invitrogen

CytKick™ Autosampler
CytKick™ MAX Autosampler
USER GUIDE

Catalog Numbers A42901, A42973

Publication Number MAN0018351

Revision A.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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Revision history MAN0018351

Revision	Date	Description
A.0	18 July 2019	New user guide

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Contents

About this guide

This user guide is for laboratory staff operating, maintaining, and analyzing data using the Attune™ NxT Acoustic Focusing Cytometer equipped with the CytKick™ Autosampler or the CytKick™ MAX Autosampler sample loading device.

IMPORTANT! For a detailed description of the Attune™ NxT Software, refer to the *Attune™ NxT Software User Guide* (Pub. No. 100024236).

For workflows and basic instructions on using the Attune™ NxT Acoustic Focusing Cytometer, refer to the *Attune™ NxT Acoustic Focusing Cytometer Quick Reference Guide* (Pub. No. 100024233) and the *Attune™ NxT Acoustic Focusing Cytometer User Guide* (Pub. No. 100024235).

CAUTION! Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Conventions

Text and keyboard conventions

Text and keyboard conventions used in this user guide are listed below. For safety alert words and symbols used in the user guide, see page 6.

Convention	Use
Italics	*Italic* text highlights new or important terms on their first appearance in the user guide. It is also used for emphasis and for user guide or reference titles. For example: *Experiment Explorer* lists *Experiments* in a hierarchal view and functions as an interface for creating new Experiments and recording data.
Bold	**Bold** text indicates user action. For example: Click **Run**.
▶	Right arrow symbol (▶) indicates a menu choice, and separates successive commands you select from a drop-down or shortcut menu. For example: Select **Show Events**▶**All Events**.
Ctrl+X	When used with key names, a plus sign means to press two keys simultaneously. For example: Click **Ctrl+P**.

Clicking

Unless explicitly stated, clicks are left mouse button clicks. If you have transposed the mouse buttons, the primary click is considered to be the left click, even though it may be physically swapped.

User attention symbols

User attention symbols used in this user guide are listed below. For safety alert words and symbols used in the user guide, see page 6.

Symbol	Use
	**Note:** Describes important features or instructions, and highlights tips that can save time and prevent difficulties.
	**IMPORTANT!** Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

Safety information

Note: See "**Appendix E: Safety**" for the complete the chemical or instrument safety information.

Safety alert words

Four safety alert words appear at points in this 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 this 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 the instruments (see "**Symbols on instruments**").

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Thermo Fisher Scientific are available to you free 24 hours a day. For instructions on obtaining SDSs, see "**Safety Data Sheets (SDS)**".

IMPORTANT! For the SDSs of chemicals not distributed by Thermo Fisher Scientific contact the chemical manufacturer.

1. Product information

System components

The CytKick™ Autosampler (Cat. No. A42901) and CytKick™ MAX Autosampler (Cat. No. A42973) are shipped with the system components listed below. All components are shipped at ambient temperature.

Component	Quantity
CytKick™ Autosampler Model AAS2 or CytKick™ MAX Autosampler Model AAS2	1
CytKick™ Autosampler Starter Kit	1

When you receive the instrument

Examine the instrument carefully for damage incurred during transit. Ensure that all parts of the instrument, including accessories listed above, are included with the product. Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

Register your instrument

Go to **thermofisher.com** to register your instrument. You will be asked to supply the serial number, your name, and your contact details. Registering your instrument ensures that you will receive notifications of software upgrades and information on new assays for use with the Attune™ NxT Acoustic Focusing Cytometer and the CytKick™ Autosampler.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Instrument exterior components

1. Sample tray door
2. Syringe pump compartment
3. Light button
4. Status indicators
5. Eject button
6. Waste bottle
7. Focusing Fluid bottle

1. Fluidics connections (from Attune™ NxT Cytometer)
2. Sample tray door (open)

The image below shows the CytKick™ Autosampler connected to the Attune™ NxT Cytometer.

1. Attune™ NxT Cytometer
2. Fluidics connections to Attune™ NxT Cytometer (from CytKick™ Autosampler)
3. Fluidics connections to CytKick™ Autosampler (from Attune™ NxT Cytometer)
4. CytKick™ Autosampler
5. Side Car bottle module with Waste and Focusing Fluid bottles

Operation principles and technical overview

The CytKick™ and CytKick™ MAX Autosamplers are sample loading devices for use with the Attune™ NxT Acoustic Focusing Cytometer. The Attune™ NxT Software controls the CytKick™ Autosampler or the CytKick™ MAX Autosampler and facilitates the acquisition of samples from multi-well plates, subsequent analysis, and batch processing for a high-throughput capability.

Instrument description

The CytKick™ and CytKick™ MAX Autosamplers are detachable instrument accessories that allow for the quick and easy processing of 96- and 384-well microtiter plates (both standard and deep well depth) with the Attune™ NxT Acoustic Focusing Cytometer.

The CytKick™ and CytKick™ MAX Autosamplers, which come with easy-to-use software for high-throughput environments, include their own on-board fluidics that can run thirty 96-well plates in the High-Throughput mode without requiring fluid replacement.

The CytKick™ and CytKick™ MAX Autosamplers are intended to operate in conjunction with the Attune™ NxT Acoustic Focusing Cytometer. Both the cytometer and the autosampler are operated by the Attune™ NxT Software installed on the connected computer. The software provides the user interface to control the instrument, and to collect, analyze, and save data. The software is capable of analysis only (without instrument and autosampler connections) on any computer that meets the minimum system requirements for post-acquisition data analysis. A software license found on a USB dongle is required to use the software on any computer.

Key features

- Acquires samples from an assortment of formats, including 96-well and 384-well plates
- Includes two modes of operation (High-Throughput using Boost mode, and standard High-Throughput mode). The High-Throughput using Boost mode is only available on the CytKick™ MAX Autosampler (Cat. No. A42973).
- Allows the customization of the plate assay parameter, including mixing, sample aspiration, and sensitivity mode
- Minimizes well-to-well carry over
- Allows easy switching between tubes and plates using software alone (no manual adjustments required)
- Features Heat Map View Analysis
- Contains on-board fluidics

Sample processing modes

The CytKick™ MAX Autosampler allows you to optimize your run protocol so that a 96-well plate can be acquired in <22 minutes in the High-Throughput using Boost Mode (page 25). Using standard plate definitions, the CytKick™ and CytKick™ MAX Autosamplers take approximately 45 minutes to acquire a 96-well plate. The following table provides a comparison of the two modes.

	High-Throughput using Boost Mode*	Standard mode
Sample volume	20 µL	40 µL
Sample rate	1000 µL/minute	500 µL/minute

*Available only on the CytKick™ MAX Autosampler (Cat. No. A42973).

Volumes

There are various volumes that need to be considered when using the CytKick™ and the CytKick™ MAX Autosamplers.

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. Dead volume is 30 µL for flow rates of up to 200 µL/minute and 50 µL for flow rates of 500–1000 µL/minute.

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

Minimum Volume: 20 µL.

Available Acquisition Volume: Well volume minus dead volume.

Mixing

Sample mixing is done by a combination of sample aspiration/dispensing and "stirring" based on the inputted sample volume, plate type, and well geometry. 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 (10 cycles maximum). The system determines the optimal sample volume to mix based on the total sample volume and plate type selected. The number of mixes defined or the amount of sample mixed affects 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, increasing the number of rinse cycles between samples results in less carryover.

2. Run samples

Workflow

Workflow overview

The workflows for running samples with or without the CytKick™ or CytKick™ MAX Autosampler follow the same general outline. Both workflows require you to create an Experiment, set up a Workspace, define a Run protocol, optimize your Experiment, and calculate Compensation. These procedures are described in detail in the *Attune™ NxT Acoustic Focusing Cytometer User Guide* (Pub. No. 100024235) and the *Attune™ NxT Software User Guide* (Pub. No. 100024236), which are available for download at **thermofisher.com**.

This section only describes the procedures and software functions that are unique to the CytKick™ and CytKick™ MAX Autosamplers.

Before you begin	(page 13)
Startup	(page 14)
Create an Experiment	(see **Workflow overview** above)
Set up a Workspace	
Define Run Protocol	
Optimize the Experiment	
Calculate Compensation	
Run Samples and collect data	
Analyze and process data	
Shutdown	(page 37)

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 NxT Focusing Cytometer, its operating principles, and the software user interface before starting your experiments.

The *Attune™ NxT Acoustic Focusing Cytometer User Guide* (Pub. No. 100024235) is available for download at **thermofisher.com**.

Before you begin

Required solutions

- **Attune™ NxT 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 1X 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.
- **10% bleach solution in deionized water** – decontaminates the fluidics lines. Prepare this solution fresh daily and use during the shutdown procedure.
- **Attune™ Debubble Solution –** a solution optimized for removing bubbles from the Attune™ NxT system.

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! You can store the reagents at colder temperatures, but running the Attune™ NxT Acoustic Focusing Cytometer with cold reagents (<15°C) affects the data quality. Before you run the instrument, ensure that all fluids have equilibrated to room temperature.

Sample requirements

The Attune™ NxT Acoustic Focusing Cytometer with the CytKick™ Autosampler is designed to handle samples in 96-well or 384-well standard or deep well plates as well as 1.5-mL and 2-mL microcentrifuge tubes. For a complete list of compatible plate types, see "Sample analysis" (page 43).

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 sample depends on the sample type and the assay.
- 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 the sample down before running it on the Attune™ NxT Cytometer.
- The maximum recommended sample concentration for 500 µL/minute and 1,000 µL/minute flow rates is 5×10^5 cells/mL.

IMPORTANT! Although running a full 384-well plate in the standard mode requires only 1.6 L of Attune™ NxT Focusing Fluid, it is necessary to have at least 1.8 L of focusing fluid in the Attune™ NxT Focusing Fluid bottle to ensure that the fluid sensor in the bottle detects the correct liquid level.

Startup

During Startup, the Attune™ NxT 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.)

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.

Before you begin

1. Check the levels in the fluidics containers of the Attune™ NxT Acoustic Focusing Cytometer and the CytKick™ Autosampler.

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

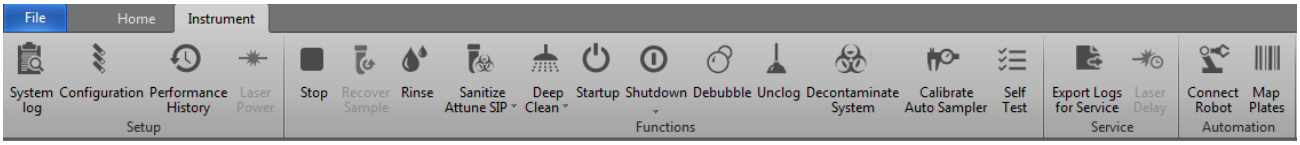
   If full, empty the waste container.

