer. This will automatically power up the Attune® Auto Sampler.

Run Startup Function

1. Click **Startup** on the upper-right corner of the Login screen.
2. Follow the instructions on the Startup prompt screen to perform the Startup operation.

   The Attune® Cytometric Software automatically initializes the pumps, and primes the fluidics lines. The status window displays the Startup operation being performed.

Note: If the daily Startup function has already been performed, click **Login** after entering your username and password.

Verify Optical Configuration

Check the optical layout of the cytometer to verify that the filters are appropriate for the sample type and fluorophores you are using.

Place your cursor over the icon in the lower-left corner to verify the optical configuration is correct. Either **Blue/Violet** or **Red/Blue** will be displayed.

If you need to change the optical configuration, refer to the instructions provided in the Attune® Acoustic Focusing Cytometer User Guide, which is available at **www.lifetechnologies.com**.

Run Performance Test

1. Check the optical layout of the Attune® Acoustic Focusing Cytometer to verify that the filters are the same as those used to perform the baseline calculations.
2. Click **Performance Test** on Main Menu
3. Verify that the lot number of the Attune® Performance Tracking Beads you are using is identical to the bead lot number used in the current baseline (If not, download the bead lot file information at **www.lifetechnologies.com** and perform baseline calculations).
4. Prepare the Attune® Performance Tracking Beads suspension.
5. Click **Run Performance Test** and follow the instructions provided by the software.
6. View Performance Test Report.

Note: For more information on Performance Tracking functions (i.e., Baseline Calculations and Performance Test), downloading bead lot information, and preparing the Attune® Performance Tracking Beads suspension, refer to the Attune® Acoustic Focusing Cytometer User Guide, available for downloading at **www.lifetechnologies.com**.

Create an Experiment

To run your samples and collect cytometric data using the Attune® Auto Sampler, you need to create a *Plate Experiment* in the Workspace. The Attune® Cytometric Software allows you to:

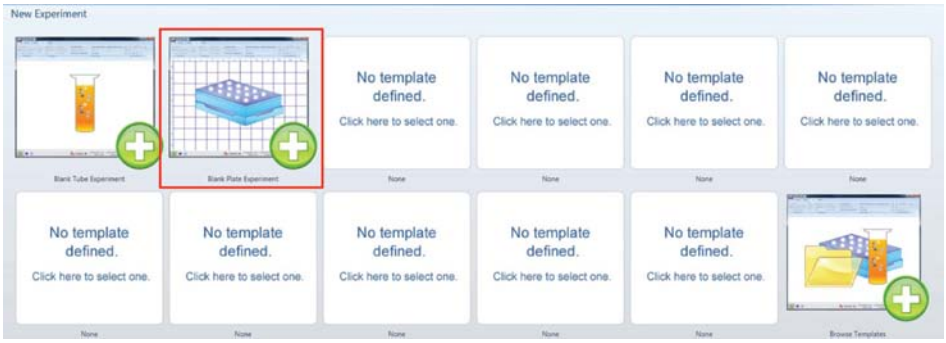
- Create a Plate Experiment using a blank template
- Create a Plate Experiment using a pre-populated template
- Create a Plate Experiment from the Experiment Explorer using the default Workspace
- Duplicate a saved Plate Experiment in the Experiment Explorer

You can perform these functions from the *Main Menu* or by using the *Experiment Explorer*.

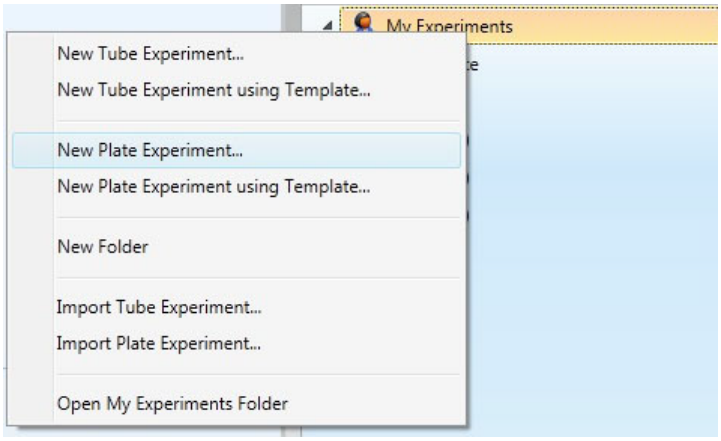
Note: A *Plate Experiment* can contain multiple *Experiments*, each with its own *Workspace*, *Instrument Settings*, and *Compensation*. Each Experiment within a Plate Experiment can have more than one *Specimen*, and each Specimen can have more than one *Sample*. Each Sample is associated with its own FCS file.

Create a Plate Experiment

1. From the Main Menu screen, click **Blank Plate Experiment** or one of the pre-populated Plate Experiment templates (if available).

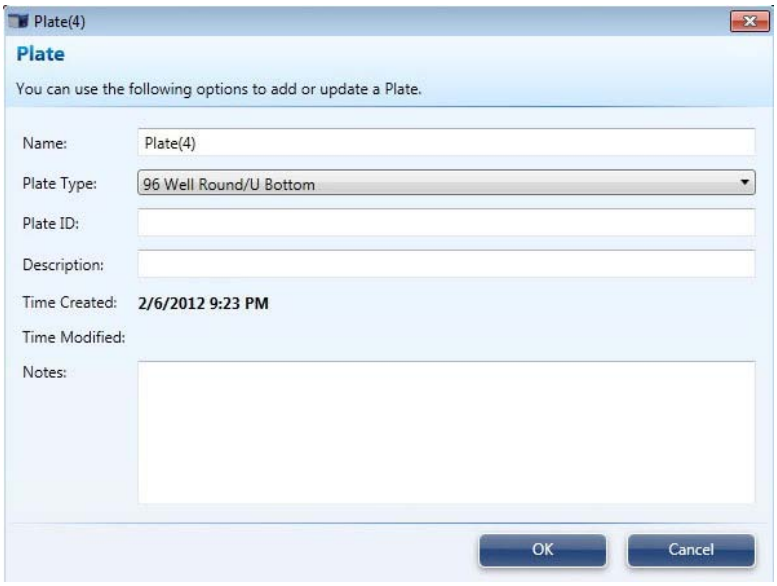


Alternatively, right-click **My Experiments** and select **New Plate Experiment**.



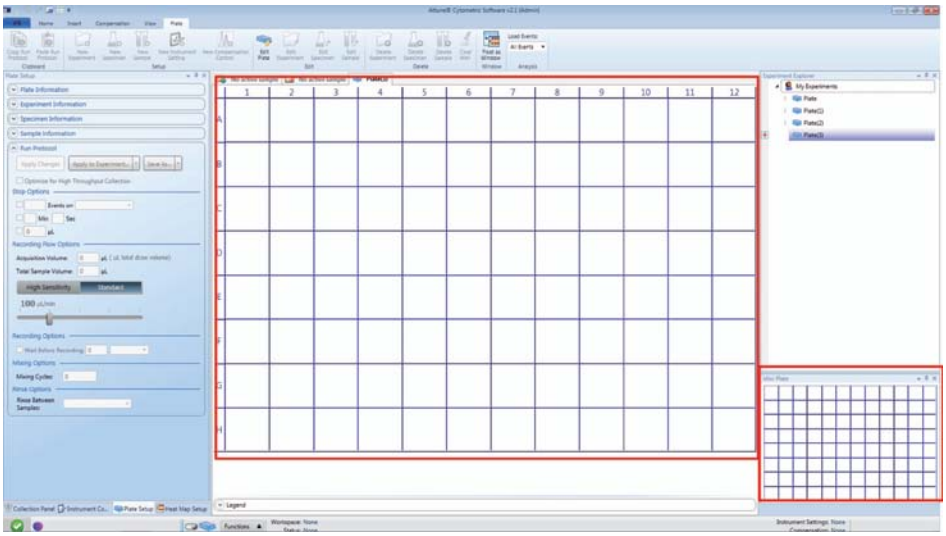
To duplicate a saved Plate Experiment, right-click on an existing plate in the Experiment Explorer and select **Duplicate Plate**.

2. In the Plate pop-up window, enter the Plate Name and Plate ID, and select the appropriate Plate Type from the drop-down menu.

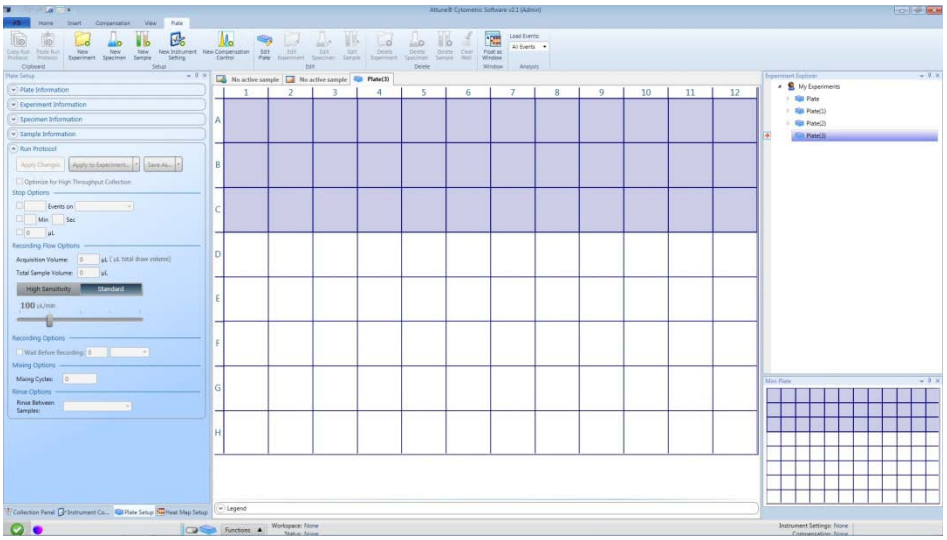


Note: We strongly recommend using round bottom plates for any assay in which homogeneous sampling and consistency of concentration is essential.

3. Click **OK**. The *Plate tab* on the *Attune® Desktop* and the Experiment Explorer display the virtual plate layout, representing the wells available on the plate (96 or 384 wells, depending on the plate type selected).



4. In the virtual plate layout, select the wells you want to include in your *Experiment*. To select the entire plate, click on the upper left hand corner of the virtual plate. To select whole columns or rows, click on the number or letter of the column or row you would like to select. To multi-select wells, columns, or rows hold down the **CTRL** key while selecting.

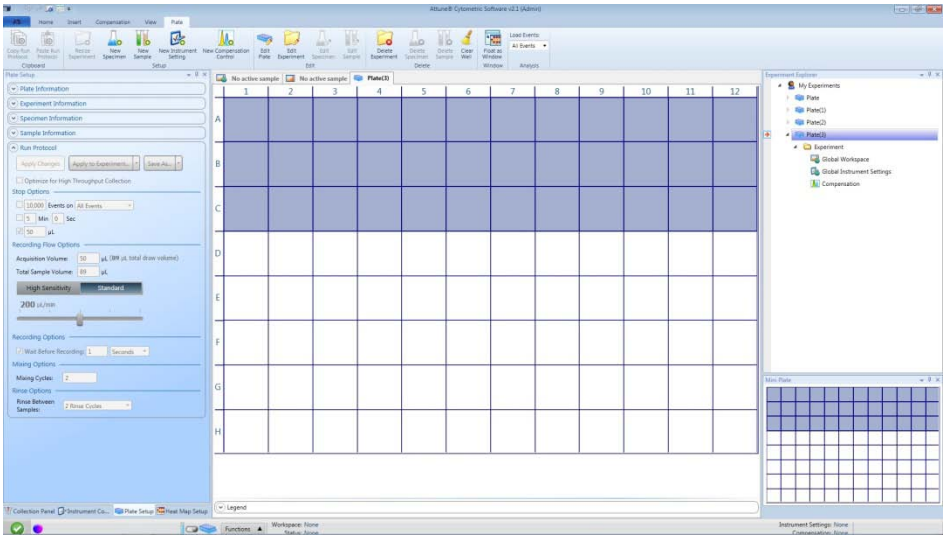


Note: The exact number of wells to select depends on how many colors you have in your experiment and how many samples you will run.

Total number of wells = Number of Compensation Samples + Number of Instrument Settings Wells + Number of Test Samples

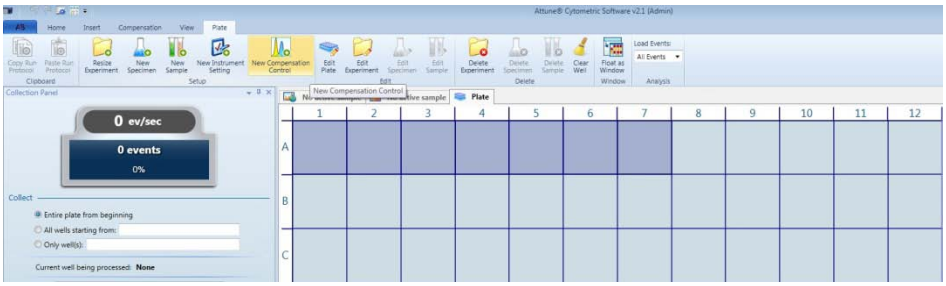
5. Click **New Experiment** on the Plate tab of the Ribbon bar.

An *Experiment* is created in the Experiment Explorer. The new Experiment is displayed in the virtual plate layout on the Attune® Desktop and the Collection Panel, and the samples associated with the Experiment have the same background color.

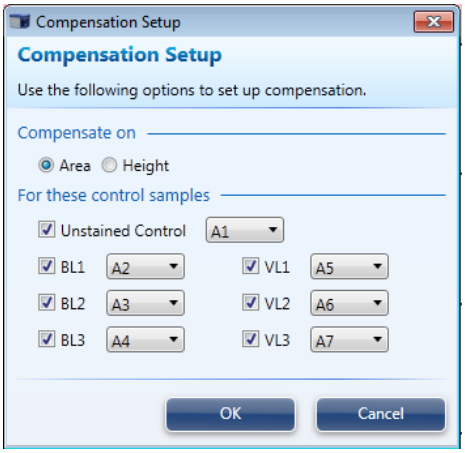


Define Compensation Control Wells

1. To define the *Compensation Control Wells*, select the desired wells from the virtual plate layout and click **New Compensation Control** on the Plate tab of the Ribbon bar.



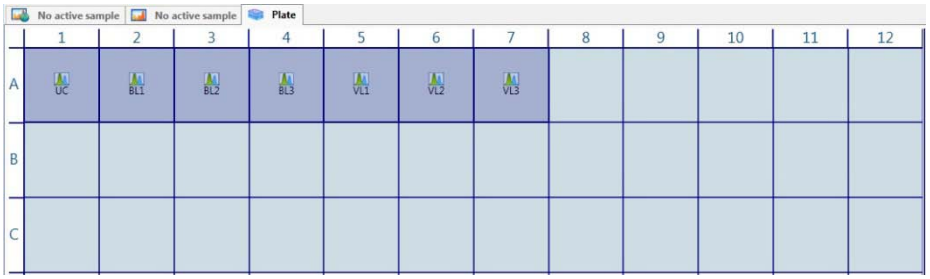
Compensation Setup window appears.



Note: If you prefer to use tubes for compensation instead of the designating specific compensation wells in your multi-well plate, you can set up compensation by clicking **Compensation Setup** in the *Compensation tab*, and then following the instruction provided by the Attune® Cytometric Software.

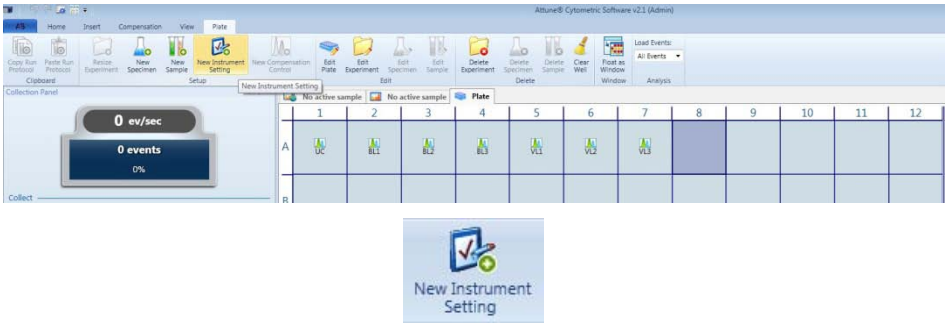
2. Choose between height and area for the compensation calculation, map the compensation controls to the desired wells using the appropriate check boxes and dropdown menus, and then click **OK**. You must select a minimum of 2 fluorescent channels or the OK button will be disabled.

   Compensation Controls Wells are displayed on the virtual plate layout in the order defined in the Compensation Setup window.



Define Instrument Settings Well

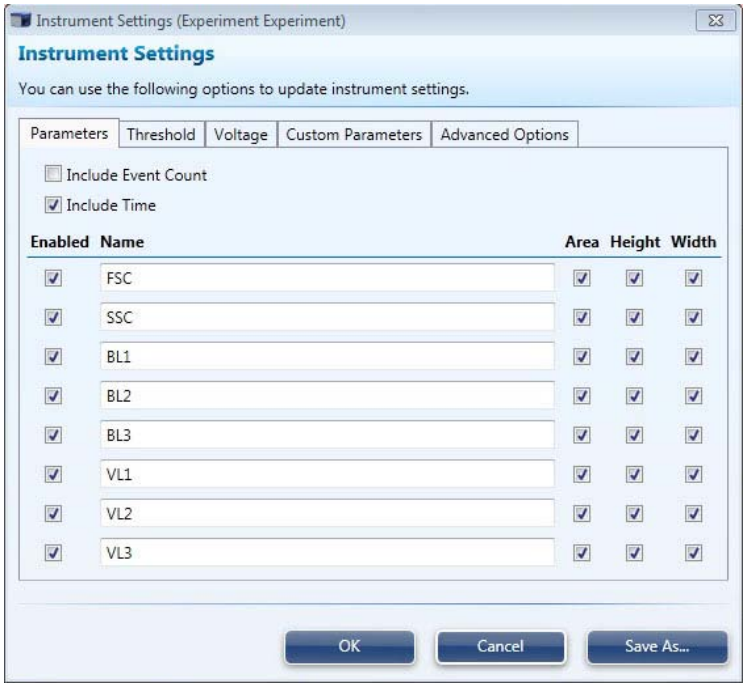
1. To define the *Instrument Settings Well*, select the desired well from the virtual plate layout to highlight and click **New Instrument Settings** on the Plate tab of the Ribbon bar.



Selected well is designated as the Instrument Settings Well.

Note: Fluorescence PMT voltages are set during compensation and they cannot be adjusted once compensation has been applied. The Instrument Settings well allows you to update threshold, scatter voltages, and depending on your access level advanced options, custom parameters, necessary in cases where the compensation controls have different scatter properties than experimental samples. When compensation is not used, the Instrument Settings well can be used to set voltages for all selected parameters. **Note that the Instrument Settings Well is not recorded during an experiment**.

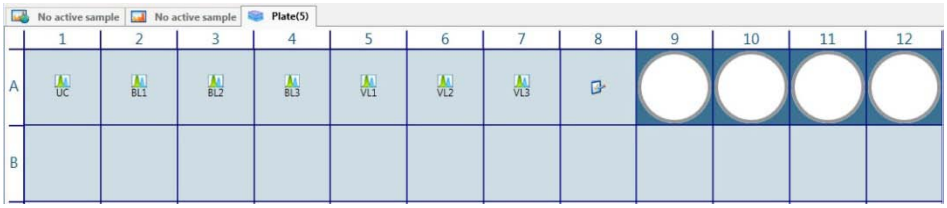
2. To update Global Instrument Settings, click **Instrument Settings** in the *View tab* or double-click **Global Instrument Settings** in the Experiment Explorer.



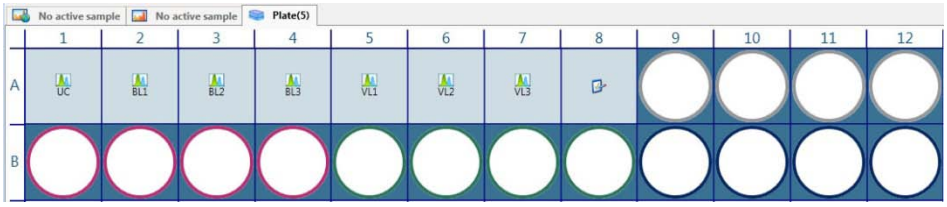
3. In the Instrument Settings window, adjust the desired parameters, thresholds, voltages, and advanced parameters, and then click **OK**. We recommend deselecting any unneeded parameters prior to setting up compensation controls to reduce the file size and speed up processing time.

Define Specimen and Sample Wells

1. To define the *Specimen* and *Sample Wells*, select the desired wells from the virtual plate layout to highlight and click **New Specimen** or **New Sample** on the Plate tab of the Ribbon bar. Selected wells are designated as Specimen or Sample Wells and are displayed on the virtual plate layout as colored circles.



2. To add additional Specimen or Sample Wells, repeat the process by selecting the desired wells and clicking **New Specimen** or **New Sample** on the Plate tab of the Ribbon bar. Each new Specimen added to the Experiment is displayed as uniquely colored circles in the virtual plate layout.

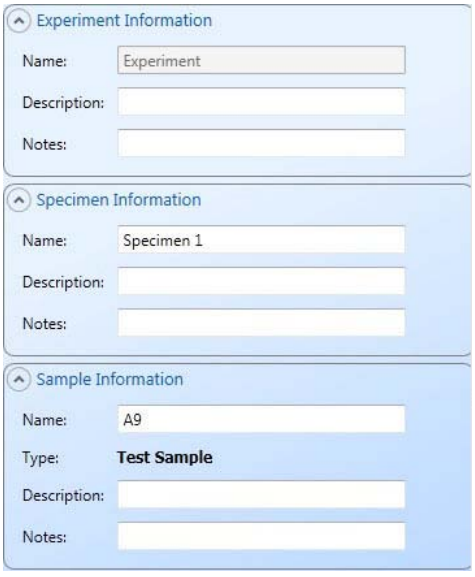


Note: There can be more than one *Experiment* in a *Plate*, each identified by a unique background color. Each Experiment can have more than one *Specimen*, and each Specimen can have more than one *Sample* with its own FCS file. Different Specimens in an Experiment are identified by different colored circles.

Add Experiment, Specimen, and Sample Information

1. To add a name, description, or notes to an Experiment, Specimen, or Sample, click to select the desired Experiment, Specimen, or Sample in the virtual plate layout, and then use the *Information tabs* in the *Plate Setup* tab to type in the appropriate information (see page 51 for more information).

   Alternatively, to rename an Experiment, Specimen, or Sample, right-click it in the Experiment Explorer, select **Rename** from the drop-down menu, and then enter the desired name.



Note: You cannot rename an active Experiment, Specimen, or Sample.

Set Up a Workspace

Workspace

Workspace displays Analysis objects (plots, gates, and statistics).

- *Workspace* types:
  - *Global Workspace* is common to all Samples in an Experiment.
  - *Local Workspace* is unique to a Sample and is denoted by an asterisk on the sample in the *Experiment Explorer*.
  - *Compensation Workspace* defines compensation controls and is pre-defined.

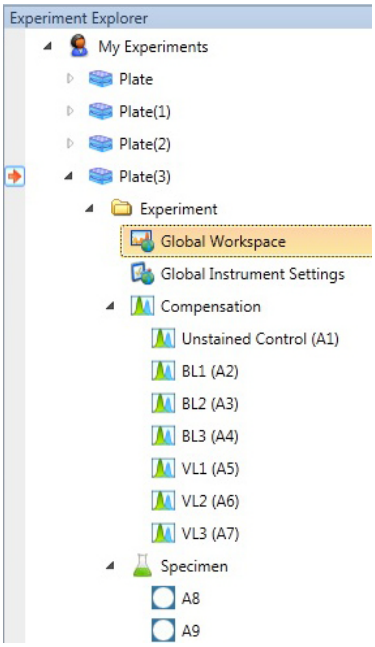
Note: For more information on working with Workspace objects and customizing the Workspace, refer to the Attune® Acoustic Cytometer User Guide, available for downloading at **www.lifetechnologies.com**.

Access the Workspace

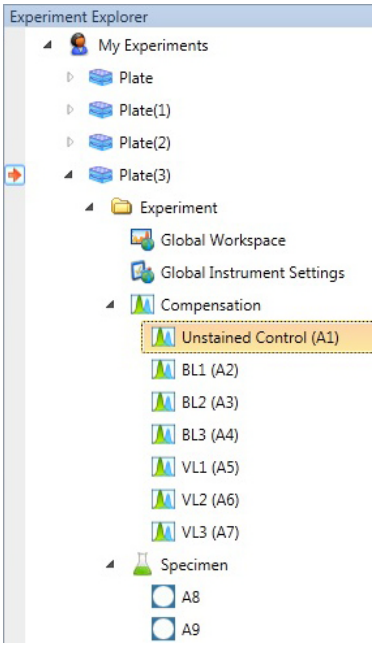
1. To access the *Global Workspace*, double-click **Global Workspace** ( ) within the Experiment in the Experiment Explorer. Alternatively, click the **Global Workspace tab** within a Sample (see "Note" below).

   To access a *Local Workspace*, click **Local Workspace tab** on the Attune® Desktop (see "Note" below).

   To access the *Compensation Workspace* double-click the specific compensation control once they have been set up in the Plate Layout view or in the Experiment Explorer.

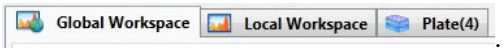


Accessing Global Workspace



Accessing Compensation Workspace

Note: You can use the *Workspace tabs* in the Attune® Desktop to access the global workspace, and create and access a local workspace for each sample.



Add Analysis Objects to Workspace

Follow the instructions below to add analysis objects (*Plots*, *Gates*, and *Statistics*) to your Workspace. For more information on working with Workspace objects and customizing the Workspace, refer to the Attune® Acoustic Cytometer User Guide, available for downloading at **www.lifetechnologies.com**.

Plots

- To insert a *Histogram*, *Dot*, or *Density Plot* to your Workspace, click **Histogram Plot, Dot Plot,** or **Density Plot** on the Insert tab of the Ribbon bar.
- To duplicate an existing plot, hold the **CTRL** key, and click and drag the desired plot. Alternatively, use **CTRL +C** and **CTRL +V** to copy and paste the desired plot.
- To resize a plot, click and drag a corner or a side of the plot.
- To change the Parameter and/or the Scale, right-click directly on the plot axis and select from the drop-down menu.

Gates

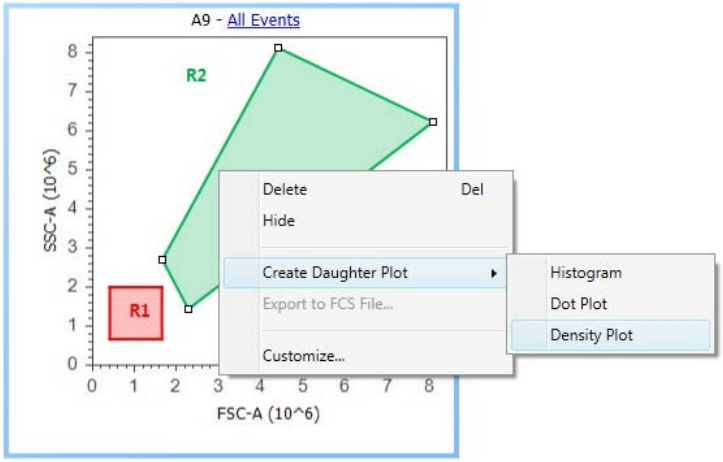
Gating Tools allow you to isolate a region in a selected plot for analysis.

- To insert a *Gate* in a plot, click the plot to select, and then select the desired gate from the *Gating Tools* on the Insert tab of the Ribbon bar. Only the types of gates that can be inserted into the selected plot will be available in Gating Tools.

Note: *Rectangular, Oval,* and *Polygon Gates* can be inserted into a *Dot Plot* or a *Density Plot*, while *Histogram* and *Bi-Marker Gates* can only be inserted into a *Histogram Plot*. A *Derived Gate* can be created as long as there is at least one gate present in the Workspace. You can create them without selecting a plot.

Daughter Plots

- To create a *Daughter Plot* from a gate in another plot, right-click the gate of interest and then select **Create Daughter Plot** to choose the type of plot to display (Dot Plot, Density Plot, or Histogram).

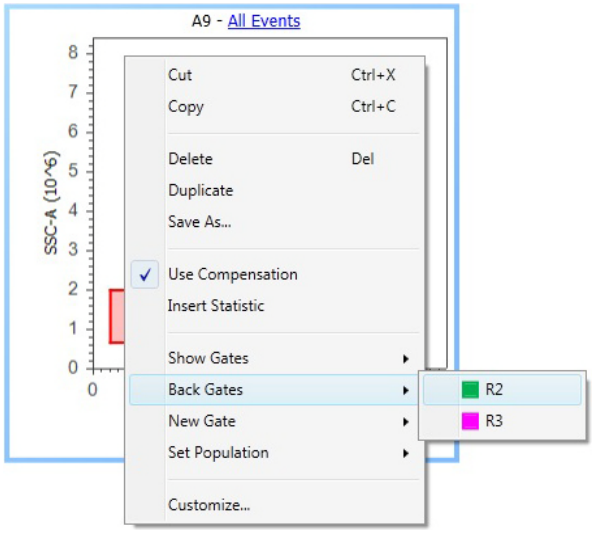


Note: Alternatively, you can right-click an existing plot, select **Set Population** from the drop-down menu, and choose the population for which to create a daughter plot.

Back Gates

Back Gates allow you to display descendent gate data on a plot. Only gates that are descendents or are at the same level within the hierarchy of the gates are available for display. Back Gates can only be displayed on Dot Plots.

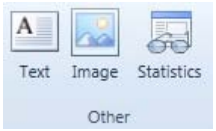
- To insert a Back Gate right-click on the Dot Plot, select **Back Gates**, and choose from the list of available gates.



Statistics

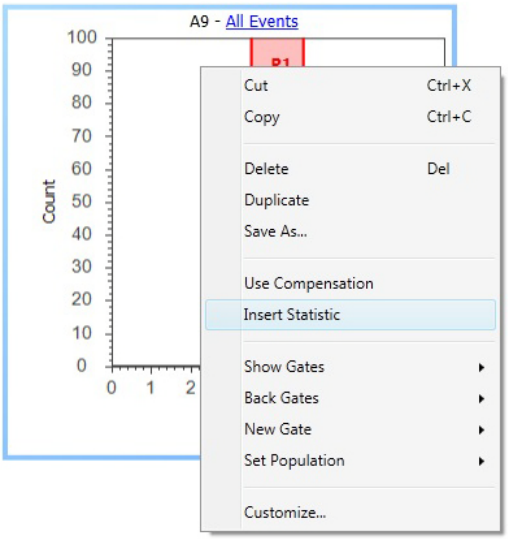
Statistics fall into two categories: *Global Statistics* show statistics for the full gating hierarchy from *All Events* down, while *Local Statistics* show statistics for a particular branch of the gating hierarchy and are plot-specific.