   Note: If you are running a full 384-well plate in the standard mode, make sure that the Attune™ NxT Focusing Fluid bottle contains at least 1.8 L of Attune™ NxT Focusing Fluid.

2. Power on the CytKick™ Autosampler first, the Attune™ NxT Cytometer next, and then the computer.
3. Open the lid to the optical compartment and verify that the optical filters are appropriate for your experiment on the Attune™ NxT Cytometer.
4. Start up the computer and log in to Windows.
5. Launch the Attune™ NxT Software.

Run Startup function

1. Sign in to the Attune™ NxT Software.
2. Click the **Startup** button located in the center of the Instrument tab on the Ribbon bar.



Alternatively, you can click the Startup button from the Collection Panel menu, underneath the progress dial.

The Startup dialog opens and provides instructions to perform the Startup operation.

3. If the tube lifter is raised, lower the tube lifter. If a plate is loaded in the Autosampler, remove the plate.
4. Click **Next**, then follow the instructions on the Startup prompt screen to perform the Startup operation, which takes less than 5 minutes to complete.

   During Startup, the Attune™ NxT Software automatically turns on the lasers and instrument systems, 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, proceed to the daily Performance Test.

Set CytKick™ and CytKick™ MAX Autosampler-specific collection options

This section describes software functions that are available when a CytKick™ or CytKick™ MAX Autosampler is installed with the Attune™ NxT Cytometer. These functions include:

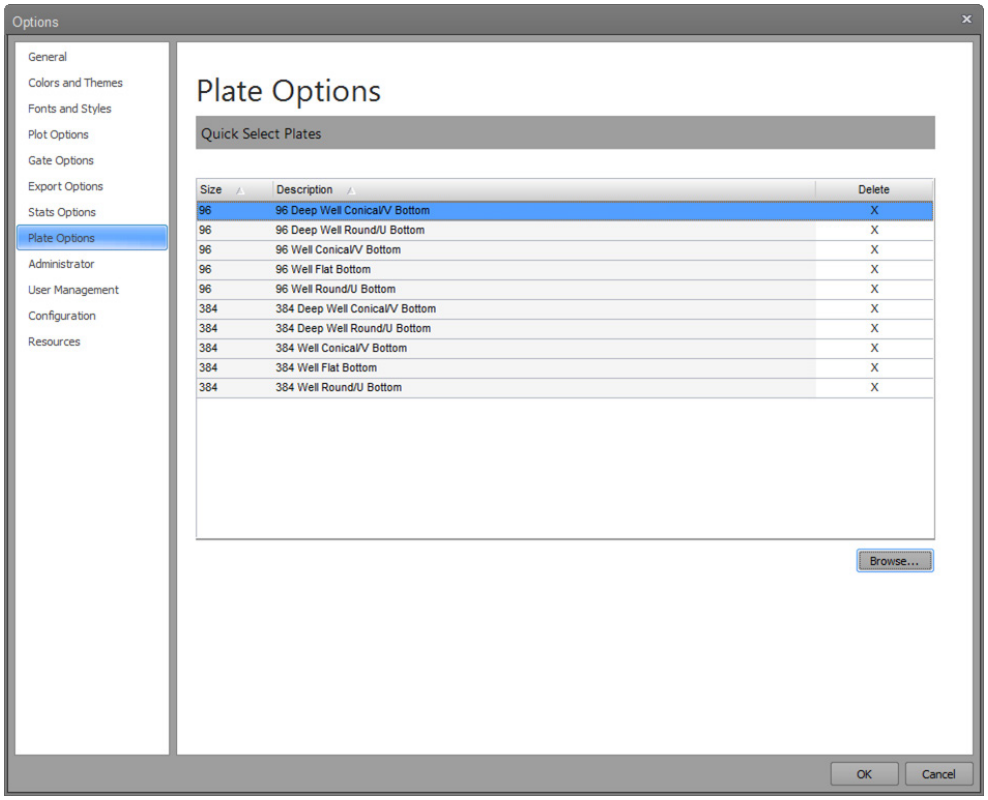
- Plate Options (page 16)
- Select Plates (page 17)
- Create/Edit Plate (page 19)
- Plate Height Sensor (page 20)
- Use Foil Cover (page 21)
- Foil Cover Check (page 22)
- Use Cooling Block (page 23)
- Optimize for High-Throughput Collection (page 24)
- Enable Boost Mode (page 25)
- Mix Mode (page 25)

The procedures and software functions common to all Attune™ NxT Cytometers (with or without an autosampler) are described in detail in the *Attune™ NxT Acoustic Focusing Cytometer User Guide* (Pub. No. 100024235) and the *Attune™ NxT Software User Guide* (Pub. No. 100024236), which are available for download at **thermofisher.com**.

Plate Options

Plate Options tab in the Options dialog contains the *Quick Select Plate*s list, which allows you to designate selected plates as favorites. The selected plates become available in the **Plate Type** dropdown in the **New Experiment** dialog and in the **Customize Plate Experiment** panel.

The Plate Options dialog is available only when a CytKick™ MAX Autosampler is connected to the Attune™ NxT Cytometer.



Note: For detailed information about the New Experiment dialog or the Customize Plate Experiment panel, refer to the *Attune™ NxT Software User Guide* (Pub. No. 100024236), which is available for download at **thermofisher.com**.

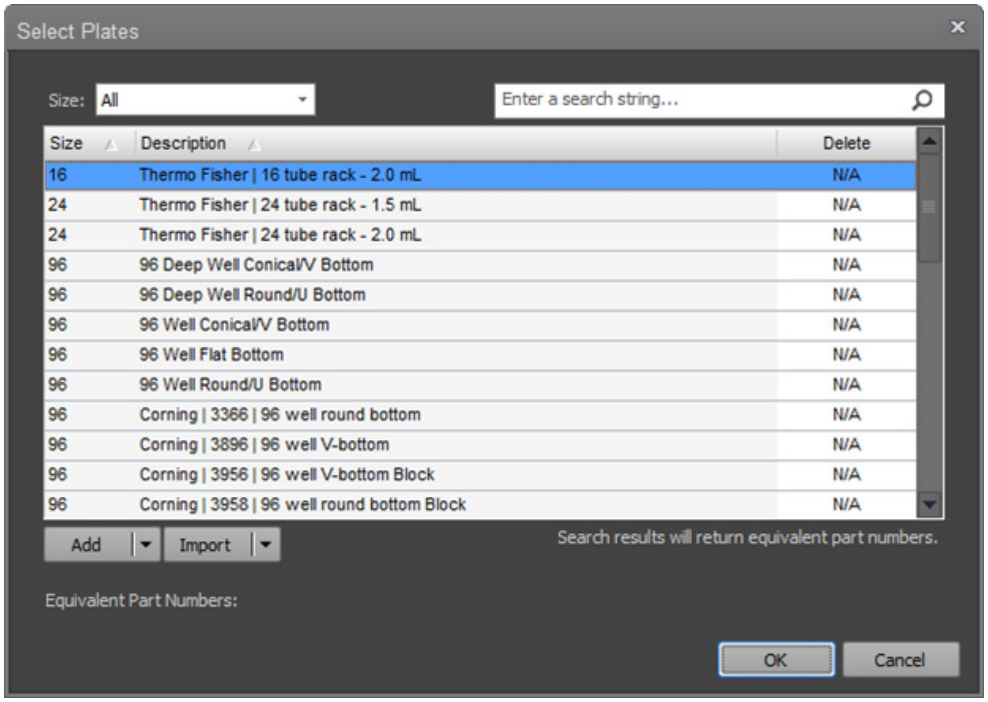
- To access the dialog, click the **Options** button on the **Quick Access** toolbar, then select the **Plate Options** tab. The Options button is enabled only when the instrument is not acquiring.
- By default the Quick Select Plates list includes the 10 defined plates available to all models. However, you can add additional plate definitions to the list using the **Select Plates** dialog (page 17).

  To launch the **Select Plates** dialog, click the **Browse** button.
- To sort the Quick Select Plates list, click on the column header. The default sort order is plate size, followed by description.
- When the mouse moves over the Delete column, the cursor changes to the hand cursor, if the plate can be deleted. To remove a Plate from the list of favorites, click the **X** in the Delete column next to the plate you wish to delete.

Select Plates

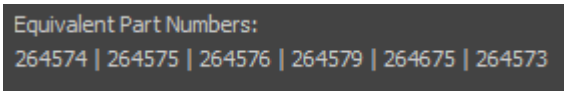
Select Plates dialog displays a list of all available plates in the database. You can add these plates to the **Quick Select Plates** list in the **Options ▶ Plate Options** dialog (page 16) or access them from the **New Experiment** dialog or the **Customize Plate Experiment** panel.

The Select Plates dialog is available only when a CytKick™ MAX Autosampler is connected to the Attune™ NxT Cytometer.



Note: For detailed information about the New Experiment dialog or the Customize Plate Experiment panel, refer to the *Attune™ NxT Software User Guide* (Pub. No. 100024236), which is available for download at **thermofisher.com**.

- The plates list has columns for **Size**, **Description**, and **Delete**. To sort the plates list by Size or Description, click on the Size or Description column header.
- To search the available plates in the database, enter the search query in the **Search** field. You can search available plates based on **Size** or **Description**.
- When you perform a search, **equivalent part numbers** where the plate definitions match are displayed in the lower left of the dialog.



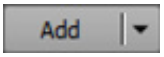
- To filter the available plate list by Size, select the desired option from the **Size** dropdown menu. Available options are **All**, **16**, **24**, **96**, and **384** (well plates).

- The **Delete** column is only visible for Administrator accounts when the dialog is launched from the Quick Select Plates list in the **Options▶Plate Options** dialog (page 16).

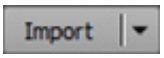
  When the mouse moves over the Delete column, the cursor changes to the hand cursor, if the plate can be deleted. You can only delete user-defined plates.

  System-defined plates display N/A in the Delete column and cannot be deleted.

  If a plate definition is in use as an existing Plate Experiment or Template, deletion is not allowed.
- To remove a Plate from the list of favorites, click the **X** in the Delete column next to the plate you wish to delete.
- **Add/Edit/Duplicate** split button allows you to add, edit, or duplicate a plate definition.

  Only Administrators, Advanced Users, and Service accounts can add, edit, or duplicate plate definitions. For users who do not have this permission, the button displays View and no dropdown menu is provided.
- To add a new plate definition to the plates list, click **Add** to open the **Create Plate** dialog (page 19).
- To edit a plate definition in plates list, select the **Edit** option on the Add/Edit/Duplicate split button to open the **Edit Plate** dialog (page 19).
- To duplicate a plate definition, select the Duplicate option on the Add/Edit/Duplicate split button. The Create Plate dialog opens with all the fields pre-populated to match those fields in the plate definition that was selected. However, the description field will be updated to have (#) appended to the description to create a unique name for the duplicated plate definition.
- **Import/Export** split button allows you to import or export a plate definition.