- To display Global Statistics, click **Statistics** on the Insert tab of the Ribbon bar without selecting a plot.



- To display Local Statistics, select the desired plot, and then click **Statistics** on the Insert tab of the Ribbon bar.

  Alternatively, right-click the plot and then select **Insert Statistics** from the drop-down menu.



Size and Position Groups of Objects in the Workspace

Size and Positions tools on the Home tab of the Ribbon bar allow you to align selected analysis objects (Plots and Statistics boxes) in the Workspace, horizontally or vertically distribute the selected objects, or to resize groups of selected objects.

1. Select one or more analysis objects by holding the **CTRL** key and clicking on the object(s). Alternatively, click and hold while dragging the cursor over multiple objects to select them as group (i.e., lasso tool).
2. Click the desired Size and Position tool on the Home tab of the Ribbon bar to align, distribute, or resize the selected objects in the Workspace.

Customize the Workspace

For information on customizing the Workspace and Workspace objects (Plots, Gates, and Statistics), refer to the Attune® Acoustic Cytometer User Guide, available for downloading at **www.lifetechnologies.com**.

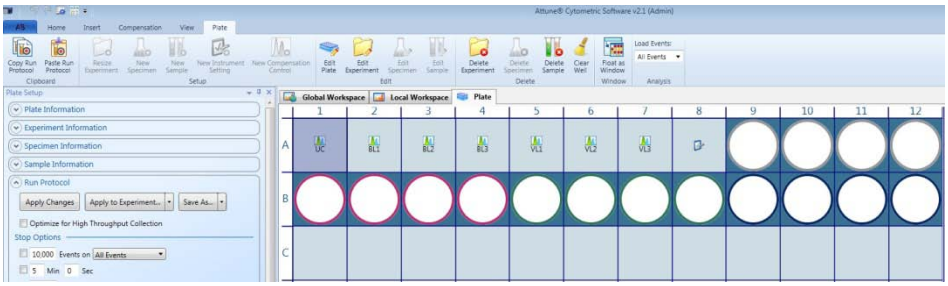
Define Run Protocol

Before processing a plate for sample collection, you need to define the *Run Protocol* for Compensation Controls, Instrument Settings Well(s), and Sample Wells. The Run Protocol allows you to define the collection criteria, collection mode, acquisition volume, recording, mixing, and rinse options.

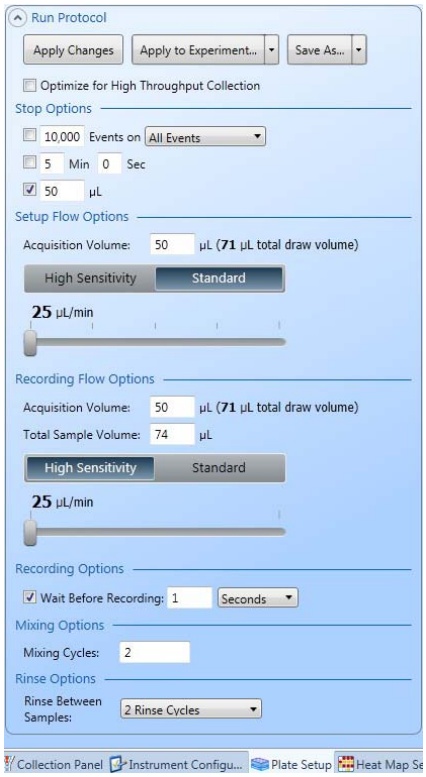
You can define the *Run Protocol* using the *Plate Setup* or the *Collection Panel* tabs.

Define Run Protocol for Compensation Controls

1. Select **Plate Setup** tab on the Attune® Desktop.
2. Click **Unstained (UC) control well** (previously defined; see page 14) on the virtual plate display to select it.



3. Make sure that the *Run Protocol* panel is expanded and displays the data acquisition options (i.e., collection criteria, collection mode, acquisition volume, and recording, mixing, and rinse options). If the Run Protocol panel is collapsed, click the **Arrow** ( ) next to Run Protocol to expand it.



4. Define the Run Protocol for the unstained compensation control by setting the following criteria:
   - *Stop Options:* number of **Events**, elapsed **Time**, and/or sample **Volume** analyzed
   - *Setup Flow Options:* **Acquisition Volume** (sample volume drawn from the well for setup), **Transit Time** (High Sensitivity or Standard), and **Flow Rate** (25–100 µL/min for High Sensitivity or 25–1000 µL/min for Standard)
   - *Recording Flow Options:* **Acquisition Volume** (sample volume drawn from the well for data recording), **Total Sample Volume** (total sample volume in the well), **Transit Time** (High Sensitivity or Standard), and **Flow Rate** (25–100 µL/min for High Sensitivity or 25–1000 µL/min for Standard)
   - *Recording Options*: time/events elapsed before starting recording
   - *Mixing Options*: **Mixing Cycles** (number of mixing cycles)
   - *Rinse Options:* **Rinse Between Samples** (number of rinses between each well)

Note: The high-throughput mode of the Attune® Auto Sampler streamlines the collection process to save time and sample. To collect your samples using an optimized protocol for high-throughput collection, check the box for **Optimize for High Throughput Collection**.

5. Click **Apply Changes**.
6. To apply the same Run Protocol to the remaining Compensation Control and Instrument Settings wells, click **Copy Run Protocol** on the Plate tab of the Ribbon bar, select the compensation control wells from the virtual plate layout, and then click **Paste Run Protocol**. You can also use the keyboard shortcuts CTRL + C (copy) and CTRL + V (paste) to apply the Run Protocol to the selected wells.

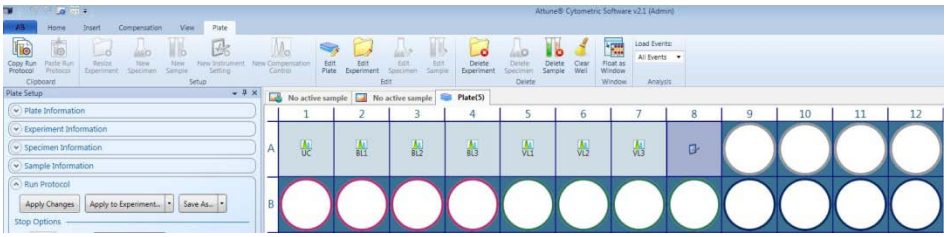
   If you wish to apply the same Run Protocol to your Sample Wells, click **Apply to Experiment**.

Note: We recommend analyzing the compensation controls with the same Run Protocol as for the samples. To allow sufficient time for adjusting voltages and gating the population(s) of interest, select a flow rate of 25 µL/minute and a minimum of 50–100 µL sample acquisition volume in the default **Standard** collection mode.

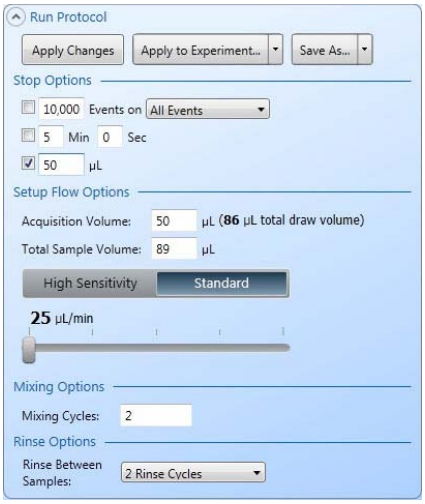
The Setup Flow Options and Recording Flow Options are set up separately to conserve the sample during setup. The Setup Flow options allow you minimize sample flow during instrument configuration. After you have adjusted the voltages during compensation setup using the Setup Flow options, the Attune® Cytometric Software records compensation settings automatically using the Recording Flow Options without further input.

Define Run Protocol for Instrument Settings Well

1. Click the **Instrument Settings Well** (previously defined; see page 15) on the virtual plate display to select it.



2. Define the Run Protocol for the Instrument Settings Well by setting the following criteria:



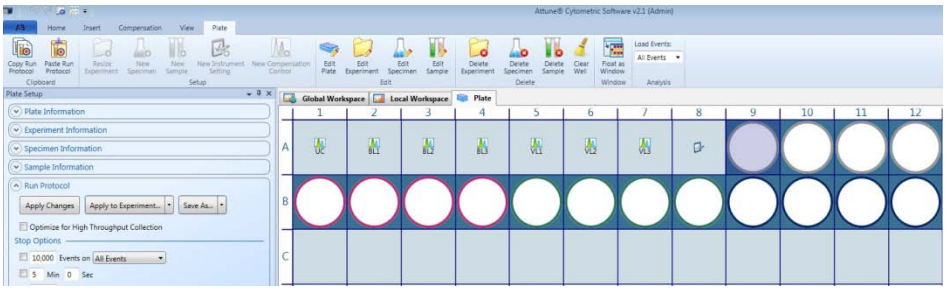
   - *Stop Options:* number of **Events**, elapsed **Time**, and/or sample **Volume** analyzed
   - *Setup Flow Options:* **Acquisition Volume** (sample volume drawn from the well for setup), **Transit Time** (High Sensitivity or Standard), and **Flow Rate** (25–100 µL/min for High Sensitivity or 25–1000 µL/min for Standard)
   - *Mixing Options*: **Mixing Cycles**
   - *Rinse Options:* **Rinse Between Samples**

> **Note:** Run Protocol for the Instrument Settings Well does not contain the Recording Flow Options, which is only available for the Compensation Control Wells and the Sample Wells. **Note that the Instrument Settings Well is not recorded during an experiment**.

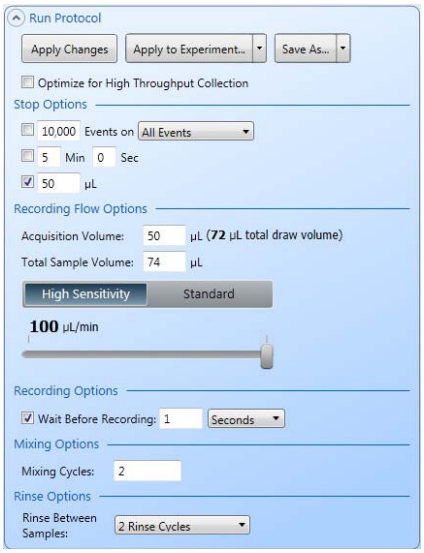
3. Click **Apply Changes**.

Define Run Protocol for Sample Wells

1. Click the first **Sample Well** (previously defined; see page 16) on the virtual plate display to select it.



2. Define the Run Protocol for the Sample Well by setting the following criteria:



   - *Stop Options:* number of **Events**, elapsed **Time**, and/or sample **Volume** analyzed
   - *Recording Flow Options:* **Acquisition Volume**, **Total Sample Volume**, **Transit Time** (High Sensitivity or Standard), and **Flow Rate** (25–100 µL/min for High Sensitivity or 25–1000 µL/min for Standard)
   - *Recording Options*: time/events elapsed before starting recording
   - *Mixing Options*: **Mixing Cycles**
   - *Rinse Options:* **Rinse Between Samples**

> **Note:** Run Protocol for the Sample Wells does not contain the Setup Flow Options, which is only available for the Compensation Control Wells and the Instrument Settings Well.

3. Click **Apply Changes**.
4. To apply the same Run Protocol to the remaining Sample Wells, click **Copy Run Protocol** on the Plate tab of the Ribbon bar, select the remaining wells from the virtual plate layout, and then click **Paste Run Protocol**.

   To apply the Run Protocol to the entire experiment, click **Apply to Experiment**.

   To modify specific Run Protocol parameters for a selection of wells, select the wells, change the desired parameters, and then click **Apply Changes**.

Setup Heat Map

The *Heat Map Setup* tab allows you to select between the *Threshold* and *Heat Map* display modes to visualize the data at a glance during or after a plate has been configured. For more information on Heat Map Setup, see page 53.

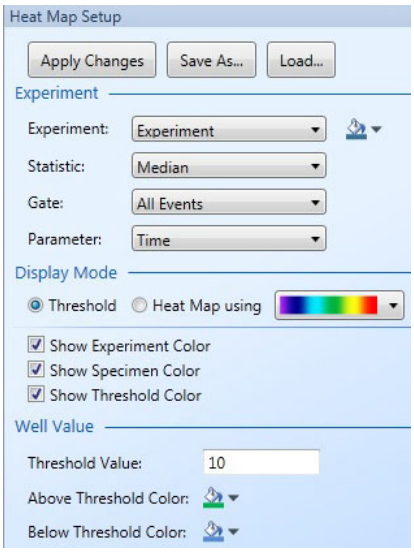
- *Threshold* mode displays each well in one of two user-defined colors based on whether the signal from the well falls above or below the set value.
- *Heat Map* mode allows you to define up to ten (10) transition values (i.e., boundaries) and assign a unique color to each range.

Note: Setting up the heat map requires that the workspace contains the appropriate gates. Heat map settings can always be adjusted after data collection is complete.

1. Select **Heat Map Setup** tab on the Attune® Desktop.
2. Under *Experiment*, using the drop-down menus, select the *Experiment* for which to set up Heat Map options, and the *Statistic*, *Gate*, and *Parameter* to use for the display.
3. Under *Display Mode*, select **Threshold** or **Heat Map**.
4. If you have selected Threshold, enter the desired *Threshold Value* and select the desired *Threshold Colors* for **Above Threshold** and **Below Threshold**.

   If you have selected Heat Map, select the transition color scheme for the heat map from the drop-down menu, and then enter the desired **Transition Values** in the corresponding fields.

   To insert additional transition points, click on the **Color Bar** and enter the desired transition value. To remove a transition point, click and drag away the corresponding **Color Marker** (i.e., arrow).
5. Click **Apply Changes**.



Note: Threshold display mode is a visual aid to determine at a glance whether the signal from a well falls above or below a set value; it does not affect data collection. As such, it is different from the *Threshold settings*, which sets the minimum signal level for each detector to eliminate unwanted events and reduce noise. For more information on Threshold settings, refer to the Attune® Acoustic Focusing Cytometer User Guide, which is available for downloading at **www.lifetechnologies.com**.

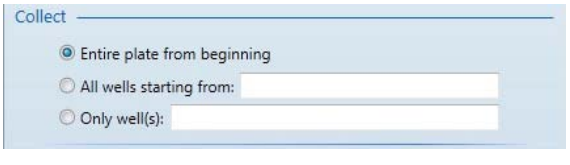
Calculate Compensation

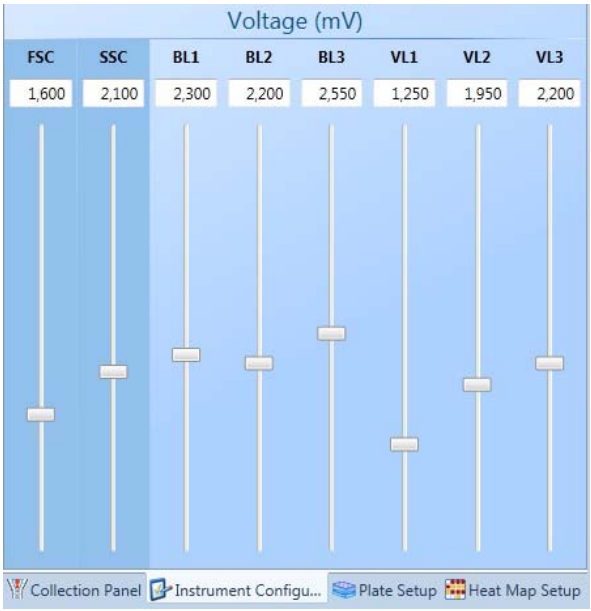
Compensation is the mathematical method used to correct the overlap of one fluorophore's emission into another fluorophore's detection channel. The Attune® Cytometric Software calculates the compensation setting automatically as it guides you through the process.