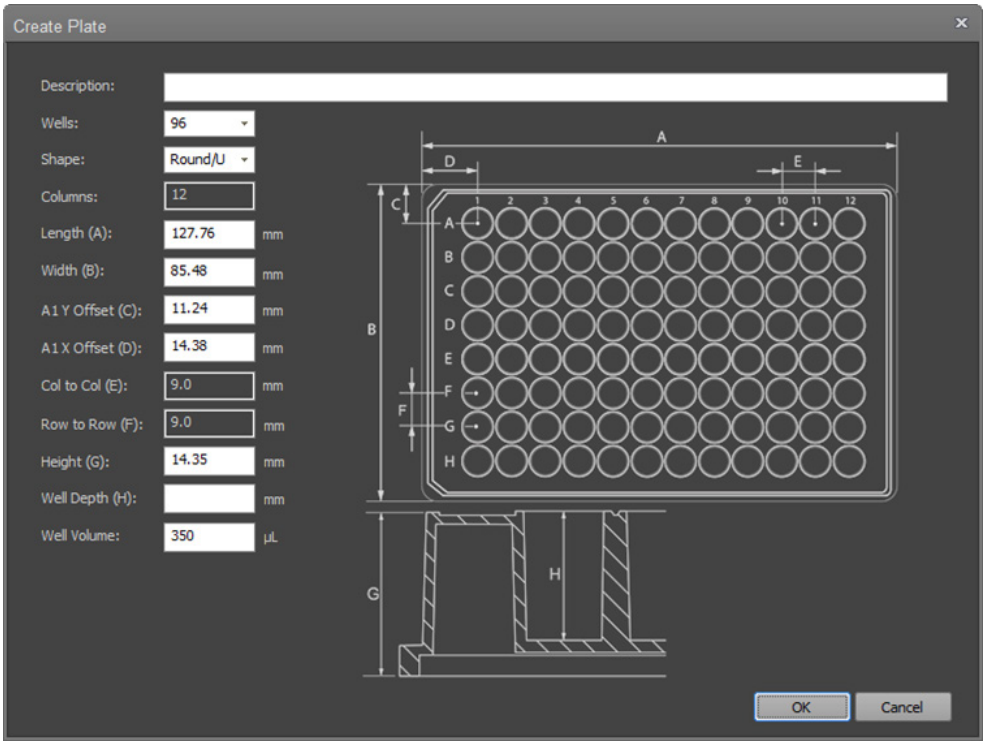
  

  This button is a split button only when launched from the Quick Select Plates list in the **Options▶Plate Options** dialog (page 16); otherwise, it is a standard button with the Import option only.
- To import a plate definition to the plates list, click **Import**. The **File Open (Import)** dialog opens, which allows you to import a file containing plate definitions.
- To export a plate definition, select the **Export** option on the Import/Export split button. The **File Save (Export)** dialog opens, which allows you to export the selected plate definitions.

Create/Edit Plate

Create/Edit Plate dialog allows you to create custom plates with specific dimensions (such as plate height and well volume) to add to the database.

The Create/Edit dialog is available only when a CytKick™ MAX Autosampler is connected to the Attune™ NxT Cytometer.



- If you have clicked **Add** in the **Select Plates** dialog (page 17) to launch the dialog, the dialog title is **Create Plate**.

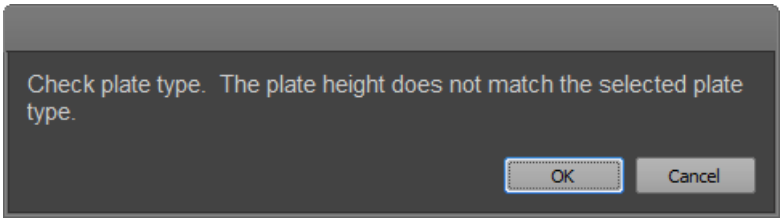
  If you have clicked **Edit** in the **Select Plates** dialog (page 17) to launch the dialog, the dialog title is **Edit Plate**.
- If a plate is used in an existing Experiment or Template, the Edit Plate dialog does not allow modification of any of the plate fields (they will all be read-only).
- The **Description** field must contain a unique description (it cannot be blank). The field allows 260 characters.
- When creating a new plate, the plate definitions have the following default values:
  - **Wells:** 96 (available options are 48, 96, and 384 wells)
  - **Shape:** Round/U bottom (available options are Round/U, Conical/V, and Flat/F bottom)
  - **Columns (read-only):** 6 for 48-well, 12 for 96-well, 24 for 384-well
  - **Length (A):** 127.76 mm
  - **Width (B):** 85.48 mm
  - **A1 Y Offset (C):** 11.24 mm for 48-well and 96-well, 8.99 mm for 384-well
  - **A1 X Offset (D):** 18.88 mm for 48-well, 14.38 mm for 96-well, 12.13 mm for 384-well

  - **Col to Col (E) (read-only):** 18.0 mm for 48-well, 9.0 mm for 96-well, 4.5 mm for 384-well
  - **Row to Row (F) (read-only):** 9.0 mm for 48-well and 96-well, 4.5 mm for 384-well
  - **Height (G):** 14.35 mm
  - **Well Depth (H):** Blank
  - **Well Volume:** 500 µL for 48-well, 350 µL for 96-well, 120 µL for 384-well
- When editing a plate, the plate definition values are pre-populated based on the known plate dimensions.

Plate Height Sensor

The CytKick™ and CytKick™ MAX Autosamplers have a plate height sensor that automatically detects the plate type (standard or deep well) at the beginning of a plate acquisition.

- The sensor checks the plate on the sample tray to ensure that the plate type corresponds with the plate definition (standard vs. deep-well plate) specified for the Experiment before a plate run starts.
- If the plate type is different from what is specified for the Experiment, the software displays a warning dialog.

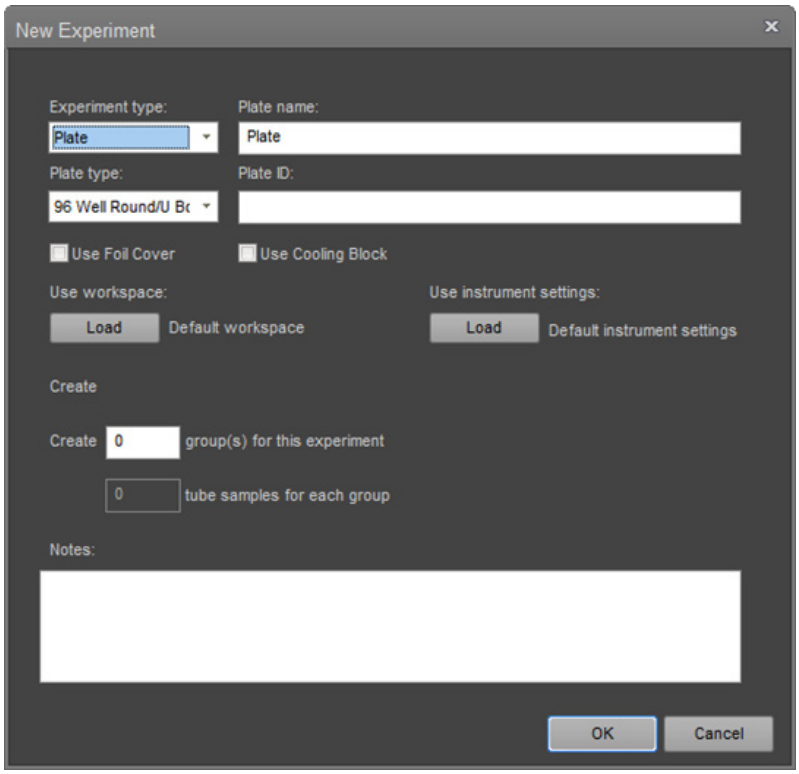


- If an incorrect plate is used, change the plate definition for the Experiment to match the plate you are using. Alternatively, change the plate you are using to match the plate definition specified for the Experiment.
- After you have corrected the error, click **OK** to re-check the plate height. If the correct plate is used, the error is cleared and the plate run starts. If the error persists, the warning dialog is displayed again.
- Click **Cancel** to abort the run without correcting the error and close the dialog.

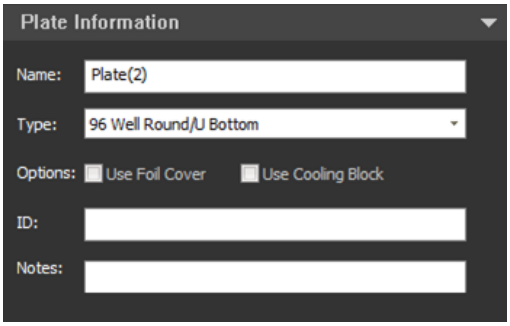
Use Foil Cover

Use Foil Cover allows you to set up a Plate Experiment using a foil cover to protect the sample plate from condensation or evaporation.

- Use Foil cover option is available only when a CytKick™ MAX Autosampler is connected to the Attune™ NxT Cytometer. Otherwise, it is not visible.
- When available, you can set the **Use Foil Cover** option in the New Experiment dialog.



Alternatively, you can select the Use Foil Cover option in the **Customize Plate Experiment** panel.

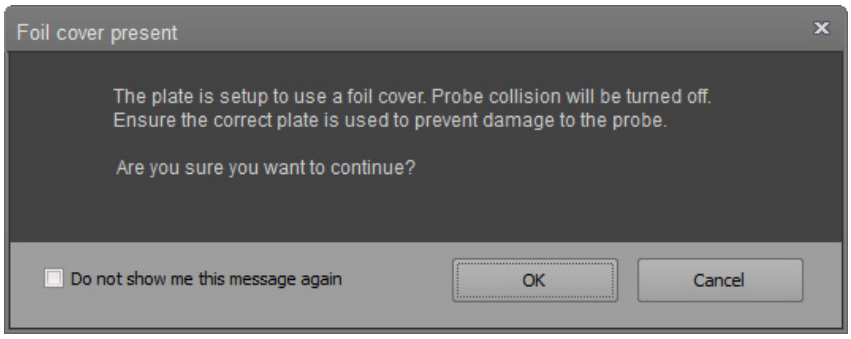


- When the **Use Foil Cover** option is enabled, the autosampler disables the probe collision sensor, which allows the use of a foil cover on the plate.

  When this option is enabled, the software displays the "Foil cover present" warning dialog (page 22) at the beginning of the plate acquisition to ensure that the correct plate is used.
- Use Foil Cover selection persists as part of a Template, and when exporting or duplicating a Plate.

Foil Cover Check

When **Use Foil Cover** is enabled (page 21), the software displays the "Foil cover present" warning dialog at the beginning of a plate acquisition.

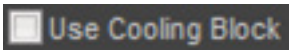


- The Foil cover present dialog is displayed when you click the **Setup Comp**, **Record Comp**, **Record All**, or **Record Plate** button on the Collection Panel, or if you attempt to **Run/Record** from a Manual well).

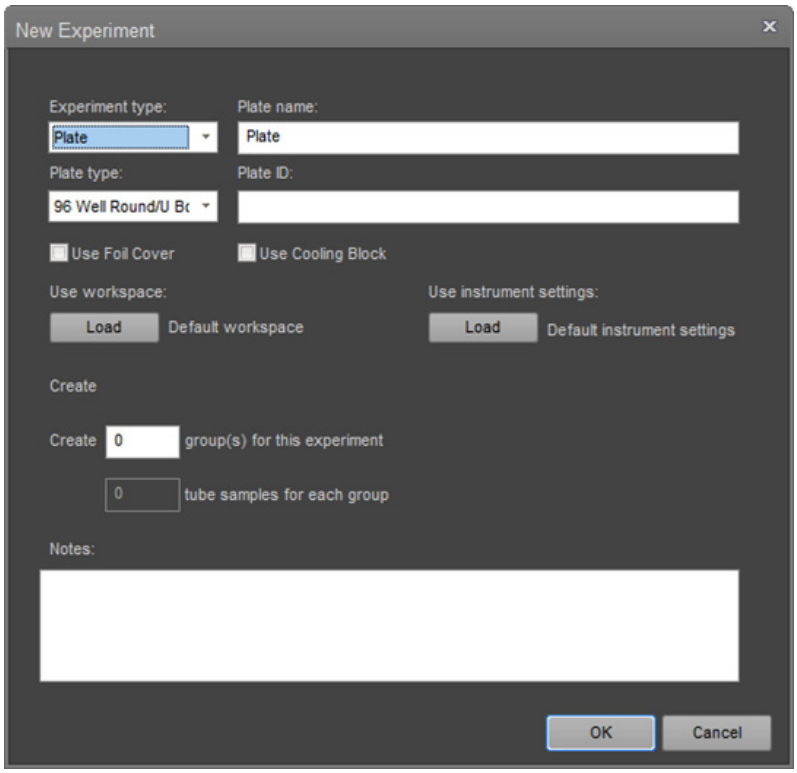
  The dialog is not displayed when you run a Tube sample or a Compensation sample from a Tube within a Plate Experiment.
- Click **OK** to close the dialog and proceed with acquisition.
- Click **Cancel** or the **X** button to abort the acquisition and close the dialog.
- Select **Do not show me this message again** to suppress dialog for the duration of the current session.

Use Cooling Block

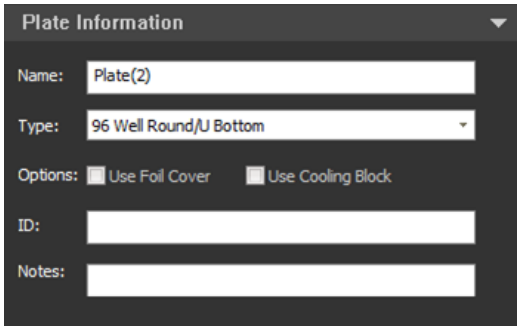
Use Cooling Block allows you to set up a Plate Experiment using a cooling block. When selected, the autosampler takes into account the extra height that the cooling block adds to the plate specification.



- This option is available only when a CytKick™ MAX Autosampler is connected to the Attune™ NxT Cytometer. Otherwise, it is not visible.
- When available, you can set the **Use Cooling Block** option in the New Experiment dialog.



You can also select this option in the **Customize Plate Experiment** panel.



- When available, this option is enabled only for standard 96-well round bottom plates (such as Thermo Scientific™ 96-well Microtiter™ Microplates, Cat. No. 2205).

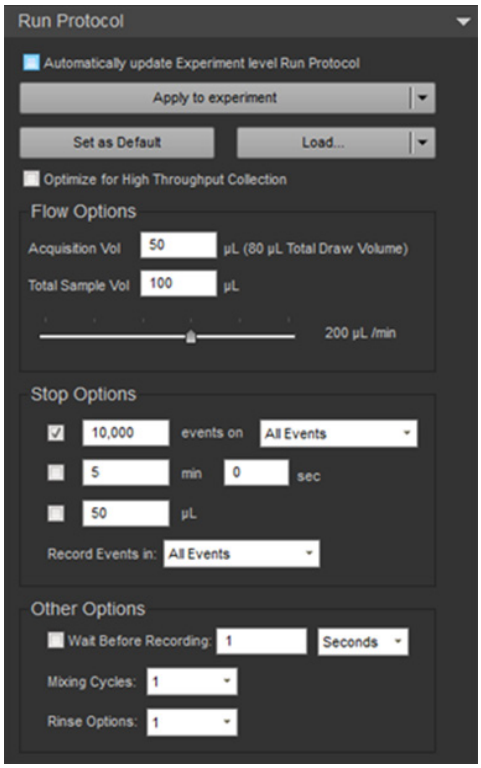
> **Note:** A standard plate is defined as a plate that is within the height range of 14.35 mm ± 0.76 mm.

- Use Cooling Block selection persists as part of a Template, and when exporting or duplicating a Plate.

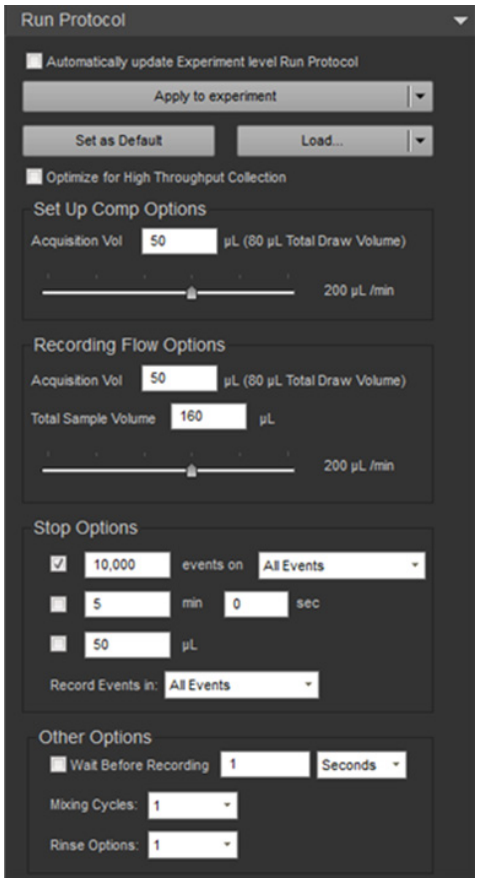
Optimize for High-Throughput Collection

Optimize for High-Throughput Collection optimizes the Run protocol for high-throughput data collection.

- This option is available only when a CytKick™ or CytKick™ MAX Autosampler is connected to the Attune™ NxT Cytometer.
- When available, you can set the **Optimize for High-Throughput Collection** option in the **Collection ▶ Run protocol** panel.



Normal and Manual well



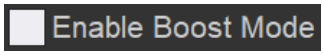
Compensation well

- The Optimize for High-Throughput Collection option is visible and enabled only for Plate collection (Normal well). It is visible but disabled for wells marked as Manual wells.
- When selected, the **Optimize for High-Throughput Collection** option optimizes the Run protocol for high-throughput data collection as follows:

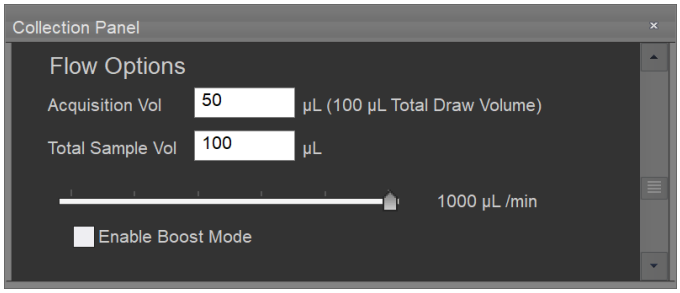
Run protocol parameter	Attune™ NxT and CytKick™ Autosampler	CytKick™ MAX Autosampler
Stop volume	40 µL	20 µL
Acquisition volume	40 µL	20 µL
Flow rate	500 µL/minute	1000 µL/minute
Mixing cycles	1	1
Rinse between samples	1	1
Wait before recording	Unchecked	Unchecked

Enable Boost Mode

Enable Boost Mode allows the processing of the Samples with reduced boost volume for high-throughput acquisition, which reduces the processing time for a standard 96-well plate from 45 minutes to 22 minutes.



- The Enable Boost Mode option is available only when a CytKick™ MAX Autosampler is connected to the Attune™ NxT Cytometer.
- When available, you can set the **Enable Boost Mode** in the **Collection ▶ Run protocol** panel under **Flow options**.

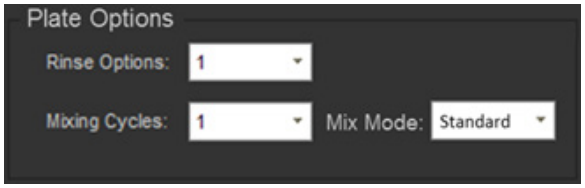


- The Enable Boost Mode option is visible only for Normal wells and Compensation wells in Plate Experiments. However, it is enabled only when the flow rate is set to 500 µL/minute or 1000 µL/minute.
- The Enable Boost Mode option is not available for Manual wells or Tube samples.

Mix Mode

Mix Mode allows you to select between **Standard** and **Gentle** mix modes.

- The Mix Mode selection is available only when a CytKick™ MAX Autosampler is connected to the Attune™ NxT Cytometer.
- When available, you can set the **Mix Mode** in the **Collection ▶ Run protocol** panel under **Other options**.



- The Mix Mode option is available only when a Plate well is selected. It is not available for Tube samples.
- When the option is set as **Standard**, the Sample is mixed using the normal mixing speed.

  When the option is set to **Gentle**, the Sample is mixed and aspirated at a slower speed.

Note: For fragile cells, viscous samples, or samples prepared in viscous buffers, use the **Gentle** mix mode.

3. System Maintenance

The Attune™ NxT Acoustic Focusing Cytometer and the CytKick™ Autosampler are designed to require minimum maintenance. However, to ensure reliability of the cytometer all its peripheral systems, 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 CytKick™ or the CytKick™ MAX Autosampler in good working condition.

Procedure	Frequency
SIP Sanitize	Daily before Shutdown
Shutdown	Daily
Visual inspection of sample injection port (SIP)	Daily
Visual inspection of fluidics bottles and connections	Daily
Visual inspection of syringe pumps	Daily
Fluidics maintenance (i.e., Rinse, Unclog, and De-bubble functions)	Daily or as needed
Computer maintenance	Monthly
Optical filter cleaning	Monthly or as needed
Fluidics decontamination	Monthly
System decontamination	3 months or as needed
Replace focusing fluid filters	3 months or after each system decontamination
Replace syringes	3 months (high volume use at >6 hours/day), 6 months (standard use), or as needed*

*The frequency of maintenance depends on how often you run the CytKick™ or the CytKick™ MAX Autosampler. On average, syringes last about 6 months with standard use.