The following example protocols are specific for calculating compensation from a sample plate. For more information on compensation calculations, including obtaining compensation values using tube experiments, refer to the Attune® Acoustic Cytometer User Guide, available at **www.lifetechnologies.com**.

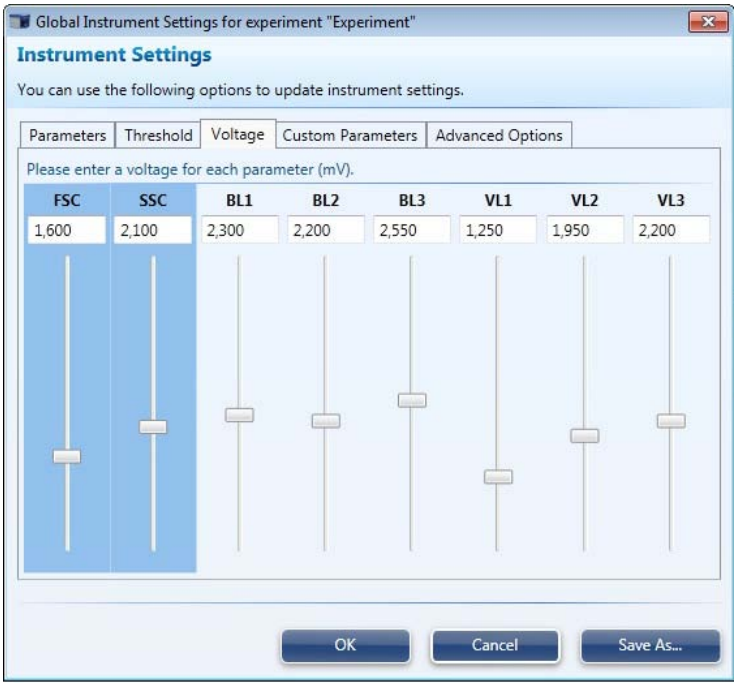
Compensation Setup

1. Ensure that the Sample plate is loaded into the Attune® Auto Sampler. If not, press the **Eject** button on the auto sampler, load the plate into the plate tray, and then close the tray door (see page 46 for the Exterior Components of the Attune® Auto Sampler).
2. On the *Collection Panel* tab, set the plate to collect **Entire plate from beginning.**
3. Click **Set Up Comp** (i.e., the Set Up/Collect button) to begin compensation setup procedure, starting with the unstained control.
4. While unstained control is running, use the **voltage slider bars** on the *Instrument Configuration* tab to optimize the voltages for the scatter parameters for FSC and SSC.

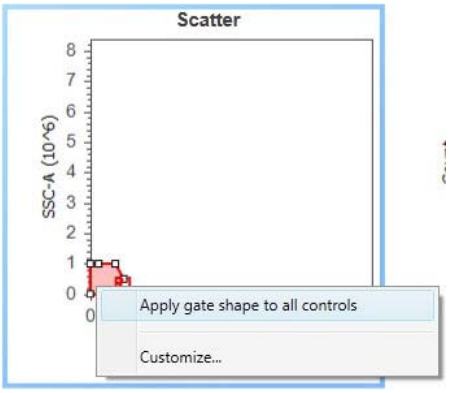




Note: Alternatively, you can click **Instrument Settings** on the View tab of the Ribbon bar to open the *Instrument Settings* window and use the *Voltage tab* to optimize the voltages. The parameter appropriate to the compensation control is highlighted during setup to signify which parameters need to be adjusted for that compensation control.



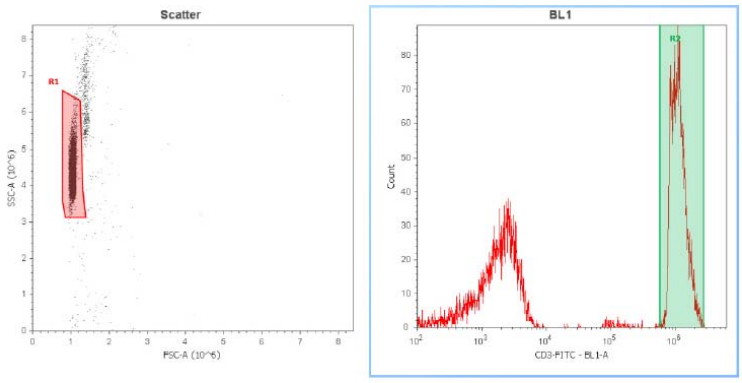
5. Set the **R1 gate** on your population of interest on the scatter plot.
6. Right-click on the gate and select **Apply gate shape to all controls**.



7. Click **Stop** on the Collection Panel when you are satisfied with the data and then click **Next** to move onto the next compensation control. The channel that will be collected next appears in parenthesis on the Next button and the previous channel is listed in parenthesis on the Prev button.

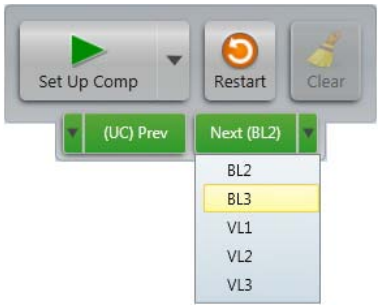
   Alternatively, allow the instrument to dispense the entire acquisition volume of the compensation control before clicking **Next** and then **Set Up Comp** to begin the set up of the next compensation well.

8. Again, using the voltage slider bars on the Instrument Configuration tab, optimize the voltage for the selected compensation control.
9. Set the **R2 gate** on the peak of interest on the histogram plot.



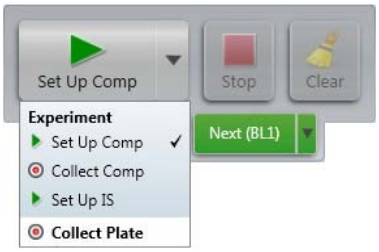
10. Repeat setup steps for the remaining compensation controls and ensure that the gates are set appropriately on the population of interest.

Note: You can jump to any step of the compensation setup using the drop-down menus on the **Prev** and **Next** buttons, provided that the instrument is not actively processing a well. **Restart** restarts the procedure from the first well and overwrites all the data previously collected.



Note: You can skip the compensation setup procedure entirely and select one of the following commands using the **Set Up/Collect** drop-down menu.

- **Collect Compensation** to record of all compensation controls automatically
- **Set Up IS** to optimize instrument settings before sample collection
- **Collect Plate** to start the collection procedure to record all samples on the plate



Collect Compensation

1. After you have set up all compensation controls, click **Next** on the Collection Panel tab. The first defined control compensation control (e.g., unstained control or UC) workspace opens.
2. Click **Collect Comp** to start the automated compensation collection procedure. The Attune® Cytometric Software acquires and records compensation controls starting with the first defined control using the Run Protocol applied to each individual well.

While the compensation controls are being recorded, ensure that gates created during compensation setup are still set on the populations of interest.

3. When the last compensation control is recorded, *Compensation Matrix* automatically opens for your review.

4. To accept the compensation matrix, click **OK**. You can also manually edit the compensation matrix values (not recommended).

Note: You can also access the Compensation Matrix by clicking **Compensation Matrix** on the *Compensation* tab of the Ribbon bar.

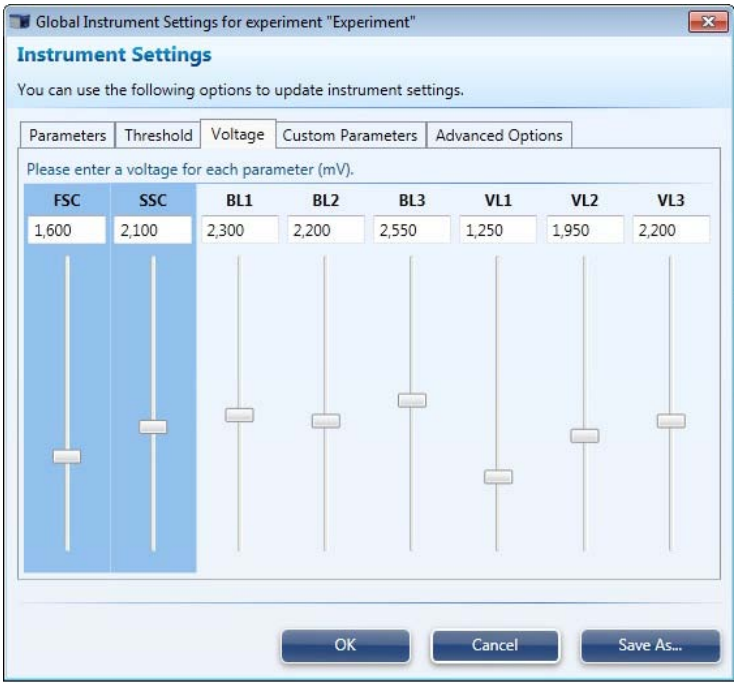
Clicking **Use Compensation** allows you to toggle between applying the recorded compensation to the experiment and turning off the compensation function.

Optimize Instrument Settings

1. Upon completion of the compensation collection and accepting the compensation matrix, click **Next** or select **Set Up IS** (if an Instrument Settings well has been defined) from the drop-down menu on the Set Up/Collect button. Global Workspace opens to allow the optimization of instrument settings. Note that the data in Instrument Settings wells will **not** be recorded.
2. Click **Set Up IS** to begin the procedure for optimizing instrument settings using the sample in the Instrument Settings well (previously defined; see page 15).

3. Click **Instrument Settings** in the View tab to open the Instrument Settings window or use the **Instrument Configuration** tab to adjust the voltages and threshold settings for the scatter channels (FSC and SSC) and the fluorescent channels (if compensation is not applied) while the sample is acquiring.

   Note that once compensation has been collected, only the scatter channels will be available for adjustment, and the channels for fluorescence voltages will be grayed out. However, if the experiment contains no compensation wells, then the fluorescence voltages will also be available for adjustment.



4. Adjust the gates in the workspace as necessary.
5. Click **Stop** when the voltages have been optimized for all available channels. Alternatively, allow the instrument to dispense the entire acquisition volume for the Instrument Settings sample.

Note: Alternatively, Instrument Settings can be set up from tubes by adding a tube sample or specimen to the experiment via Experiment Explorer. To add a tube specimen, right click on the Experiment folder in the Experiment Explorer and select **New Tube Specimen**. To add a new tube sample, right-click on the Specimen in the Experiment Explorer and select **New Tube Sample**. Instrument settings collected from tube must be performed prior to collecting the plate.

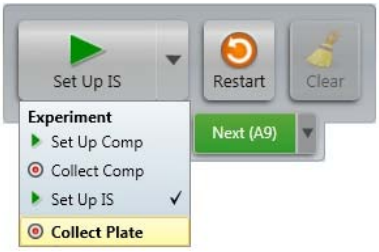
Collect Samples

After you have calculated the compensation and optimized instrument settings, you are ready to run your samples to acquire and record data.

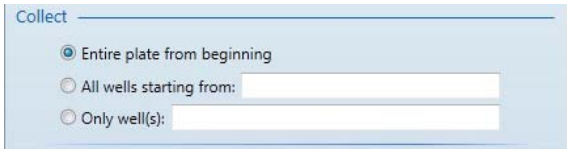
The following example protocols are specific for running samples from a sample plate. For information on collecting from sample tubes (i.e., Tube mode), refer to the Attune® Acoustic Cytometer User Guide, available at **www.lifetechnologies.com**.

Collect Samples (Plate Collection Mode)

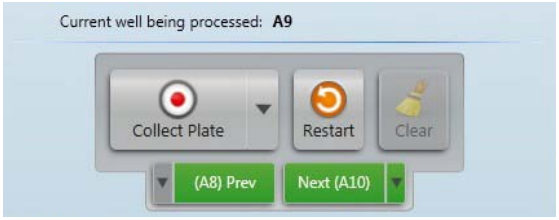
1. After you have completed the procedure for optimizing instrument settings, click **Next** to move to sample wells or select **Collect Plate** from the drop-down menu on the Set Up/Collect button.



   If you have set up multiple experiments on the same plate and wish to collect only from specific wells, you can enter your selection in the **All wells starting from** or the **Only well(s)** fields.



2. Click **Collect Plate** to start the automated sample collection procedure. The Attune® Cytometric Software acquires and records samples starting with the first designated sample well using the appropriate Run Protocol applied to that well.



   The well that is being acquired is indicated in several locations on the software interface (Collection Panel, Mini Plate, and the Plate tab) and that the data for that well is visible on the global workspace.

   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.

3. Click **Pause** to temporarily halt sample collection, if needed. The instrument completes the collection of the current well and then pauses the collection procedure.

When the instrument is paused between wells, you can make changes to the Run Protocols, Heat Map settings, and Workspace objects for the remaining wells.

Click **Clear** to erase all collected data from that well without pausing the collection procedure.

To resume the data collection from the point where it was paused, click **Resume**.

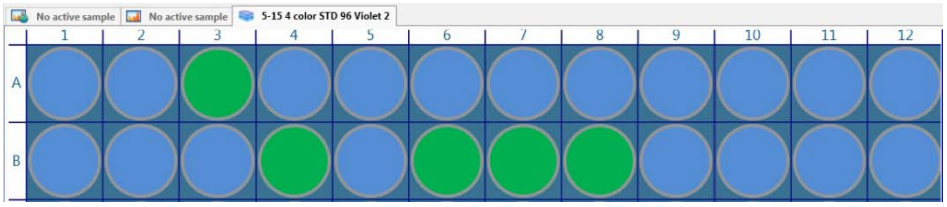
Click **Restart** to restart collection from the first well and overwrite all collected data including compensation controls and Instrument Settings wells.

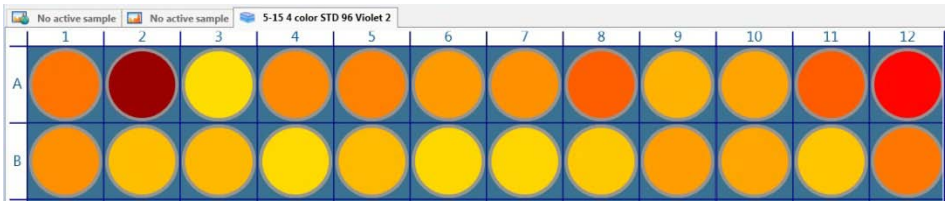
Note: If you click on another Plate or Tube Sample within the Plate Experiment, or adjust collection criteria when sample collection is paused, the software will warn you that the sample will be discarded if this action is completed. To avoid losing your sample, you can choose to run the *Sample Recovery* function from the Functions menu of the lower taskbar (see page 33).

4. Sample collection automatically stops after all the selected samples in the plate are collected and the Attune® Cytometric Software automatically saves the data in a unique FCS file.

Once data is recorded in a given well, the well color changes according to the Threshold or Heat Map value (previously defined; see page 25). Note that Heat Map settings can be adjusted in the *Heat Map Setup tab* before or after collection (see page 53 for more information on Heat Map Setup).

The examples below show the wells in Threshold (top) and Heat Map (bottom) display modes at the completion of sample collection.