Daily maintenance

Daily Shutdown

Daily shutdown involves executing the **Shutdown** function (page 37). The Shutdown procedure ensures that all sample fluid and dyes have been removed from the fluidics lines and the pumps have been decontaminated and filled with Attune™ Shutdown solution to prevent the formation salt crystals.

Depending on the Shutdown option selected (Quick, Standard, or Thorough), the Shutdown procedure can take up to 75 minutes, but most of the steps are automated and under computer control. At the end of the shutdown procedure, the cytometer and its peripherals are automatically powered down.

Visual inspection

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

Fluidics maintenance

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

- *Unclog* function is a back flush operation to remove clogs from the sample probe and flow cell (page 30).
- *Rinse* function is used for system cleaning between sticky samples. This function requires user-supplied bleach or detergent (page 28).
- *De-bubble* function is used for clearing bubbles in the fluidics lines (page 31).

Sanitize between uses

Run the **Sanitize Attune SIP** procedure (page 28) to sanitize the CytKick™ or the CytKick™ MAX Autosampler between uses.

This procedure is intended for a quick cleaning of the instrument to minimize cross-contamination.

For a more thorough decontamination, perform the Decontaminate System procedure (page 32).

Note: For monthly and periodic maintenance of the system, refer to the Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide, which is available for download at **thermofisher.com**.

Sanitize SIP

Run the *Sanitize Atttune SIP* function to quickly wash and sanitize the:

- Attune™ NxT Cytometer Sample Injection Port (SIP) and sample lines

  OR

- Autosampler SIP and sample lines

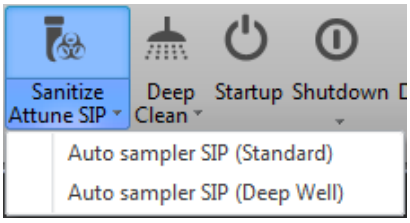
When running the Sanitize SIP function for the CytKick™ and CytKick™ MAX Autosamplers, you have the option of sanitizing the SIP for standard or deep-well plates.

Run the Sanitize SIP function daily before Shutdown (page 37) and in between samples as needed.

Note: It is especially important to perform the Sanitize SIP function when running sticky samples, DNA stains, or beads.

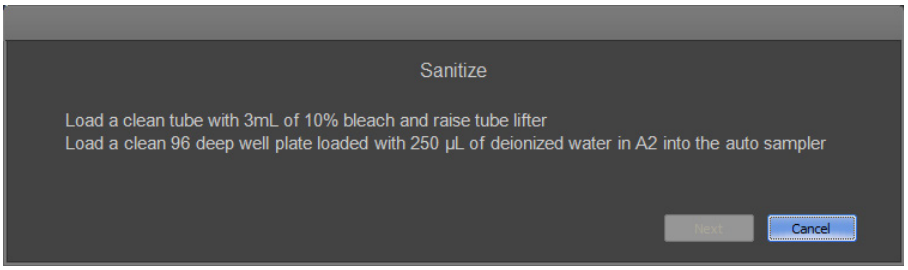
Run Sanitize Autosampler SIP (Standard) function

1. On the Instrument ribbon, click **Sanitize Attune SIP**.



2. From the dropdown, select **Autosampler SIP (Standard)** (if you have been using standard plates).

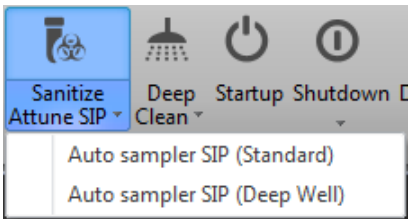
   The *Sanitize dialog* appears and provides instructions to perform the Sanitize Autosampler SIP procedure for standard plates.



3. Load a clean tube with 3 mL of 10% bleach to the Attune™ NxT Cytometer SIP and raise the tube lifter.
4. Add 250 µL of deionized water to the A2 well of a clean 96-well deep well plate, then load the plate into the autosampler.
5. Click **Next** to run the Sanitize SIP function.

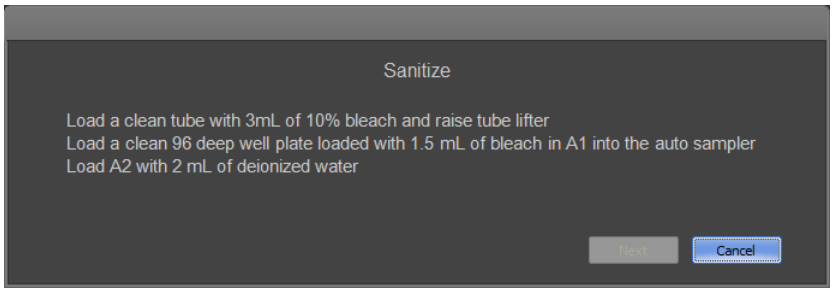
Run Sanitize Autosampler SIP (Deep Well) function

1. On the Instrument ribbon, click **Sanitize Attune SIP**.



2. From the dropdown, select **Autosampler SIP (Deep Well)** (if you have been using deep-well plates).

   The *Sanitize dialog* appears and provides instructions to perform the Sanitize Autosampler SIP procedure for deep-well plates.



3. Load a clean tube with 3 mL of 10% bleach to the Attune™ NxT Cytometer SIP and raise the tube lifter.
4. Add 1.5 mL of 10% bleach to the A1 well and 2 mL of deionized water to the A2 well of a clean 96-deep well plate, then load the plate into the autosampler.
5. Click **Next** to run the Sanitize SIP function.

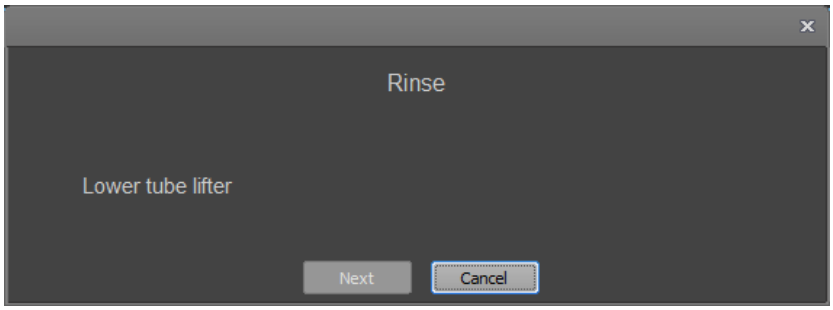
Rinse

Run the Rinse function

The *Rinse* function rinses the sample lines.

1. On the Instrument ribbon, click **Rinse**.

   The *Rinse dialog* appears and provides instructions to perform the Rinse procedure.



2. If the tube lifter is raised, lower the tube lifter.
3. Click **Next** to run the Rinse function.

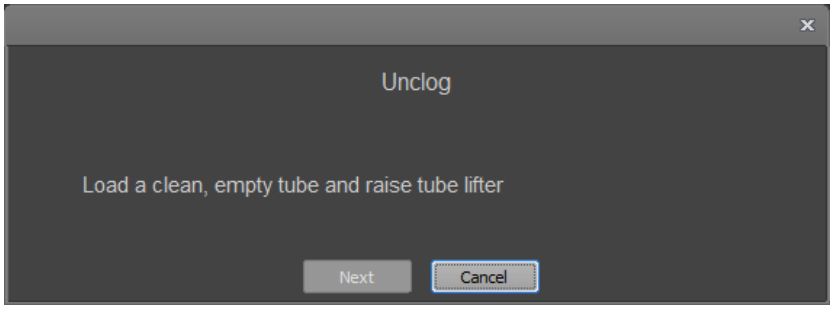
Unclog

The *Unclog* function is a back flush operation to remove clogs from the sample probe and flow cell.

Run the Unclog function

1. On the Instrument ribbon, click **Unclog**.

   The *Unclog dialog* appears and provides instructions to perform the Unclog procedure.



2. Load a clean, empty tube into the instrument, then raise the tube lifter.
3. Click **Next** to run the Unclog function.
4. When the function completes, lower the tube lifter.
5. Click **Next** to close the dialog and automatically perform a Rinse.

De-bubble

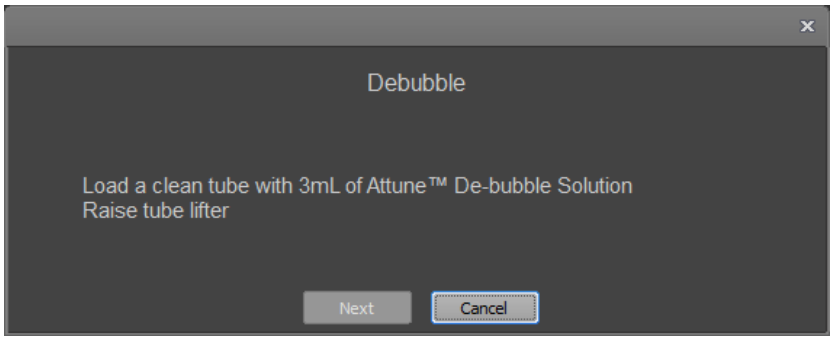
The *De-bubble* function is used for clearing bubbles in the fluidics lines of the cytometer and flow cell.

Run the De-bubble function

1. On the Instrument ribbon, click **De-bubble**.

   The *De-bubble dialog* appears and provides instructions to perform the De-bubble procedure.
2. If the tube lifter is raised, lower the tube lifter.
3. Click **Next** to automatically perform a Rinse.
4. When the rinse is complete, fill a clean tube with at least 1.5 mL of Attune™ Debubble Solution.



5. When prompted, load the tube into the instrument, then raise the tube lifter.
6. Click **Next** to start the De-bubble function.
7. When the function completes, lower the tube lifter.
8. Click **Next** to close the dialog and automatically begin a Rinse.

Decontaminate system

The *Decontaminate System* function of the Attune™ NxT Software is used for the automated decontamination of the Attune™ NxT Acoustic Focusing Cytometer and the CytKick™ Autosampler fluidics.

Perform the Decontaminate System 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 Shutdown 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 before it becoming idle

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

IMPORTANT! Perform the Decontaminate System procedure on a monthly basis, if you intend to leave the Attune™ NxT 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. Instrument hardware may be contaminated by biohazardous material. Using fresh 10% bleach solution in deionized water is the only procedure we recommend for decontaminating the Attune™ NxT Cytometer and the CytKick™ Autosampler.

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 fluidics containers with deionized water.
2. Ensure that all fluidics lines and sensor cables are connected.

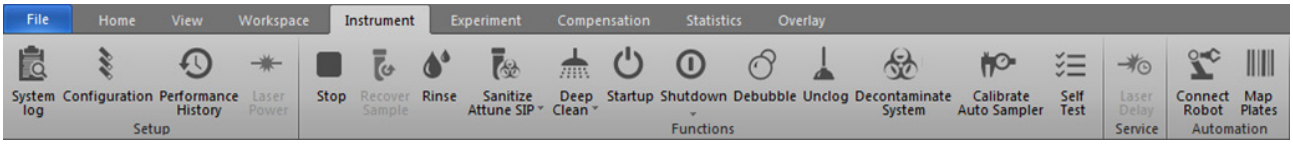
Note: For the location of the fluidics compartment and instructions on filling the fluidics bottles refer to the Attune™ NxT Acoustic Cytometer User Guide, which is available for download at **thermofisher.com**

Run Decontaminate System function

The Attune™ NxT Software provides instructions to perform the *Decontaminate System* function. The Decontaminate System function for the CytKick™ Autosampler is broken into four phases and it can take up to 45 minutes. However, most of the procedure is performed automatically.

Follow all the instructions provided by the instrument and click **Next** between each phase of the System Decontamination procedure. During the procedure, the software provides real-time updates on the Decontaminate System function being executed.

1. Click the **Decontaminate System** button located in the Instrument tab, then follow the prompts in the *Decontamination dialog*



2. Click **Next** to start Decontamination Phase 1. When prompted:
   a. Rinse all fluidics bottles with deionized water.
   b. Fill the Attune™ NxT Cytometer and CytKick™ Autosampler focusing fluid bottles with 500 mL of 10% bleach.

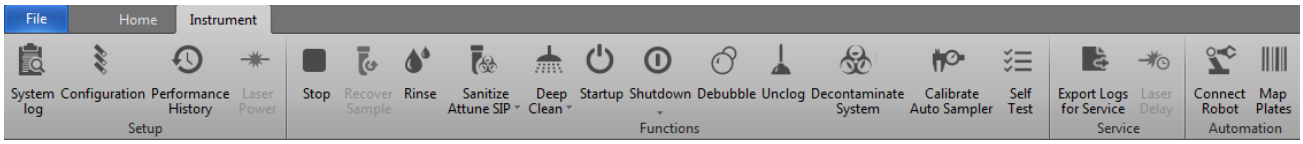
      Leave all other bottles empty.
   c. Reconnect all fluidics lines and bottle cables.
3. Click **Next** to start Decontamination Phase 2. When prompted:
   a. Load a clean, empty standard 96-well plate into the CytKick™ Autosampler.
   b. Load a clean, empty tube on the SIP of the Attune™ NxT Cytometer, and then raise the tube lifter.
4. Click **Next** to start Decontamination Phase 3. When prompted:
   a. Rinse the Attune™ NxT Cytometer and CytKick™ Autosampler focusing fluid bottles with deionized water
   b. Fill both focusing fluid bottles with 500 mL of deionized water.
   c. Reconnect all fluidics lines and bottle cables.
   d. Load a clean, empty tube on the SIP of the Attune™ NxT Cytometer, and then raise the tube lifter.
5. Click **Next** to start Decontamination Phase 4. When prompted:
   a. Replace the focusing fluid filters with fresh filters.
   b. Rinse all fluidics bottles with deionized water.
   c. Replace all fluids in all fluidics bottles with the appropriate solutions.
   d. Reconnect all fluidics lines and bottle cables.
   e. Lower the tube lifter, then remove the plate from the CytKick™ Autosampler.

Calibrate Autosampler

Calibrate Autosampler function sets the probe position relative to the plate tray to ensure that the probe consistently measures from the same spot in each well. Calibrate Autosampler function also calibrates the probe line volume to ensure that the sample is properly positioned for analysis. The calibration procedure takes approximately 2 minutes to complete.

The Calibrate Autosampler button is located in the Instrument ribbon. It is only visible if there is an autosampler present, and enabled when the instrument status is idle.

The Calibrate Autosampler function is disabled when moving between wells or when a plate run is paused.



Note: The CytKick™ and CytKick™ MAX Autosamplers are pre-calibrated before the units are shipped and the instruments auto re-calibrate every month.

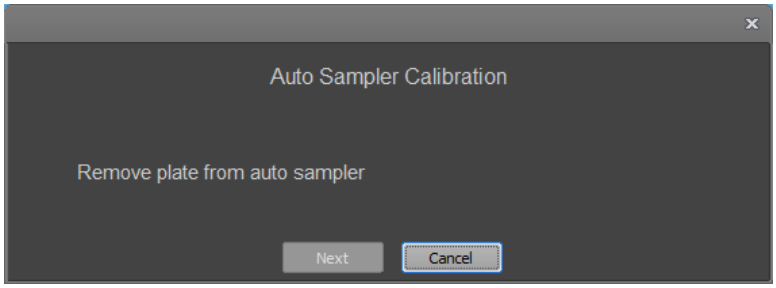
Autosampler calibration is needed only if one of these conditions has occurred:

1. The probe has been replaced by a Thermo Fisher Scientific service representative, the fluid lines between the Attune™ NxT Cytometer and the autosampler has been replaced, or the system has sustained a bump.
2. The CytKick™ or the CytKick™ MAX Autosampler has been disconnected fluidically from the Attune™ NxT Cytometer.

Run the Calibrate Autosampler function

1. On the Instrument ribbon, click **Calibrate Autosampler**.

   The *Calibrate Autosampler dialog* appears and provides instructions to perform the Calibrate Autosampler procedure.

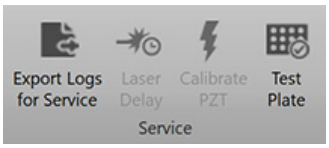


2. If a plate is loaded in the autosampler, remove the plate.
3. Click **Next** to run the Calibrate Autosampler function, then follow the instructions provided by the dialog.

Test Plate

Test Plate function allows you to validate a plate to ensure that the autosampler probe position is in the correct location in all four corners of the plate.

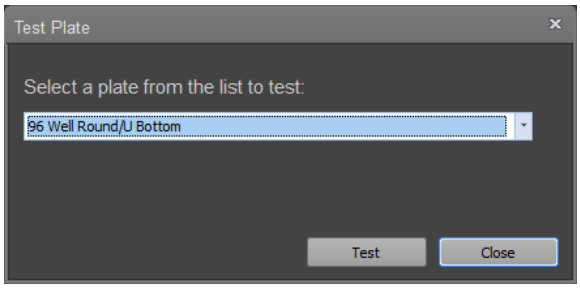
The Test Plate button is located in the Service group of the Instrument ribbon. It is visible only when a CytKick™ or CytKick™ MAX Autosampler is connected to the Attune™ NxT Cytometer and powered on.



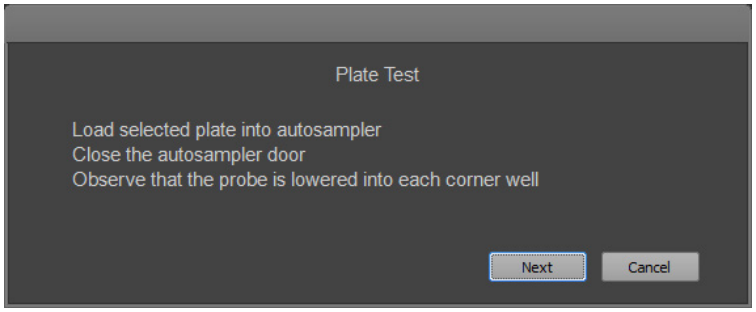
The button is enabled only when the instrument status is idle. The button is disabled when the instrument is paused or set to Automation mode.

Run the Test Plate function

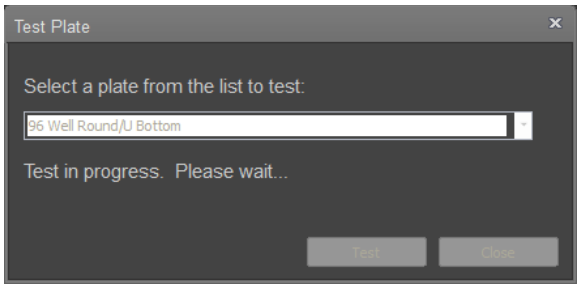
1. On the Instrument ribbon, click **Test Plate** to open the *Test Plate* dialog.



2. Select the plate you wish to test from the dropdown list, then click **Test**. Plate Test instructions appear.

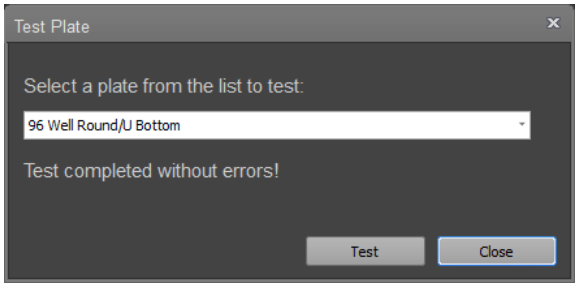


3. Load the selected into the autosampler, close the autosampler door, then click **Next** to start the Test Plate procedure.
4. The autosampler iterates through each corner of the selected plate/tube rack and lowers the probe to the well depth as defined in the selected plate definition. The autosampler cycles through each corner of the plate three times during the procedure.



Note: You cannot cancel the Test Plate procedure once it has started.

5. At the successful completion of the test, the Test Plate dialog displays the "Test completed without errors!" message.



   If a probe collision with the test plate occurs, the Test Plate dialog displays an error message.

6. At the completion of the test, click **Close** to close the Test Plate dialog.

Shutdown

The *Shutdown* function of the Attune™ NxT Software facilitates the automated shutdown of the instrument and its peripherals. The Shutdown procedure removes all sample fluid and dyes from the system, decontaminates the fluidics lines and sample pumps, and fills them with Attune™ Shutdown Solution to prevent the formation of salt crystals.

Depending on the Shutdown option selected (Quick, Standard, or Thorough), the automated Shutdown procedure can take up to 75 minutes.

If the CytKick™ Autosampler 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

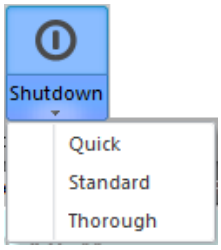
1. Check the levels in the fluidics bottles.
2. Ensure that the wash and shutdown solution bottles are full. If empty, fill the appropriate bottles with Attune™ Wash or Attune™ Shutdown Solution.
3. Empty the waste bottles on the Attune™ NxT Acoustic Focusing Cytometer and the CytKick™ Autosampler.

Note: For the location of the fluidics compartment and instructions on filling the fluidics bottles refer to the Attune™ NxT Acoustic Cytometer User Guide, which is available for download at **thermofisher.com**.

Shutdown options

There are three options available for the Shutdown function:

- **Quick** –Quick option uses 5 wash cycles and takes 25 minutes to complete.
- **Standard** –Standard option uses 15 wash cycles and takes 55 minutes to complete.
- **Thorough** –Thorough option uses 25 wash cycles and takes 75 minutes to complete.



For daily use, we recommend the Standard Shutdown function.