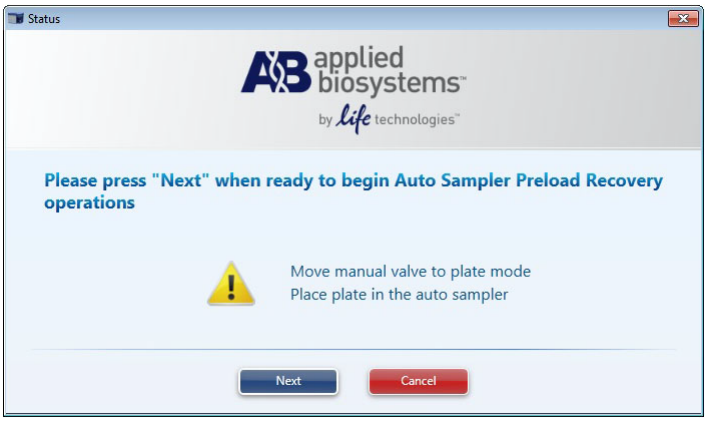


Note: For more information on data collection using the Attune® Acoustic Cytometer, refer to the Attune® Acoustic Cytometer User Guide, available at **www.lifetechnologies.com**.

Sample Recovery (Plate Collection Mode)

The *Sample Recovery* function of the Attune® Acoustic Focusing Cytometer, allows the return of unused sample volume back to the well (or the tube; see below) from which it was initially drawn. For example, if you have initially set the Total Sample Volume to 150 µL, but have gathered sufficient data after collecting only 50 µL of the sample, you can recover the unused 100 µL of the sample by running the Sample Recovery function.

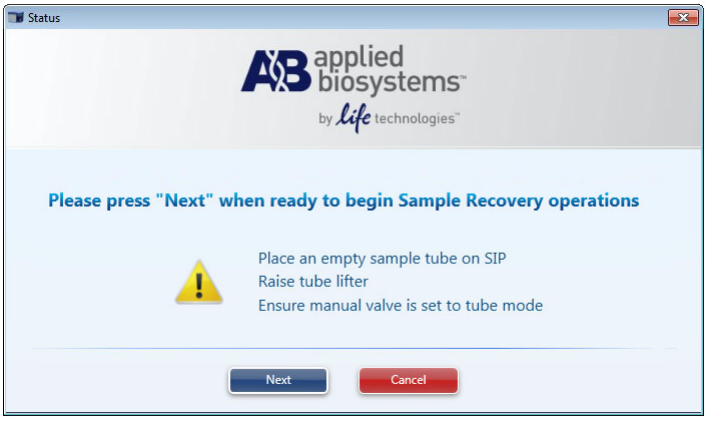
1. Select **Sample Recovery** from the Functions menu of the lower taskbar. The *Sample Recovery* prompt screen appears.



2. Ensure that the manual valve is set to **Plate** mode and follow the instructions on the screen to recover your sample.

Sample Recovery (Tube Collection Mode)

1. Select **Sample Recovery** from the Functions menu of the lower taskbar. The *Sample Recovery* prompt screen appears.



2. Place an empty sample tube on the Sample Injection Port (SIP) and raise the tube lifter.
3. Ensure that the manual valve is set to **Tube** mode and follow the instructions on the screen to recover your sample.

Note: You can run the Sample Recovery function only if the volume of the sample to be recovered is more than 40 µL.

You can run the tube-based Sample Recovery function only if the tube lifter has not been dropped yet and/or no other function requiring a rinse cycle has been processed.

Analyze and Process Data

Data Analysis and Processing

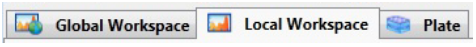
1. To analyze and process data globally, open the **Global Workspace** for any sample well by double-clicking the well in the Plate tab or the Mini Plate, or double-clicking the appropriate file in Experiment Explorer.

   Adjust gates and add or remove plots as desired.

   Right-click the border area of a plot and choose **Customize** to adjust the axes, display information, and other plot-specific settings, as needed. Multiple plots can be customized at the same time by selecting multiple plots then right-clicking and selecting **Customize**.

   All plots containing modified parameters will be automatically updated after each change and for all subsequent samples in the global workspace.

2. To analyze and process data locally (i.e., only for the selected well), click the **Local Workspace** tab. Any changes made within the local workspace will not be propagated to other samples' workspaces.



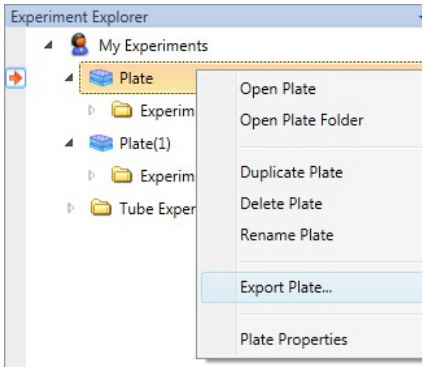
3. Run Protocol is displayed at bottom of Collection Panel, however this protocol cannot be changed via the Collection Panel; it will have to be modified on the Plate Setup tab.

Note: For more information on data analysis and processing using the Attune® Cytometric Software, refer to the Attune® Acoustic Cytometer User Guide, available at **www.lifetechnologies.com**.

Exporting Plates

1. Right-click the **Plate** you want to export in the Experiment Explorer.
2. From the drop-down menu, select **Export Plate**.
3. Select the storage location and click **Save**.

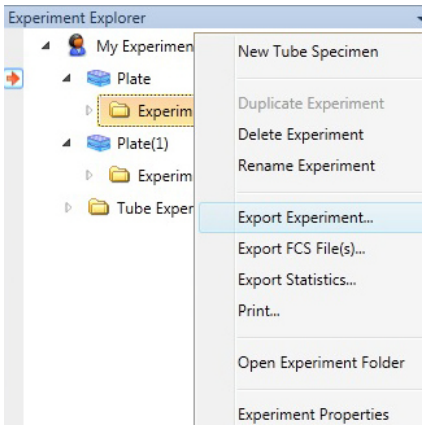
   All the Experiments in the Plate, along with their associated Workspaces, Instrument Settings, Compensation Controls, all sample-specific information will be exported.



Exporting Experiments

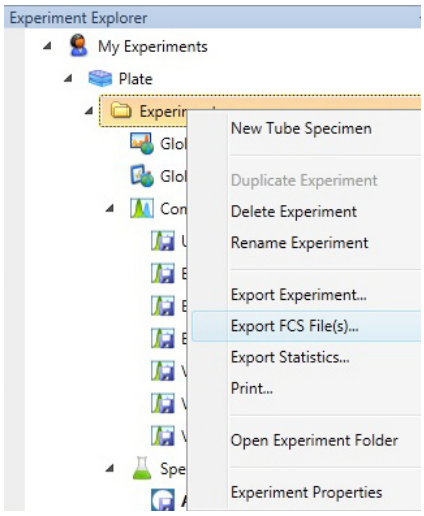
1. Right-click the **Experiment** you want to export in the Experiment Explorer. From the drop-down menu, select **Export Experiment**.
2. Select the storage location and click **Save**.

   Workspace, Instrument Settings, Compensation Controls, as well as all sample-specific information in the selected Experiment(s) will be exported.



Batch Processing: Exporting FCS Files

1. Select the **Experiment, Specimen,** or **Sample files** to export.
2. Right-click and select **Export FCS File(s)** from the drop-down menu.
3. Select the storage location and click **Save**. You can export FCS using the FCS 3.0 or FCS 3.1 format.



Batch Processing: Exporting Statistics

1. Select the **Experiment, Specimen,** or **Sample files** to export.

   Right-click and select **Export Statistics** from the drop-down menu.

   Export Statistics window opens.
2. Select to export the statistics using **Global Workspace** or **Local Workspace**.
3. Select to export the statistics as a **Single File** (combines statistics for all samples in a single file) or as **Individual Files** (exports statistics for each sample as a separate file), and then click **OK**.
4. Select the storage location and click **Save**. Statistics files are exported as Comma Separated Values Files (*.csv).

Batch Processing: Printing

1. Select the **Plate(s), Experiment(s), Specimen(s),** or **Sample files** to print.
2. Right-click and select **Print** from the drop-down menu.

   Print window opens.
3. Select to print using **Global Workspace** or **Local Workspace**, and specify the printer or select to create a PDF.
4. Click **OK** to print.

Shutdown

The *Shut Down* function of the Attune® Cytometric Software facilitates the automated shutdown of the instrument. The function ensures that all sample fluid and dyes have been removed from the system the fluidics lines and the two pumps have been decontaminated and filled with Attune® Shutdown Solution.

The automated shutdown procedure for systems with the Attune® Auto Sampler is broken into three phases and can take up to 30 minutes. Make sure to follow all the instructions provided by the instrument during shutdown.

If the Attune® Auto Sampler is not powered on, the system will perform a tube based shutdown.

IMPORTANT! Perform the following shutdown procedures at least once a day, even if the instrument is intended for continuous use. Proper cleaning of the instrument ensures its consistent and accurate operation.

CAUTION! BIOHAZARD. Cytometer hardware may be contaminated by biohazardous material. Using fresh 10% bleach solution in deionized water is the only procedure we recommend for decontaminating the cytometer.

IMPORTANT! 10% bleach is defined as a 1 in 10 dilution (1 part bleach to 9 parts deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

Check Fluid and Waste Levels

1. Check the levels in the fluid tanks.
2. Ensure that the wash and shutdown solution tanks are at least half-full. If empty, fill the appropriate tanks with Attune® Wash or Attune® Shutdown Solution.
3. Empty the waste tank on both the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler.

Note: For the location of the fluidics compartment and instructions on filling the fluid tanks refer to the Attune® Acoustic Cytometer User Guide, available for downloading at **www.lifetechnologies.com**.

IMPORTANT! Powering the instrument on and off within 30 minutes can decrease the laser lifetime. The Shut Down function powers off the laser and the instrument automatically. If you interrupt the script, you will need to force exit, then restart the script and let it run to completion.

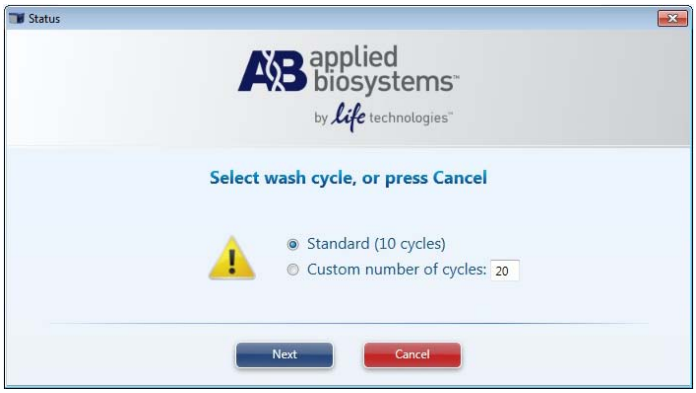
Run Shut Down Function

The Attune® Cytometric Software provides instructions to perform the *Shut Down* operation. The Shut Down operation is broken into three phases in systems with the Attune® Auto Sampler, and it can take up to 30 minutes; however, most of the operation is performed automatically. Make sure to follow all the instructions provided by the instrument during the Shut Down cycle. During the operation, the software provides real-time updates on the shutdown function being executed.

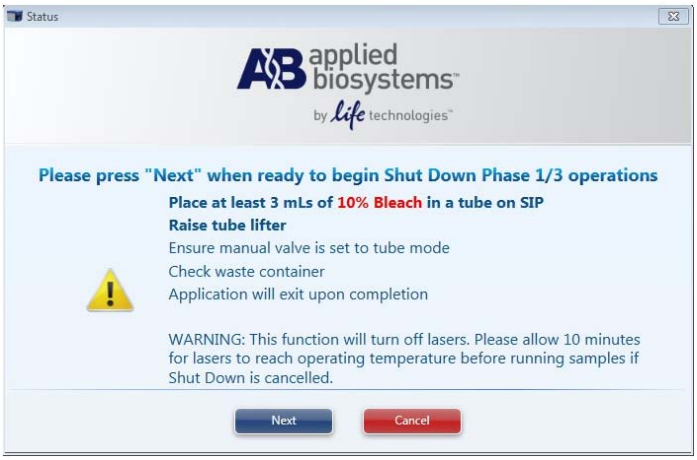
1. **Log Out** of the Attune® Cytometric Software, and click **Shut Down**.

   The *Shut Down* prompt screen appears.

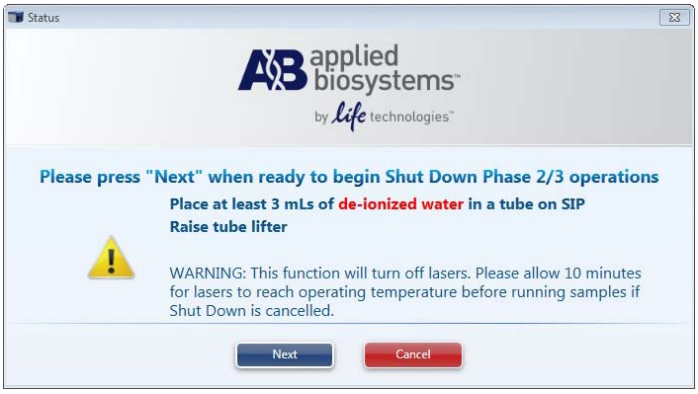
   

2. Select **Standard** for 10 wash cycles or **Custom** to enter the desired number of wash cycles. Click **Next**.
3. When prompted, place 3 mL of 10% bleach solution in a tube on the sample injection port (SIP) and raise the tube lifter.

4. Ensure that the manual valve is set to **tube mode** and click **Next**.

5. When prompted, place 3 mL of deionized water in a tube on the SIP, lift the tube filter, and click **Next**.



6. When prompted, place an empty 96-well round bottom plate in the Auto Sampler and close the door.

7. Move the manual valve to **plate mode** and click **Next**.
8. At the end of the shutdown operation, the Attune® Cytometric Software automatically powers down the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler. The Attune® Auto Sampler is in a low power standby mode and will automatically power on if the software is started and then the Attune® Acoustic Focusing Cytometer is powered on. The eject button of the Attune® Auto Sampler will blink in the standby mode until the instrument is turned off using power switch located at the rear of the Attune® Auto Sampler.

IMPORTANT! If you cancel the shutdown, allow at least 10 minutes for the lasers to reach operating temperature before running any samples. You will also need to re-run Startup.

IMPORTANT! If you intend to leave the Attune® Acoustic Focusing Cytometer in the shutdown state for longer than two weeks, perform *System Decontamination* (page 39) instead of running the *Shut Down* function. System decontamination cleans the fluidics systems of the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler and leaves the fluidics systems in deionized water to prevent salt crystals from clogging them.

System Decontamination

The *System Decontamination* function of the Attune® Cytometric Software facilitates the automated decontamination of the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler fluidics. This function, which is broken into four automated phases, is only available to system administrators and takes approximately 45 minutes.

Perform the *System Decontamination* operation:

- as a monthly maintenance routine to prevent and reduce microbial growth within the instrument
- if the system is likely to be idle for more than two weeks (run it in place of the Shut Down function)
- if the instrument has been idle for more than two months
- if the instrument has been idle for more than two weeks without decontamination run prior to it becoming idle

The system decontamination procedure is broken into four phases (mostly automated) and takes approximately 45 minutes. Make sure to follow all the instructions provided by the instrument during system decontamination. Note that this function is only available to system administrators.

IMPORTANT! Perform the following decontamination procedure on a monthly basis, or if you intend to leave the Attune® Acoustic Focusing Cytometer in the shutdown state for longer than two weeks or if you plan to ship the instrument for service. Proper decontamination of the instrument ensures its consistent and accurate operation, and reduces potential health hazards.

CAUTION! BIOHAZARD. Cytometer hardware may be contaminated by biohazardous material. Using fresh 10% bleach solution in deionized water is the only procedure we recommend for decontaminating the cytometer.

IMPORTANT! 10% bleach is defined as a 1 in 10 dilution (1 part bleach to 9 parts deionized water) of 5.25% sodium hypochlorite in deionized water. This gives a final concentration of 0.5% sodium hypochlorite equivalent to 5,000 ppm of available chlorine.