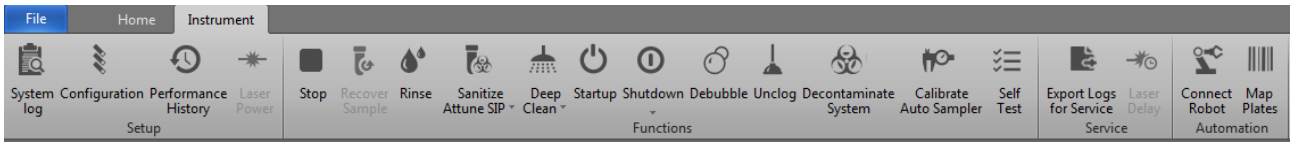
IMPORTANT! The Shutdown function powers off the laser and the Attune™ NxT Cytometer automatically. If you cancel the Shutdown function while it is running, exit and restart the Attune™ NxT Software, complete the Startup function, then restart the Shutdown function and let it run to completion.

Run Shutdown function

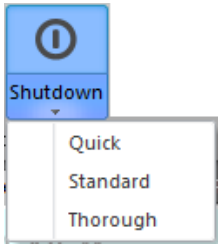
The Attune™ NxT Software provides instructions to perform the *Shutdown* operation. The Shutdown operation is broken into three phases in systems with the CytKick™ Autosampler, and it can take more than 40 minutes to complete. However, most of the operation is performed automatically.

Make sure to follow all the instructions provided by the instrument during the Shutdown cycle. During the operation, the software provides real-time updates on the shutdown function being executed.

1. Before the Shutdown procedure, run the Sanitize SIP function (page 28) using the Attune™ Flow Cell Cleaning Solution (Cat. No. A43635).
2. Click the **Shutdown** button located in the Instrument tab.



The dropdown menu provides three options for Shutdown (**Quick**, **Standard**, and **Thorough**). Select the appropriate option. For daily use, we recommend the Standard Shutdown option.



3. The *Shutdown* prompt screen appears.
4. When prompted, place 3 mL of 10% bleach solution in a tube on the sample injection port (SIP), then raise the tube lifter.
5. When prompted, place 3 mL of deionized water in a tube on the SIP, lift the tube filter, then click **Next**.
6. When prompted, place an empty 96-well round bottom plate in the CytKick™ Autosampler, then close the door.
7. Move the manual valve to **plate mode**, then click **Next**.
8. At the end of the Shutdown operation, the Attune™ NxT Software automatically powers down the Attune™ NxT Acoustic Focusing Cytometer and the CytKick™ Autosampler.

   The CytKick™ Autosampler remains in a low power standby mode and will automatically power on if the Attune™ NxT Software is started and the Attune™ NxT Acoustic Focusing Cytometer is powered on.

   The eject button of the CytKick™ Autosampler blinks in the standby mode until the CytKick™ Autosampler is turned off using the power switch located at the back of the instrument.

IMPORTANT! If you cancel the Shutdown function while it is running, exit and restart the Attune™ NxT Software, complete the Startup function, then restart the Shutdown function and let it run to completion.

IMPORTANT! If you intend to leave the Attune™ NxT Acoustic Focusing Cytometer in the shutdown state for longer than two weeks, run the *System Decontamination* function (page 32) instead of the Shutdown.

System Decontamination cleans the fluidics systems of the Attune™ NxT Acoustic Focusing Cytometer and the CytKick™ Autosampler and leaves the fluidics systems in deionized water to prevent salt crystals from clogging them.

Appendix A: Troubleshooting

This section includes the following topics:

- Tips to help you troubleshoot issues with CytKick™ Autosampler
- Technical Assistance Information

Note: For troubleshooting issues encountered with the Attune™ NxT Cytometer, including cytometer operation, performance tracking, and sample preparation, refer to the *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide*, which is available for download at **thermofisher.com**

CytKick™ Autosampler troubleshooting

Observation	Possible causes	Recommended solutions
No sample being analyzed	No power to the CytKick™ Autosampler	Attach the power plug and turn the CytKick™ Autosampler ON.
	Fluidics are not connected	Attach the fluidics connectors from the CytKick™ Autosampler to the Attune™ NxT Cytometer.
	Fluidics are leaking	Check for leaks at the connectors on the Attune™ NxT Cytometer.
	No plate in the CytKick™ Autosampler	Place a plate in the sample tray of the CytKick™ Autosampler.
	Incompatible plate type	See compatible plate types in "System specifications" (page 43). Contact Technical Support if the problem persists.
	Instrument is clogged	Run Unclog function. Contact Technical Support if the problem persists.
	Empty fluid container	• Check for empty fluid bottle (Focusing fluid, Wash, or Shutdown solution) on the Attune™ NxT Cytometer or the CytKick™ Autosampler. • Ensure that the fill lines and fluid level detectors are plugged in completely.
	CytKick™ Autosampler is powered OFF	Power ON the CytKick™ Autosampler.
	USB cable not connected	Ensure that the USB cable is plugged into the instrument and the computer.
	Sample plate is not selected	Select the sample plate.
	Sample volume is less than specified for the system	Total Draw Volume displayed in the software is the **absolute minimum** sample volume required. Any deviation to less than this volume in a well (e.g. pipetting error) can lead to bubbles drawn into system.
Red light blinks	Error occurred in system	• Power the instrument OFF and ON. • Run the Calibrate Autosampler function.
After initial power ON, the tray is ejected with the well plate	Well plate is present during power ON of the instrument	Remove the well plate from the tray during the power ON cycle.
Computer is not communicating with the CytKick™ Autosampler	USB cable not fully plugged in	Verify that the USB cable connection is in place in the back of the CytKick™ Autosampler and the computer.
	Faulty USB cable	Replace USB cable. Contact Technical Support if the problem persists.
	USB port changed from the original port	Try a different USB port on the computer. If the problem persists, reinstall the USB drivers.

Observation	Possible causes	Recommended solutions
CytKick™ Autosampler and/or computer has no power	Power supply not plugged into the appropriate outlet	Ensure that the CytKick™ Autosampler, Attune™ NxT Cytometer, and the computer are plugged into the appropriate outlet.
	No power at the outlet	Ensure 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 syringes on the Attune™ NxT Cytometer and the Autosampler for leaks and tighten the syringes if necessary. Be careful not to over tighten.
	Loose fittings	Check for loose fittings on both ends of the tubing between the Attune™ NxT Cytometer and the CytKick™ Autosampler. Open the syringe pump compartment door and check for loose fittings on the CytKick™ Autosampler syringe pump.
	Defective sample syringe on the CytKick™ Autosampler	Replace the sample syringe on the CytKick™ Autosampler.
	Defective sample syringe on the Attune™ NxT Cytometer	Replace the sample syringe on the Attune™ NxT Cytometer.
	Fluidic valve or tubing failure within the Attune™ NxT Cytometer	Verify that the sample can be properly analyzed on the Attune™ NxT Cytometer in tube mode and contact Technical Support.
Sample aspirated, then backfilled into sample well	Clog in the sample line	• Run Unclog function. Contact Technical Support if the problem persists. • If persistent, designate rinse wells throughout plate between samples and/or increase rinses between wells. • Ensure sample size is within system specification (< 50 micrometers).
	CytKick™ Autosampler Waste bottle is not connected	Check to ensure that the quick disconnect fitting to the CytKick™ Autosampler Waste bottle is properly connected. The fitting should click when properly connected.
	CytKick™ Autosampler Focusing Fluid bottle is not connected	Check to ensure that the quick disconnect fitting to CytKick™ Autosampler Focusing Fluid bottle is properly connected. The fitting should click twice (a light click followed by a louder click) when properly connected.
	CytKick™ Autosampler Focusing Fluid bottle is drawing air	Fill the Focusing Fluid bottle if low
	Fluidic system failure in the Attune™ NxT Cytometer	Contact Technical Support.

Observation	Possible causes	Recommended solutions
Long delay between sample aspiration and events appearing on screen (normally events appear in ~10 seconds); there are gaps or large spikes in the Count vs. Time plots	Sample syringe is leaking	Ensure that the sample syringe is sealed properly in the Attune™ NxT Cytometer and the CytKick™ Autosampler.
	Loose fittings	Check for loose fittings on both ends of the tubing between the Attune™ NxT Cytometer and the CytKick™ Autosampler. Open the syringe pump compartment door and check for loose fittings on the CytKick™ Autosampler syringe pump.
	Incompatible plate type	See compatible plate types in "System specifications" (page 43). Contact Technical Support if the problem persists.
	Partial clog in the fluidics system	Run Unclog function. Contact Technical Support if the problem persists.
Sample probe is not centered in the sample well	Probe is bent or faulty, or the system is out of calibration	Run Calibrate Autosampler function. If the problem persists after calibration, contact Technical Support.
	Incorrect plate type selected	Select appropriate plate type.
Focusing fluid syringe pump does not shut off	Software glitch or the Focusing fluid reservoir level sensor is malfunctioning	Turn OFF and restart the CytKick™ Autosampler and the Attune™ NxT Cytometer. If the problem persists, turn OFF the CytKick™ Autosampler and contact Technical Support.
Rinse fluid pump does not shut off	Software glitch or the Rinse (Waste) fluid reservoir level sensor is malfunctioning	Turn OFF and restart the CytKick™ Autosampler and the Attune™ NxT Cytometer. If the problem persists, turn OFF the CytKick™ Autosampler and contact Technical Support.
Fluid is leaking from the base of the CytKick™ Autosampler or into the interior drip tray	1-mL syringe seal is broken	Replace the sample syringe on the CytKick™ Autosampler.
Inconsistent results experienced between wells	Sample volume loaded into each well is not adequate	Total Draw Volume displayed in the software is the **absolute minimum** sample volume required. Any deviation to less than this volume in a well (e.g. pipetting error) can lead to bubbles drawn into system and inconsistent results.
	Inconsistent sample preparation	Verify sample preparation and well loading is consistent across the plate.
	Sample concentration exceeds system specifications	Verify sample concentration is not in excess of system requirements.
Large amount of debris is seen in data	CytKick™ Autosampler has been idle for an extended time	Run the Startup function on the Attune™ NxT System three times. Run the De-bubble function two times with Attune™ Debubble solution. Run the Rinse function two times.
	Recent replacement of a fluidics line component	

Appendix B: Technical overview

System specifications

Physical characteristics

Footprint (H × W × D): Approximately 41 × 43 × 33 cm (16 × 17 × 13 in)

Weight: Approximately 16.9 kg (37.2 lb) with empty bottles

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

Operating humidity: <80% non-condensing

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

Space requirements

Minimum space requirements (H × W × D): 74 × 39 × 39.5 cm (29.1 × 39 × 15.6 in), when attached to Attune™ NxT Flow Cytometer

Minimum clear height: 74 cm (29.1 in) above the mounting

Minimum width: 43 cm (17 in); when attached to the Attune™ NxT Flow Cytometer, the total width is 99 cm (39 in)

Minimum depth: 39.5 cm (15.6 in) provides 33 cm (13 in) for the cytometer unit, and 6.5 cm (2.6 in) behind the unit for ventilation

Fluidics

Fluid storage: External to CytKick™ Autosampler, with level sensing

Total fluid volume: 2000 mL per container; capable of running thirty 96-well plates in Boost Mode

Sample analysis

Compatible plate types: 96-well, standard depth (flat, round, V-bottom)
96-well, deep-well (round and V-bottom)
384-well, standard depth (flat, round, and V-bottom)
384-well, deep-well (round and V-bottom)
48-well, flat bottom
1.5-mL and 2-mL microcentrifuge tubes (with optional adapter)
Foil on 96-well standard depth (round)
96-well standard depth (round) on Cooling Block accessory

Processing time: <45 minutes for 96-well plate, using the High-Throughput mode (CytKick™ and CytKick™ MAX models)
<22 minutes for 96-well plate, using the High-Throughput with Boost mode (CytKick™ MAX model only)

Carry-over: <0.5% in standard mode using 2 wash cycles
<1% in Boost Mode

Mixing Cycles: Each well is mixed via aspiration of sample

Wash Cycles: User defined number of wash cycles (up to 10 wash cycles)

Minimum Sample Volume: 50 µL (70 µL at 1000 µL/minute)

Dead Volume: Does not exceed 30 µL (except 50 µL at 500–1000 µL/minute)

Minimum Sample Acquisition Volume: 20 µL

Software

Attune™ NxT Software Version 3.2 or higher required.