Prepare for System Decontamination

1. Rinse out all fluid containers.
2. Fill the shutdown solution tank with 200 mL of **10% bleach**. Ensure that the wash solution tank is empty.
3. Fill focusing fluid tanks on both the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler with 450 mL of **10% bleach**.
4. Ensure that all fluid lines and sensor cables are connected.

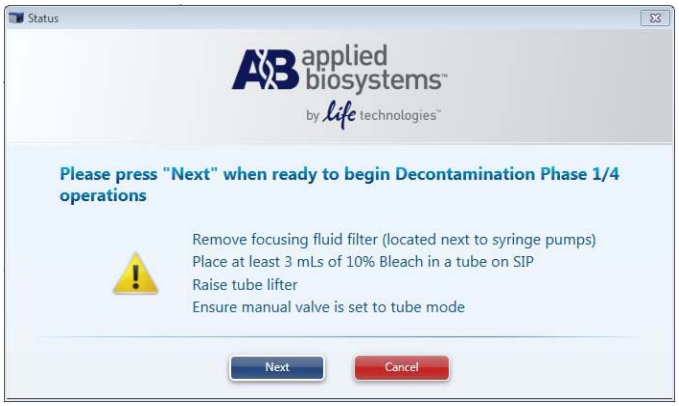
Note: For the location of the fluidics compartment and instructions on filling the fluid tanks refer to the Attune® Acoustic Cytometer User Guide, available for downloading at **www.lifetechnologies.com**.

Run System Decontamination Function

The Attune® Cytometric Software provides instructions to perform the *System Decontamination* operation. The System Decontamination operation is broken into four phases and it can take up to 45 minutes; however, most of the operation is performed automatically. Make sure to follow all the instructions provided by the instrument during the System Decontamination procedure. During the operation, the software provides real-time updates on the shutdown function being executed.

1. Select **System Decontamination** from the *Functions* menu. 

   Make sure that the appropriate tanks are filled with 10% bleach and all fluid lines and sensor cables are connected (see Prepare for System Decontamination, page 39).
2. Click **Next** to start Decontamination Phase 1.
3. When prompted, remove focusing fluid filter located next to the syringe pump on the Attune® Acoustic Focusing Cytometer, place at least 3 mL of 10% bleach in a tube on SIP, raise the tube filter, and click **Next**.



4. When prompted, move the manual valve to **Plate** mode, and then click **Next** to start Decontamination Phase 2.
5. When prompted, rinse the focusing fluid tanks on both the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler and fill them with 450 mL of deionized water.
6. Move the manual valve to **Tube** mode, place at least 3 mL of 10% bleach in a tube on SIP, raise the tube filter, and click **Next** to start Decontamination Phase 3.
7. When prompted, move the manual valve to **Plate** mode, and then click **Next** to start Decontamination Phase 4.
8. After Decontamination Phase 4 is complete, replace the focusing fluid filter with a fresh filter, and perform a full system reset by unplugging the instrument and leaving it unplugged for at least 30 seconds.
9. Replace all fluids in all tanks with the appropriate solutions.

2. System Maintenance

The Attune® Acoustic Focusing Cytometer is designed to require minimum maintenance. However, to ensure reliability of the cytometer, you must perform basic preventative maintenance procedures on a regular basis, as listed below.

CAUTION! BIOHAZARD. All biological samples and materials that come into contact with them have the potential to transmit infectious diseases and are considered biohazardous. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Never pipette by mouth.

Maintenance Schedule

The table below lists the routine maintenance procedures that keep the Attune® Acoustic Focusing Cytometer and all its peripheral systems in good working condition.

Procedure	**Frequency**
Shutdown	Daily
Visual inspection of sample injection port (SIP)	Daily
Visual inspection of fluidics tanks and connections	Daily
Visual inspection of syringe pumps	Daily
Fluidics maintenance (i.e., De-bubble, Unclog, and Wash functions)	Daily and as needed
Computer maintenance	Monthly
Optical filter cleaning	Monthly and as needed
Fluidics decontamination	Monthly
System Decontamination	Monthly and as needed
Changing focusing fluid filter	Monthly
Replacing syringes	As needed*

*The frequency of maintenance depends on how often you run the cytometer. On average, syringes last about 6 month.

Daily Maintenance

Daily Shutdown

Daily shutdown involves executing the **Shutdown** function. This function ensures that all sample fluid and dyes have been removed from the fluidics lines and the two pumps have been decontaminated and filled with Attune® shutdown solution to prevent the formation salt crystals.

The shutdown procedure can take up to 30 minutes, but most of the steps are automated and under computer control. At the end of the shutdown procedure, the cytometer is automatically powered down.

Visual Inspection

Visually inspect the sample injection port, fluidics tanks and connections, and the syringe pumps for any leakage. If you notice any leaks in the fluidics lines, contact your service representative. Decontaminate any spills by wiping the area with 10% bleach solution.

Fluidics Maintenance

Daily fluidics cleaning involves executing the **De-bubble**, **Unclog**, and **Wash** functions as needed.

- *De-bubble* is a user-initiated function for clearing bubbles in the fluidics lines of the cytometer.
- *Unclog* function is a user-initiated back flush operation to remove clogs from the sample probe and flow cell.
- *Wash* is a user-initiated system cleaning between sticky samples. This function requires user supplied bleach or detergent.

Sanitize Between Uses

Follow the procedure below to sanitize the Attune® Auto Sampler between uses. Note that this procedure is intended for a quick cleaning of the instrument to minimize cross-contamination. For a more thorough decontamination, perform the System Decontamination procedure (see page 39).

1. Run a well of Milli-Q® water.
2. Run a well of freshly prepared 10% bleach (1 part 5.25% sodium hypochlorite to 9 parts of deionized water).
3. Repeat a well of Milli-Q® water.

Note: For monthly and periodic maintenance of the system, refer to the Attune® Acoustic Cytometer User Guide, available for downloading at **www.lifetechnologies.com**.

Appendix A: Ordering Information

The following reagents and consumables have been specifically formulated for use with the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler, and are available separately from Life Technologies. Ordering information is provided below. For more information, go to **www.lifetechnologies.com** or contact Technical Support.

Product	Amount	Cat. no.
Attune® Focusing Fluid, 1X Solution	1 × 1 L 6 × 1 L	4449790 4449791
Attune® Focusing Fluid, 10X Solution	1 × 1 L	4449792
Attune® Wash Solution	500 mL	4449755
Attune® 10X Shutdown Solution	250 mL	4454955
Attune® Performance Tracking Beads	50 measurements	4449754
Attune® 10 mL syringe	1 each	4452819
Attune® 1 mL syringe	1 each	4452079
Attune® Focusing Fluid Filter	1 each	4456564
Attune® Auto Sampler PLUG,1/4-28 Teflon	1 each	4476990
Attune® Auto Sampler Bottle Assembly Focusing Fluid	1 each	4477847
Attune® Auto Sampler Bottle Assembly Waste	1 each	4477850
Attune® Auto Sampler 10 mL Syringe	1 each	4478686
Attune® Emission Filter Holder Blade	1 each	4465834
Attune® Dichroic Filter Holder Blade	1 each	4465832
Attune® Bottle assembly, Wash, 250 mL	1 each	90032053
Attune® Bottle assembly, Waste, 1 L	1 each	90032053
Attune® Bottle assembly, Sheath, 1 L	1 each	90039273
Attune® Bottle assembly, Shutdown, 250 mL	1 each	90039274

Appendix B: Instrument Description

System Components

The Applied Biosystems® Attune® Auto Sampler is shipped with the system components listed below. All components are shipped at ambient temperature.

Component	Quantity
Attune® Auto Sampler	1
Power cord kit, universal voltage C13 2.5 m RC	1
Attune® Cytometric Software (CD)	1
Attune® Cove Base	1
Attune® Auto Sampler Base	1
96-well plates	1 box
Attune® Auto Sampler Quick Reference Card	1
Attune® Auto Sampler User Guide	1
Attune® Cytometric Software V2.1 Release Notes	1

Product Use

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

System Specifications

Physical Characteristics

Footprint (H × W × D): Approximately 16″/40 cm × 11.5″/29 cm × 11.5″/29 cm

Space requirements (H × W × D): 29″/74 cm × 13.8″/35 cm × 23.1″/58.5 cm above the mounting surface

Weight: Approximately 35 lb/15.9 kg

Operating temperature: 15–30°C (50–95°F)

Operating humidity: <80% non-condensing

Electrical requirements: 100–240VAC, 50/60 Hz, <300 W

Fluidics

Fluid storage: Within instrument with level sensing

Total fluid volume: 800 mL per container; capable of running four (4) 96-well plates in standard mode with 2 washes/well

Sample Analysis

Compatible plate types: 96-well, standard depth (flat, round, V-bottom)
96-well, deep-well (flat, round, and V-bottom)
384-well, standard depth (flat, round, and V-bottom)
384-well, deep-well (round and V-bottom)

Processing time: <45 minutes for 96-well plate, using high-throughput mode
<60 minutes for 96-well plate, using standard mode, 2 wash cycles
<260 minutes for 384-well plate, using standard mode, 2 wash cycles

Carry-over: <0.5% in standard mode using 2 wash cycles

Mixing Cycles: Each well is mixed via aspiration of sample (not shaking).

Wash Cycles: User defined number of wash cycles (up to 3 wash cycles)

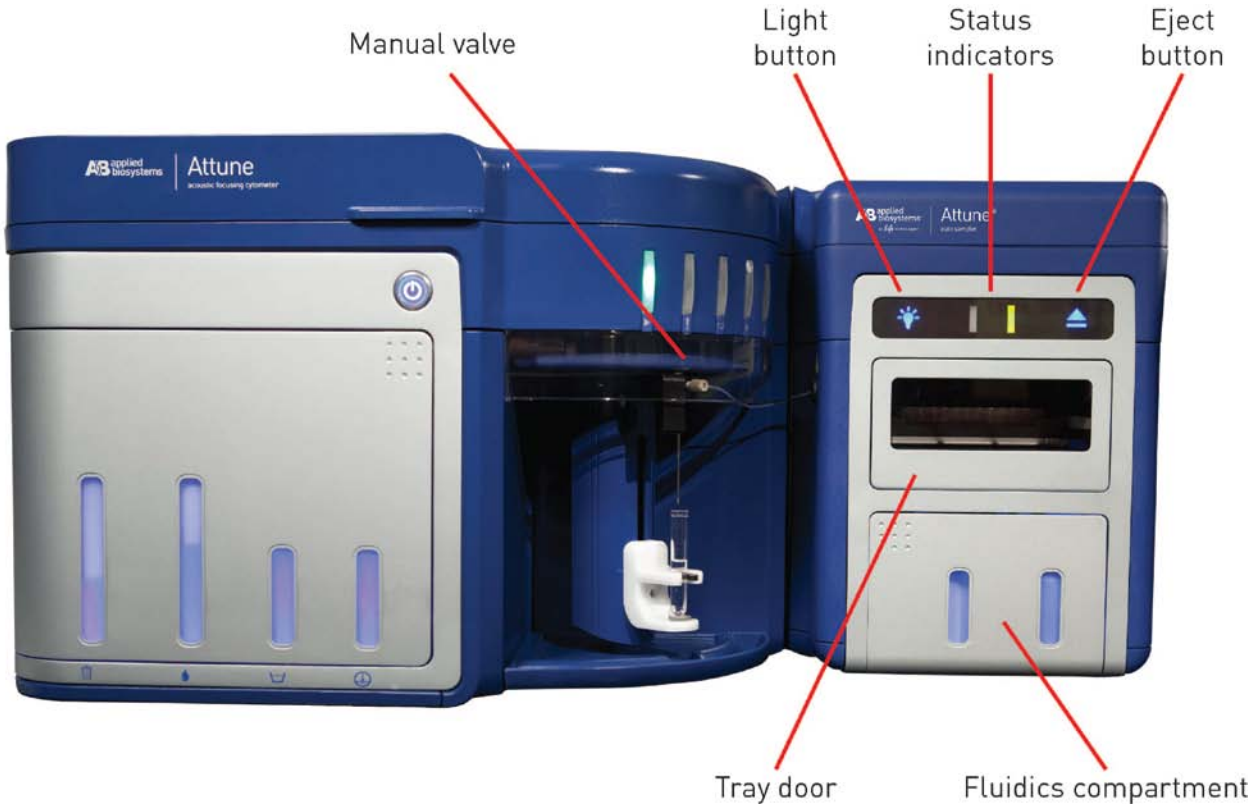
Minimum Sample Volume: Does not exceed 50 µL for 96-well plates

Minimum Dead Volume: Does not exceed 30 µL

Computer and Software

- Windows® 7 64-bit operating system
- Attune® Cytometric Software Version 2.1 or higher required

Instrument Exterior Components



Operation Principles and Technical Overview

The Attune® Auto Sampler is a sample loading device for use with the Attune® Acoustic Focusing Cytometer. The Attune® Cytometric Software controls the auto sampler and facilitates the acquisition of samples from multi-well plates, subsequent analysis, and batch processing for a high-throughput capability.

Instrument Description

The Attune® Auto Sampler is a detachable instrument accessory that allows for the quick and easy processing of 96- and 384-well microtiter plates (both standard and deep well depth) with the Attune® Acoustic Focusing Cytometer. The Attune® Auto Sampler, which comes with easy-to-use software for high-throughput environments, includes its own on-board fluidics that can run four 96-well plates or one 384-well plate without requiring fluid replacement, and can process a 96-well microtiter plate in less than 60 minutes in the standard mode with less than 1% carryover.

The Attune® Auto Sampler is intended to operate in conjunction with the Attune® Acoustic Focusing Cytometer. Both the cytometer and the auto sampler connect to a PC via USB cables and are operated by the Attune® Cytometric Software installed on the connected PC. The software provides the user interface to control the instrument, and to collect, analyze, and save data. The software is also capable of analysis only (without instrument and sampler connections) on any PC that meets the minimum system requirements for post-acquisition data analysis.

Key Features

- Acquires samples from 96-well and 384-well plates
- Includes two modes of operation (High-throughput and Standard)
- Allows the customization of the plate assay parameter, including mixing, sample aspiration, and sensitivity mode
- Minimizes well-to-well carry over
- Supports multiple experiments on a single plate
- Allows easy switching between tubes and plates by toggling a valve
- Features Heat Map View Analysis
- Contains on-board fluidics

Modes

The Attune® Auto Sampler can process a 96-well plate in approximately 60 minutes in standard mode using 2 wash cycles and 45 minutes in high-throughput mode using pre-defined settings. The table below provides a comparison of the two modes.

	High-throughput	Standard
Sample Volume	40 µL	50 µL-well volume
Number of Mixes	0–1	0–60
Number of Rinses	0–1	0–30
Rinse volume	200 µL	200 µL

Volumes

There are many different volumes that need to be considered when using the auto sampler.

Well Volume: Total volume a well can hold when completely full

Draw Volume: Volume drawn from the well that is necessary to provide the user-defined acquisition volume

Dead Volume: Volume aspirated to fill the fluidics lines up to the analysis point

Total Sample Volume: Total sample volume in each well necessary for efficient mixing of the sample

Minimum Volume: 40 µL for high-throughput mode and 50 µL for standard mode

Available Acquisition Volume: Well volume minus dead volume

Mixing

Mixing of the sample is done by sample aspiration and dispensing. Mixing effectiveness depends on the amount of sample aspirated and the viscosity of the sample. The number of mixing cycles can be defined by the user (60 cycles maximum). The system determines the optimal volume of sample to mix based on the total sample volume and plate type selected. The number of mixes defined or the amount of sample mixed will affect the time to process the plates. In the high-throughput mode, mixing has been optimized to enable maximal sample throughput.

Note: Mixing efficiency can vary depending upon the type of plate used. We strongly recommend using round bottom plates for any assay in which homogeneous sampling and consistency of concentration is essential.

Carry Over

The number of rinses between samples can be defined to help minimize carryover. In general, large number of wash cycles between samples results in the less carryover.

Appendix C: Attune® Cytometric Software

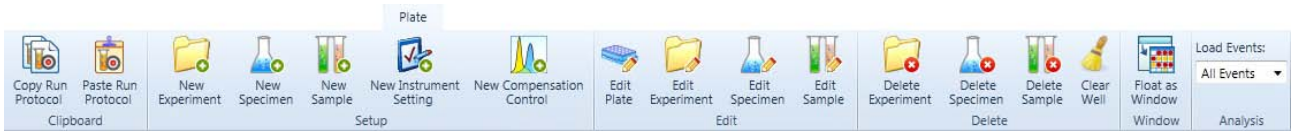
Attune® Auto Sampler Functions

The functions of the Attune® Acoustic Focusing Cytometer and the Attune® Auto Sampler are controlled by Attune® Cytometric Software. This appendix describes the unique features of the Attune® Cytometric Software as it pertains to the Attune® Auto Sampler.

Note: For detailed information about the Attune® Cytometric Software, refer to the Attune® Acoustic Focusing Cytometer User Guide, which is available for downloading at **www.lifetechnologies.com**.

Plate Ribbon

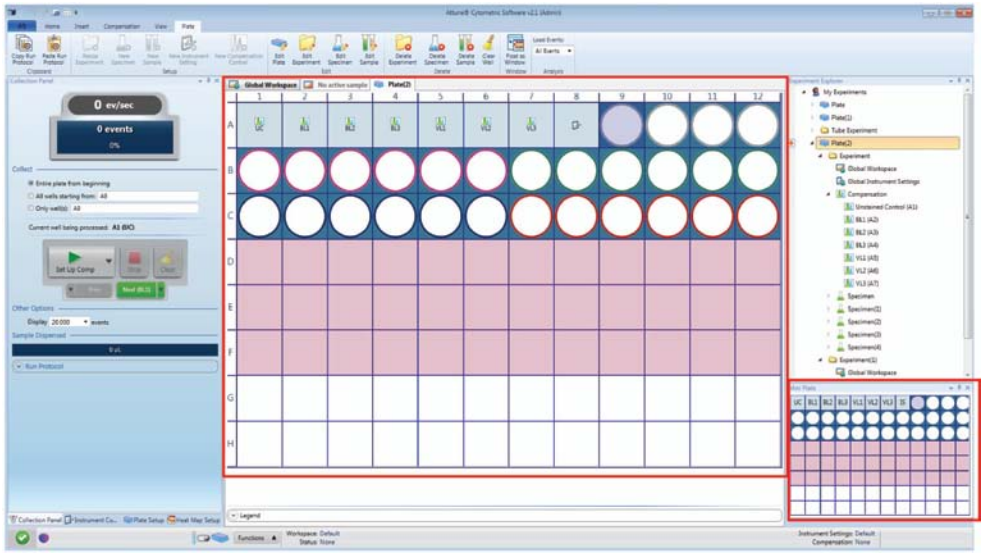
The *Plate Ribbon* facilitates the setup and editing of plate-based experiments. It is organized into six sections: *Clipboard*, *Setup*, *Edit*, *Delete*, *Window*, and *Analysis*.



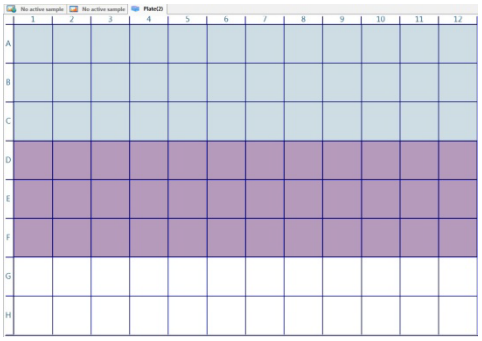
- *Clipboard* allows you to copy and paste Run Protocols.
- *Setup* allows you to create new Experiments, Specimens, Samples, Instrument Settings, and Compensation Controls.
- *Edit* allows you to add or update Plate, Experiment, Specimen, or Sample information.
- *Delete* allows you to delete entire Experiment(s), Specimen(s), and individual Sample(s) with or without their associated files, as well as clearing wells (i.e., removing it from the Experiment)
- *Window* allows you to display the virtual plate layout as a floating window or a tab docked on the Attune® Desktop.
- *Analysis* allows you to select the number of events to be analyzed.

Plate View

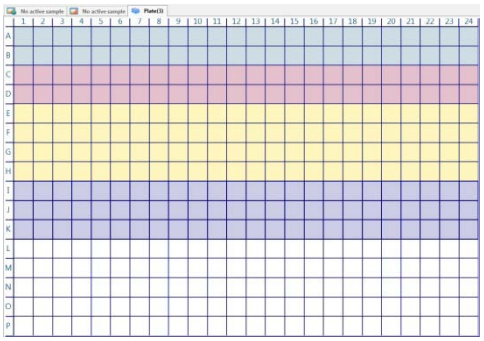
The *Plate view* displays a virtual layout of the sample plate, and it is used to define the well order for compensation control wells, instrument setting well(s), and sample well(s). The plate view is available when a plate-based experiment is open, and it is displayed in the *Plate tab* on the *Attune® Desktop*. A preview of the virtual plate layout is also available as the *Mini Plate* below the *Experiment Explorer.*



Once selected, the plate layout view shows a preview of the plate type selected. Each experiment is displayed in a different color on the plate layout.



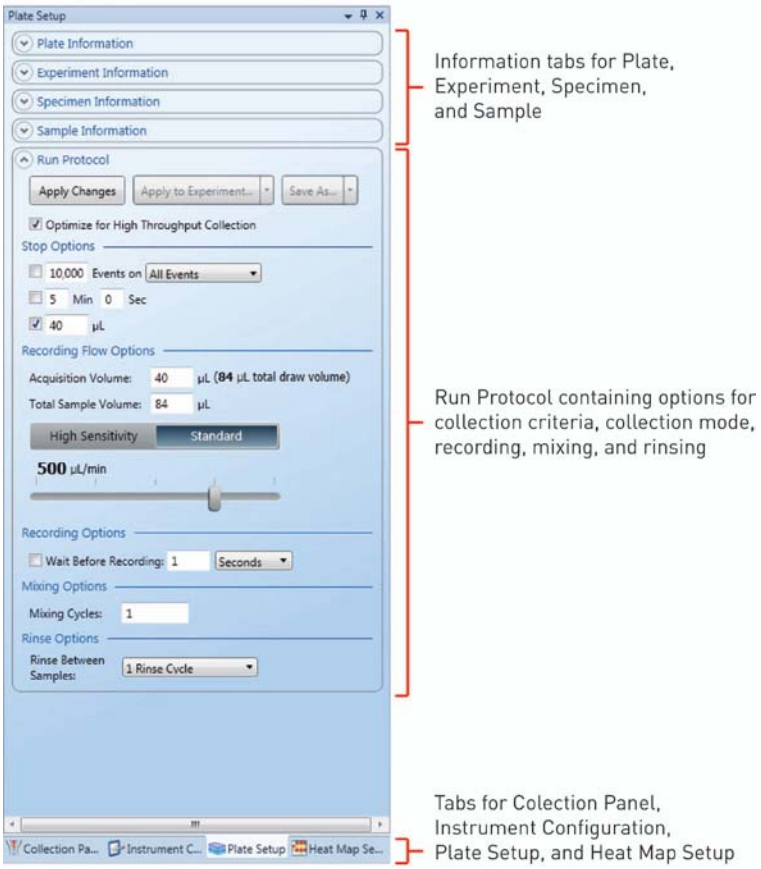
96-well plate



384-well plate

Plate Setup Tab

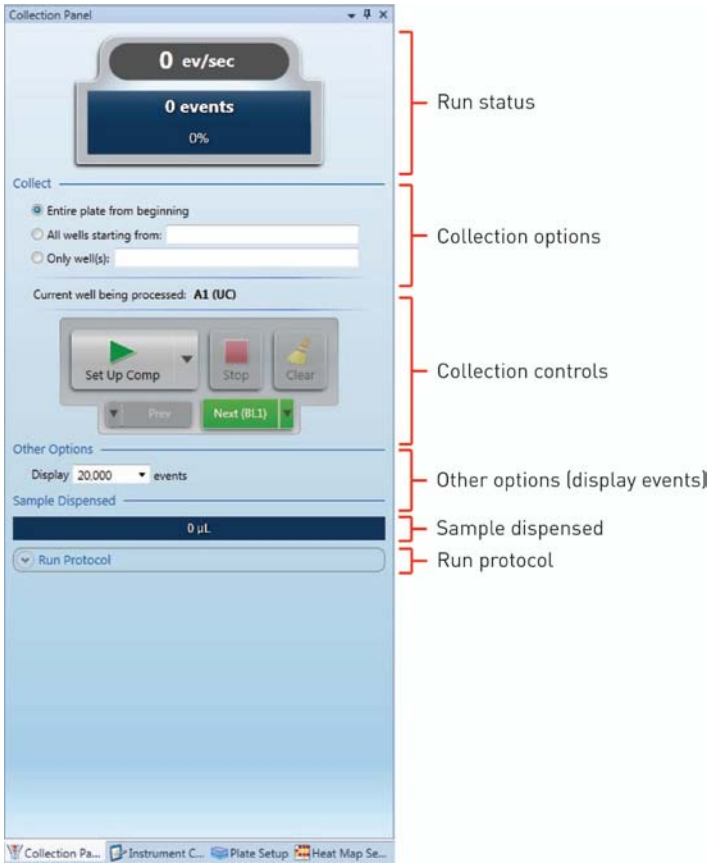
The *Plate Setup* panel consists of five collapsible sections. The first four of these sections (*Plate Information, Experiment Information, Specimen Information*, and *Sample Information*) are used for adding or updating Plate, Experiment, Specimen, or Sample information, while the *Run Protocol* section is used for defining the collection options.



- *Plate Information* allows you to input and edit plate name, plate ID, a short description, and notes, and to select the *Plate Type* from a drop-down menu.
- *Experiment Information, Specimen Information*, and *Sample Information* allow you to enter and edit name, a short description, and notes for the selected Experiment, Specimen, or Sample.
- *Run Protocol* allows you to define the collection criteria, collection mode, acquisition volume, and recording, mixing, and rinse options.

Collection Panel Tab

The *Collection Panel* tab contains the virtual plate layout, run status, collection options, collection controls, and the collapsible *Run Protocol*. It allows you to define collection criteria and well collection order, initiate data collection, and monitor the run progress during sample collection.



- *Run status* displays the cumulative count and event rate of all events that register above the set threshold, and the progress bar provides an ongoing update of completion as defined in the Run Protocol.
- *Collection options* allows you choose between collecting data from the entire plate, from part of the plate starting from a specific well, or from only selected well(s). During a run, the well from which the sample is drawn is also indicated here.
- *Collection controls* allow you to prompt the Attune® Acoustic Focusing Cytometer to run your samples and record flow cytometric data using eight basic commands: *Run*, *Record*, *Pause*, *Stop*, *Clear*, *Save*, *Previous Sample*, and *Next Sample.*
- *Run Protocol* allows you to view the collection criteria, collection mode, acquisition volume, and recording, mixing, and rinse options.

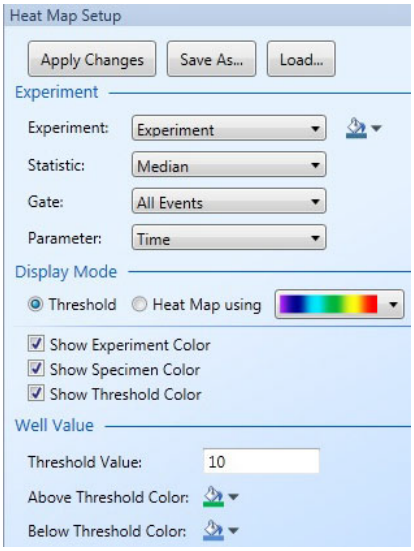
Heat Map Setup Tab

The *Heat Map Setup* tab allows you to select between the *Threshold* and *Heat Map* display modes to visualize the data at a glance after a plate is collected.

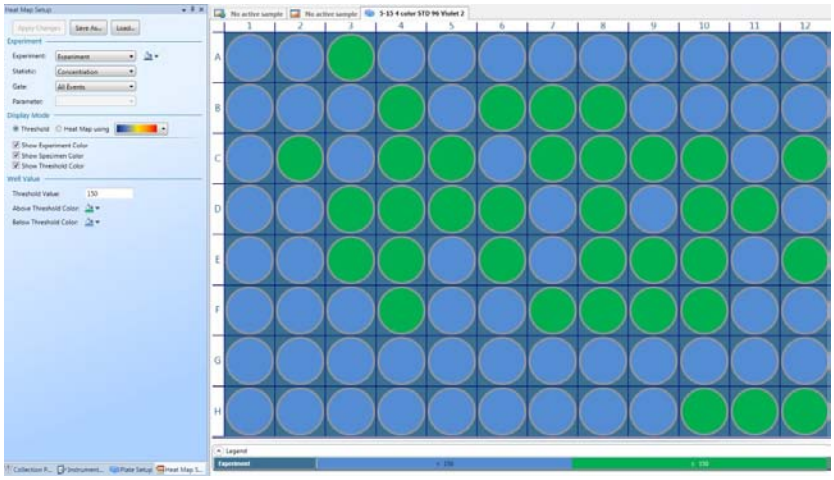
- *Threshold* mode displays each well in one of two user-defined colors based on whether the signal from the well falls above or below the set value.
- *Heat Map* mode allows you to define up to ten (10) transition values (i.e., boundaries) and assign a unique, user-defined color to each range.

Note: Threshold display mode is a visual aid to determine at a glance whether the signal from a well falls above or below a set value; it does not affect data collection. As such, it is different from the *Threshold settings*, which sets the minimum signal level for each detector to eliminate unwanted events and reduce noise. The threshold value set in Threshold settings instructs the software to record and analyze only the events with parameter values above the set threshold. For more information on Threshold settings, refer to the Attune® Acoustic Focusing Cytometer User Guide, which is available for downloading at **www.lifetechnologies.com**.

Threshold Display



1. Select **Heat Map Setup** tab on the Attune® Desktop.
2. Select from the drop-down menus under *Experiment*:
   - **Experiment** and **Fill Color**
   - **Statistic** (Event Count, Median, Mean, Event Percent Parent, Event Percent Total, Standard Deviation, Coefficient of Variation, Concentration)
   - **Population** (All Events or specific gated populations)
   - **Parameter**
3. Under *Display Mode*, select **Threshold**.
4. Under *Well Value*, enter **Threshold Value** and select **Above** and **Below Threshold Colors**.
5. Click **Apply Changes**. Each well in the plate view will display the appropriate color depending on whether the signal from the well falls above or below the set threshold value.