Appendix C: Installation

The installation of the CytKick™ and CytKick™ MAX Autosamplers is performed by Thermo Fisher Scientific field service representatives. The installation instructions in this section are provided for informational purposes only.

Install the CytKick™ Autosampler

Space requirements

Minimum space requirements (H × W × D): 74 × 39 × 39.5 cm (29.1 × 39 × 15.6 in), when attached to Attune™ NxT Flow Cytometer

Minimum clear height: 74 cm (29.1 in) above the mounting

Minimum width: 43 cm (17 in); when attached to the Attune™ NxT Flow Cytometer, the total width is 99 cm (39 in)

Minimum depth: 39.5 cm (15.6 in) provides 33 cm (13 in) for the cytometer unit, and 6.5 cm (2.6 in) behind the unit for ventilation

Connect the CytKick™ Autosampler

1. Uncrate the CytKick™ Autosampler and place it on the bench next to the Attune™ NxT Acoustic Focusing Cytometer.
2. Remove the shrink wrap from the CytKick™ Autosampler and inspect the instrument for any sign of damage. Remove the tape and bag from the two external fluidics lines and disconnect the lines from the union fitting.
3. Connect the fluidics lines from the CytKick™ Autosampler into the two ports on the Attune™ NxT Cytometer; it does not matter which line goes into which port on the cytometer (page 9).

   Note: Watch for leaks at the connector when the instrument is first run to verify proper seal at the fittings.

4. Open the sample tray door and remove the protective shipping foam from the plate tray compartment. The sample tray door is spring loaded, but it is easy to open from either the left or right tray door corners.
5. Connect the power supply cord to the CytKick™ Autosampler and then plug it in to the electrical outlet. Follow the labels on the back of the instrument to get the plug into the correct orientation.

6. Connect the USB cable into the back of the CytKick™ Autosampler and then plug the other end of the USB cable into the back of the Attune™ NxT Acoustic Focusing Cytometer.

7. Fill all of the fluidics bottles and ensure that the waste bottle is empty for both the Attune™ NxT Acoustic Focusing Cytometer and the CytKick™ Autosampler.
8. Power on the CytKick™ Autosampler, and then the Attune™ NxT Acoustic Focusing Cytometer.
9. Launch the Attune™ NxT Software and sign in.

Prime the system fluidics

Priming the system fluidics is critical to proper instrument performance. After completing the CytKick™ Autosampler installation, follow the priming procedure below.

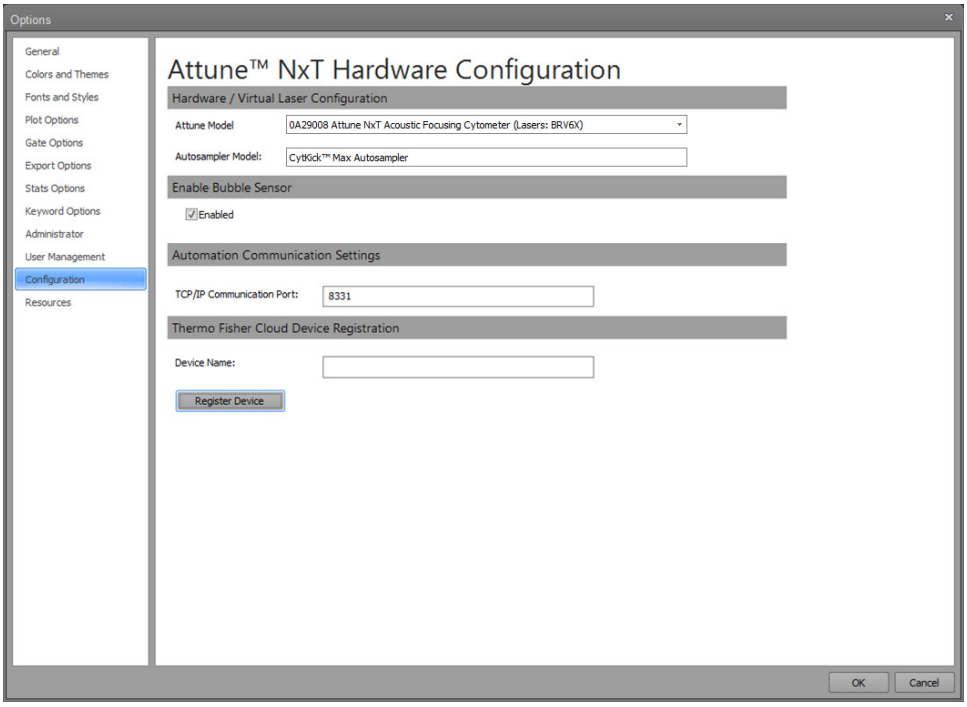
1. Run the **Startup** procedure (page 14).
2. Run the **De-bubble** procedure (page 31).
3. Run the **Autosampler Calibration** function (page 34).

Note: For instructions on decontaminating the CytKick™ Autosampler and preparing it for shipment, refer to the *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide* (Pub. No. 100024234), which is available for download at **thermofisher.com**.

Configure the Attune™ NxT Software for use with the CytKick™ Autosampler

After connecting to the Attune™ NxT Cytometer, the autosampler declares its configuration and automatically sets the correct **Autosampler Model** using an onboard dongle and the autosampler firmware.

When working in analysis-only conditions, you can set the correct **Autosampler Model** in the **Configuration ▶ Hardware/Virtual Laser Configuration** options dialog.



The selection of the correct autosampler model aids in the creation of Experiments when working in analysis-only conditions (i.e., when not connected to an Attune™ NxT Cytometer) by maintaining correct instrument settings, channel mapping, and run protocol options.

Appendix D: Ordering information

The following reagents and consumables have been specifically formulated for use with the Attune™ NxT Cytometer and CytKick™ and CytKick™ MAX Autosamplers. They are available from Thermo Fisher Scientific. Ordering information is provided below. For more information, go to **thermofisher.com** or contact Technical Support.

Product	Amount	Cat. No.
Attune™ NxT Focusing Fluid, 1X Solution	1 × 1 L 6 × 1 L	4488621 4449791
Attune™ NxT Focusing Fluid, 1X Solution	1 × 10 L	A24904
Attune™ Wash Solution	500 mL	A24974
Attune™ 1X Shutdown Solution	250 mL	A24975
Attune™ NxT Flow Cell Cleaning Solution	30 mL	A43635
Attune™ Performance Tracking Beads	3 mL	4449754
Attune™ 1 mL syringe	1 each	100022591
Attune™ NxT Focusing Fluid Filter	1 each	100022587
CytKick™ Autosampler PLUG,1/4-28 Teflon	1 each	4476990
CytKick™ Autosampler Bottle Assembly – Focusing Fluid	1 each	100054670
CytKick™ Autosampler Bottle Assembly – Waste	1 each	100054669
CytKick™ Autosampler 1 mL Syringe	1 each	100054593
CytKick™ Autosampler Tubing Assembly	1 each	100054481
Attune™ Emission Filter Holder Blade	1 each	4465834
Attune™ NxT Blank Dichroic Filter Holder	1 each	100022651
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 E: 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 This document 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 this 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 the instruments (see "**Safety symbols**").

Symbols on instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on the 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 the instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "**Safety labels on instruments**"). 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 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.thermofisher.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 the 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 Thermo Fisher Scientific 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 Thermo Fisher Scientific.
	**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 Thermo Fisher Scientific 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 "**Safety Data Sheets (SDS)**").

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 "**Safety Data Sheets (SDS)**").
- 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 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 (see "**Safety Data Sheets (SDS)**").
- 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™ NxT 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 Attune™ NxT Acoustic Focusing Cytometer and the CytKick™ Autosampler have an installation (overvoltage) category of II, and are 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™ NxT Acoustic Focusing Cytometer has seven different laser configurations, using one or more of the following excitation lasers:

Blue 488 nm, 20 mW laser; Violet 405 nm, 50 mW laser; Red 637 nm, 100 mW laser; Yellow 561 nm, 50 mW laser, and Green 532 nm, 50 mW laser.

Under normal operating conditions, the Attune™ NxT 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 a Thermo Fisher Scientific 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
- European safety and EMC standards
- Australian EMC standards

U.S. and Canadian Safety Standards

The **CSA C/US Mark** signifies that the product meets applicable U.S. and Canadian standards, including those from CSA, CSA America, ANSI, ASME, ASSE, ASTM, NSF and UL.

European Safety and EMC Standards

The **CE Mark** symbolizes that the product conforms to all applicable European Community provisions for which this marking is required. Operation of the instrument is subject to the conditions described in this manual.

The protection provided by the instrument may be impaired if the instrument is used in a manner not specified by Thermo Fisher Scientific.

Australian EMC standards

The **C-Tick Mark** indicates conformity with Australian and New Zealand standards for electromagnetic compatibility.

Documentation and support

Obtaining support

Technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including user guides, manuals, and protocols

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Thermo Fisher Scientific Technical Support and Sales facilities.

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 (refer to *Attune™ NxT Acoustic Focusing Cytometer Maintenance and Troubleshooting Guide*; Pub. No. 100024234).

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **thermofisher.com/support**.

IMPORTANT! For the SDSs of chemicals not distributed by Thermo Fisher Scientific, contact the chemical manufacturer.

Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website.

Go to **thermofisher.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

IMPORTANT! Wiping the computer supplied with the Attune™ NxT Acoustic Focusing Cytometer (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.