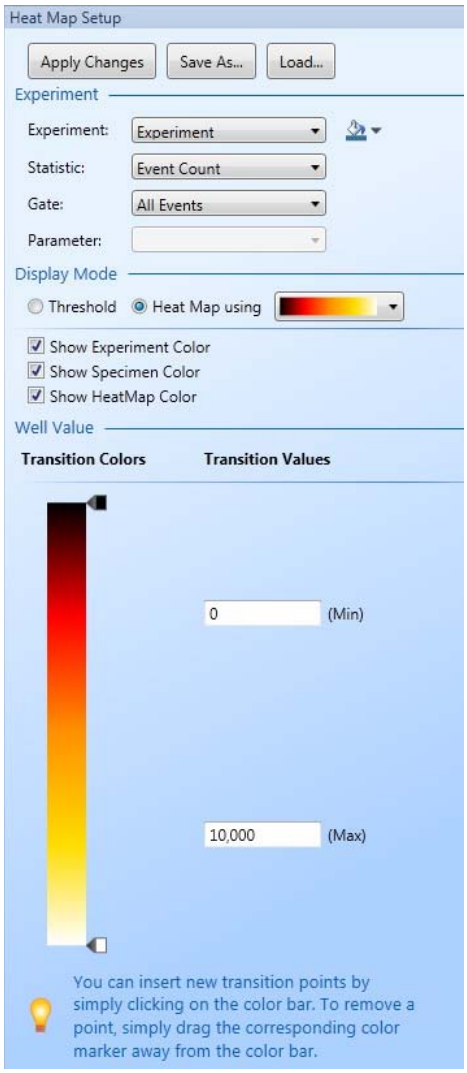


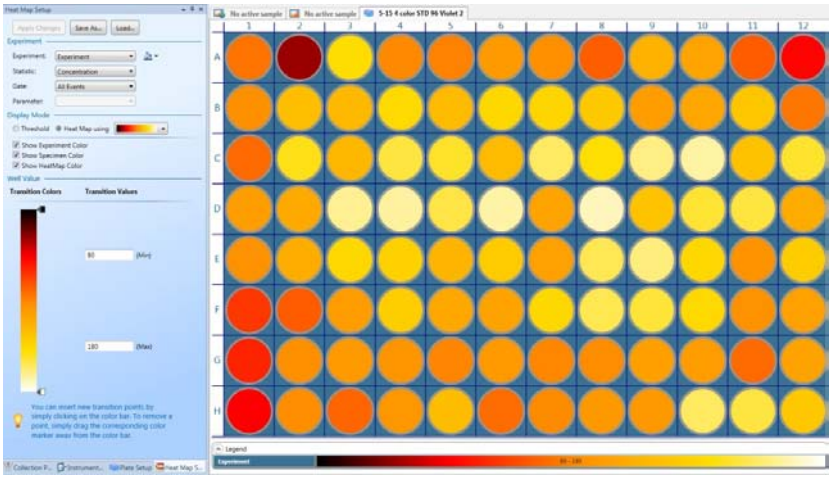
Heat Map Display

1. Select **Heat Map Setup** tab on the Attune® Desktop.
2. Select from the drop-down menus under *Experiment*:
   - **Experiment** and **Fill Color**
   - **Statistic** (Event Count, Median, Mean, Event Percent Parent, Event Percent Total, Standard Deviation, Coefficient of Variation, Concentration)
   - **Population** (All Events or specific gated populations)
   - **Parameter**
3. Under *Display Mode*, click **Heat Map using** and select the transition colors for the heat map from the drop-down menu.
4. Under *Well Value*, enter **Transition Values** and select **Transition Colors** using the color marker on the color bar.

   To insert a new transition point, click on the color bar and enter the transition value.

   To remove a transition point, drag the corresponding color marker away from the color bar.
5. Click **Apply Changes**. Each well in the plate view will display the appropriate color depending on whether the signal from the well falls within the set boundaries.





Appendix D: Troubleshooting

This section includes the following topics:

- Tips to help you troubleshoot your experiment
- Technical Assistance Information

Note: For Attune® Cytometric Software Troubleshooting, refer to the Attune® Cytometric Software Release Notes or contact Technical Support.

Attune® Auto Sampler Troubleshooting

Observation	Possible Causes	Recommended Solutions
No sample being analyzed	No power to instrument.	Attached the power plug and switch on the instrument.
	Fluidics are not connected	Connect fluidic connectors to Attune System.
	Fluidics are leaking	Check for leaks at the connectors at the Attune System.
	No plate in Auto Sampler	Place plate in instrument.
	Clog in Sample line.	Unclog sample lines from instrument.
	Focusing fluid tank is empty	• Fill the focusing fluid tank. • Ensure that the fill lines and fluid level detectors are plugged in completely.
	Instrument is powered off	Turn on the instrument.
	USB cable not connected	Ensure that the USB cable is plugged in to the instrument and the computer.
	Sample plate is not selected	Select the sample plate.
Red light blinks	Error occurred in system.	• Power the instrument off and on. • Perform plat calibration.
After initial power on, the tray is ejected with the well plate	Well plate is present during power on of the instrument	Ensure no well plate is present in the tray during power on cycle.
Computer is not communicating with the instrument	USB cable not fully plugged in	Examine the USB plug in the back of the instrument and the computer.
	Faulty USB cable	Contact Technical Support.
	USB port changed from the original port	Try a different USB port. If the problem persists, reinstall the USB drivers.
Instrument and/or computer has no power	Power supply not plugged into the appropriate outlet	Ensure that the instrument and/or computer are plugged into the appropriate outlet.
	No power at the outlet	Make sure that the outlet is functioning properly and the circuit breaker is not tripped.
	Faulty power supply	Contact Technical Support.
Sample is not aspirating	Loose sample syringe	Check the sample syringe for leaks and tighten if necessary. Be careful not to over tighten.
	Defective sample syringe	Replace sample syringe.
	Pinch in valve tubing at Attune System	Contact Technical Support.
	Defective pinch valve in Attune System	Contact Technical Support.

Observation	Possible Causes	Recommended Solutions
Sample aspirated, then backfilled into sample well	Clog in the sample line	Unclog fluid line.
	Pinch valve error in Attune System	Contact Technical Support.
	Pump error	Contact Technical Support.
Long delay between sample aspiration and events appearing on screen (normally events appear in ~10 seconds)	Sample syringe is leaking	Ensure that the sample syringe is sealed properly.
	Partial clog in the fluidics system	Unclog fluid line.
Sample probe is not centered in the sample well	SIP tube is bent	Contact Technical Support.
	SIP tube is faulty	Contact Technical Support.
Focusing fluid pump does not shut off	Focusing fluid reservoir level sensor is malfunctioning	Turn off the instrument and contact Technical Support.
Rinse fluid pump does not shut off	Rinse (Waste) fluid reservoir level sensor is malfunctioning	Turn off the instrument and contact Technical Support.
Fluid is leaking from the base of the instrument or into the bottle bay drip tray	Crack in fluidics tank	Replace the damaged fluidics tank.
	Snap fitting is broken or dripping	Contact Technical Support.
	1 mL syringe seal is broken	Replace sample syringe.

Appendix E: Limited Product Warranty

Limited warranty. Life Technologies warrants that all standard components of its Attune® Acoustic Focusing Cytometer will be free of defects in materials and workmanship for a period of one (1) year after the date of installation, and the software will be free of substantial programming errors or defects when properly installed. However, this warranty will not last longer than 15 months from the date of shipment. Life Technologies also warrants that the instrument (including software) will perform in accordance with its published specifications when delivered. Life Technologies will repair or replace, at its discretion, all defective components during this warranty period. Life Technologies reserves the right to use new, repaired, or refurbished instruments or components for warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period. Unless a different written warranty is included with product literature, Life Technologies warrants that each consumable supplied with the instrument will meet its specifications stated in its published catalogs and any associated supplementary terms relating to the product. This warranty lasts from the delivery of the consumable until either the consumable's expiry or "use by" date, or, if no expiry or "use by" date is specified, for 12 months from the delivery of the consumable, provided, however, that the warranty will not last for more than thirty (30) days after Customer opens consumable's original container. Life Technologies does not warrant that the operation of the instrument or its operating software will be uninterrupted or error free. Warranty claims must be made within the applicable warranty period.

Warranty exceptions. The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the specifications or not in conformance with the instructions; use in combination with software or products not supplied or authorized by Life Technologies; modification or repair of the product not authorized by Life Technologies, relocation or movement of the instrument by Customer or any third party not acting on behalf of Life Technologies; or intrusive activity, including without limitation computer viruses, hackers or other unauthorized interactions with instrument or software that detrimentally affects normal operations. Without limiting the above mentioned, computer hardware, monitors, accessories, software or other products not purchased from or supplied by Life Technologies ("Non-LIFE Product") are not covered under the foregoing warranty even if such Non-LIFE Product is integral to functional use of a Life Technologies product.

Warranty limitations. THE WARRANTIES IDENTIFIED ABOVE ARE LIFE TECHNOLOGIES' SOLE AND EXCLUSIVE WARRANTIES WITH RESPECT TO SUCH PRODUCTS AND ARE IN LIEU OF ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, ALL OF WHICH OTHER WARRANTIES ARE EXPRESSLY DISCLAIMED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, OR REGARDING RESULTS OBTAINED THROUGH THE USE OF ANY PRODUCT (INCLUDING, WITHOUT LIMITATION, ANY CLAIM OF INACCURATE OR INCOMPLETE RESULTS), WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF DEALING OR USAGE OF TRADE. WITHOUT LIMITING THE FOREGOING, IN NO EVENT SHALL LIFE TECHNOLOGIES BE LIABLE FOR CONSEQUENTIAL, INDIRECT, PUNITIVE, INCIDENTAL, OR OTHER SPECIAL DAMAGES SUSTAINED BY THE BUYER OR ANY OTHER PERSON OR ENTITY, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT LIFE TECHNOLOGIES IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, INCLUDING WITHOUT LIMITATION, DAMAGES ARISING FROM OR RELATED TO LOSS OF USE, LOSS OF DATA, OR FOR LOSS OF REVENUE OR OTHER FINANCIAL LOSS. NO EMPLOYEE OR REPRESENTATIVE OF LIFE TECHNOLOGIES HAS ANY AUTHORITY TO MODIFY THE TERMS OF THIS LIMITED WARRANTY STATEMENT AND ANY SUCH MODIFICATION MADE BY ANY EMPLOYEE OR REPRESENTATIVE OF LIFE TECHNOLOGIES WILL NOT BE BINDING ON LIFE TECHNOLOGIES, UNLESS IN A WRITING SIGNED BY AN EXECUTIVE OFFICER OF LIFE TECHNOLOGIES. THIS WARRANTY IS LIMITED TO THE BUYER OF THE PRODUCT FROM LIFE TECHNOLOGIES AND IS NOT TRANSFERABLE. THE FOREGOING LIMITATIONS OR EXCLUSIONS OF WARRANTIES, LIABILITY, REMEDIES, OR DAMAGES SET FORTH ABOVE SHALL NOT APPLY TO THE EXTENT PROHIBITED BY LAW.

Appendix F: Safety

This section includes the following topics:

- Safety conventions used in this document
- Symbols on instruments
- Safety labels on instruments
- General instrument safety
- Chemical safety
- Chemical waste safety
- Electrical safety
- Physical hazard safety
- Biological hazard safety
- Laser safety
- Workstation safety
- Safety and electromagnetic compatibility (EMC) standards
- SDSs

Safety Conventions Used in this Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems® user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action:

Definitions

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for **IMPORTANT**! safety alerts, each safety alert word in an Applied Biosystems® document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard icons that are affixed to Applied Biosystems® instruments (see "Safety Symbols" on page 63).

Symbols on Instruments

Electrical Symbols on Instruments

The following table describes the electrical symbols that may be displayed on Life Technologies instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following table describes the safety symbols that may be displayed on Life Technologies instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "Safety Labels on Instruments" on page 64). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Indicates the presence of an ultraviolet light and to proceed with appropriate caution.

Environmental Symbols on Instruments

The following symbol applies to all Life Technologies electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your Customer Service representative for equipment pick-up and recycling. See **www.appliedbiosystems.com** for a list of customer service offices in the European Union.

Safety Labels on Instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Life Technologies instruments in combination with the safety symbols described in the preceding section.

Hazard Symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT!** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Applied Biosystems.
	**DANGER!** Class 3B visible and/or invisible laser radiation present when open. Avoid exposure to beam.	**DANGER!** Rayonnement visible ou invisible d'un faisceau laser de Classe 3B en cas d'ouverture. Evitez toute exposition au faisceau.
	**CAUTION!** Moving parts. Crush/pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

General Instrument Safety

WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Life Technologies may result in personal injury or damage to the instrument.

Moving and Lifting the Instrument

CAUTION! PHYSICAL INJURY HAZARD The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and Lifting Stand-alone Computers and Monitors

WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See "SDSs" on page 72.

Cleaning or Decontaminating the Instrument

CAUTION! Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.

Chemical Safety

Chemical Hazard Warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

General Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “SDSs,” page 72)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

Chemical Waste Hazard

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposal.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical Safety

DANGER! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the Attune® Acoustic Focusing Cytometer without its instrument panels in place. Do not remove instrument panels.
High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

DANGER! ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

DANGER! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

DANGER! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage Rating

The Applied Biosystems® Attune® Acoustic Focusing Cytometer has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical Hazard Safety

Moving Parts

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Biological Hazard Safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories*

  (stock no. 017-040-00547-4; **www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm**)
- Occupational Safety and Health Standards, Bloodborne Pathogens

  (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at:

  www.cdc.gov

In the EU:

- Check your local guidelines and legislation on biohazard and biosafety precaution, and the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition

  www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Laser Safety

Laser Classification

The Attune® Acoustic Focusing Cytometer blue/violet configuration uses a 488nm, 20mW laser and a 405nm, 50mW laser, and the blue/red configuration uses a 488nm, 20mW laser and a 638nm, 50mW laser. Under normal operating conditions, the Attune® Acoustic Focusing Cytometer is categorized as a Class I laser product. When safety interlocks are disabled during certain servicing procedures and/or input/output optics covers are removed, the laser can cause permanent eye damage, and, therefore, is classified under those conditions as a Class 3B laser.

Laser Safety Requirements

To ensure safe laser operation:

- The system must be installed and maintained by an Applied Biosystems® Technical Representative.
- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating, you may be exposed to laser emissions in excess of the Class 3B rating.
- Do not remove safety labels.

Additional Laser Safety Information

Refer to the user documentation provided with the laser for additional information on government and industry safety regulations.

WARNING! LASER HAZARD. Lasers can burn the retina, causing permanent blind spots. Never look directly into the laser beam. Remove jewelry and other items that can reflect the beam into your eyes. Do not remove the instrument top or front panels. Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the top or front panels are removed for service.

WARNING! LASER HAZARD. An overheated laser can cause severe burns if it comes in contact with the skin. DO NOT operate the laser when it cannot be cooled by its cooling fan. Always wear appropriate laser safety goggles.

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

- U.S. and Canadian safety standards
- Canadian EMC standard
- European safety and EMC standards
- Australian EMC standards

U.S. and Canadian Safety Standards

The Attune® Acoustic Focusing Cytometer has been tested to and complies with standard:

UL 61010-1/CSA C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

FDA "Radiation Control for Health and Safety Act of 1968 Performance Standard 21 CFR 1040.10 and 1040.11," as applicable.

Canadian EMC Standard

This instrument has been tested to and complies with ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."

European Safety and EMC Standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards EN 61010-1:2006, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

EN 60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User Guide."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

SDSs

SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to *new* customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely. Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

You can obtain from Life Technologies the SDS for any chemical supplied by Life Technologies. This service is free and available 24 hours a day.

To obtain SDSs:

1. Go to **www.lifetechnologies.com**, click **Support**, and then select **SDS.**
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, and then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
    - **Open** – To view the document
    - **Print Target** – To print the document
    - **Save Target As** – To download a PDF version of the document to a destination that you choose

IMPORTANT! For the SDSs of chemicals not distributed by Life Technologies contact the chemical manufacturer.

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