Agilent

NovoSampler® Pro Operator's Guide

Prologue

I.　Revision History

Version	Revision Date
1.0	2015.12
1.1	2016.08
1.2	2017.05
1.3	2018.08
1.4	2019.10

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The NovoExpress® Software provides users with the ability to control data collection and analysis on the NovoCyte® Flow Cytometer, NovoCyte Quanteon™ Flow Cytometer, NovoCyte Advanteon™ Flow Cytometer with NovoSampler®, NovoSampler® Pro and NovoSampler® Q. The software contains features to control sample acquisition, data analysis, report generation, and quality control testing. The NovoExpress® Software is the property of ACEA Biosciences, Inc. Purchase of the software grants the user a non-exclusive personal license. The NovoExpress® Software cannot be copied or modified in any way without the written consent of ACEA Biosciences, Inc.

II.　Contact Information

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International Distributors	Please contact ACEA Biosciences, Inc., or visit www.aceabio.com for a complete list of international distributors

III.   Trademark

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IV.   Intended Use

The NovoSampler Pro is an optional sample handling module to the NovoCyte system. The NovoSampler Pro is used with the NovoCyte Flow Cytometer to achieve automatic collection of sample in compatible sample plates or tubes.

V.   Using this Guide

This manual describes the operation and maintenance of NovoSampler Pro. The features of the NovoSampler Pro are controlled through the NovoExpress Software. The use of the software to control the NovoSampler Pro will be described in this guide.

This manual contains the following chapters:

► Overview and Installation: Introduction to the NovoSampler P   ro.

► Software Overview: Description of the NovoExpress software interface and functions related to the NovoSampler Pro.

► Sample Acquisition: Description of performing the instrument QC test, setting up work list, performing automatic sample acquisition, and analyzing data.

► Maintenance: Description of the maintenance on the NovoSampler Pro.

► Troubleshooting: Description of errors, possible causes, and solutions.

The appendix lists the supplies and accessories of NovoSampler Pro.

VI.   Conventions

Text and Keyboard Conventions

The following conventions are used in this guide:

Format	Description
Sequence of numbers ① ②	Sequences of numbers are used to describe the sequence of steps to be carried out.
Italic Font, gold	This font is used to refer to another section in the manual with additional information.
Italics Font	This font is used to describe an operation of the software using a button, icon, or function. Tips and additional information are also in this font.
→	The arrow indicates selection steps. For example, click *File → Print*, means to select the *Print* function from the *File* menu.
Ctrl+X	When using a keyboard shortcut, + means to press the two keys simultaneously. For example, Ctrl+C means to hold down the Ctrl key (Control) and press the C key.

Symbols

The following table lists the symbols used in this guide:

Symbol	Meaning	Description
	Biohazard	This flag indicates that the operation may have potential for biological infection. Protective measures should be taken.
	Warning	This flag indicates improper or unsafe operation could result in material damage, data loss, mild or severe personal injury, or even death.
	Shock Hazard	This flag indicates that the operation has a risk of electric shock.
	Important	Important information.
	Additional Information	Provides additional information about the current topic or product.
► ► ►	Form	Next to continue this form.
■	The End of the Table	This is the end of the table.

VII.   Safety and Limitations

The NovoCyte System is intended for use by trained laboratory professionals. The NovoSampler Pro is designed with measures to protect the safety of the operator. However please use the instrument in strict accordance with the manual for operation, maintenance, and repair. Failure to follow instructions in the manual can result in failure or damage to the instrument's safety features.

Electrical Safety

To prevent electrical shock and instrument damage, follow the following

electrical safety guidelines.

► Unless otherwise specified, always turn off the flow cytometer using the power switch and unplug the power cord before performing maintenance on the NovoSampler Pro.

► Use the proper power supply, and promptly replace any damaged electrical wires, plugs, or cables.

► If liquid enters inside the system or the system leaks, turn off the power immediately and contact the customer service department of ACEA Biosciences, Inc.

Biosafety

⚠️☣️ *All biological samples and materials that come into contact with the instrument or components of the instrument can potentially transmit infectious diseases. To avoid exposure to biohazardous materials, follow the following biosafety guidelines.*

► Take reasonable precautions in handling waste in accordance with the relevant local standards. Avoid having waste come into contact with the mouth or eyes.

► Do not directly touch the samples or waste. Wear appropriate protective equipment including gloves, clothing, and eyewear. Never pipette by mouth. If the sample accidentally comes in contact with skin, follow working standards and immediately consult a health professional.

► Users must wear gloves when handling samples as biohazardous material may come into contact with the outside of the sample tube.

► Users must wear gloves when handling the orbital shaker or rack as biohazardous material may remain on the surface.

General Safety

⚠️ *While the system is running, do not touch the sample injection probe. Do not put your fingers or hands near the opening of the housing of the sample injection probe.*

⚠️ *To prevent accidents and ensure high quality data, do not move the instrument after the installation of the NovoSampler Pro by an ACEA professional. If the instrument needs to be moved after the installation, please contact ACEA for re-installation. The instrument will be re-installed and verified for normal operation.*

⚠️ *The sample injection probe is a moving part and may cause injury. To prevent accidents, do not reach into the NovoSampler Pro while the instrument is running. When placing or removing samples, do not place your hand below the sample injection probe.*

⚠️ *The NovoSampler Pro contains moving parts which may cause injury. To prevent accidents, ensure that the cover to the NovoSampler Pro is closed during sample acquisition. The NovoSampler Pro contains a detector to sense if the lid is open to ensure the user's safety, but the user should confirm that the NovoSampler Pro has completely stopped running before opening the cover.*

VIII. Technical Support

If there are operating or technical questions, please refer to the sections relating to the instrument operation in this guide. If a problem occurs, refer to the troubleshooting section in this guide for solutions. For additional technical support, please contact your local ACEA Biosciences representative or distributor. When contacting ACEA Biosciences, be sure to provide the following information:

▶ The product name, product model and serial number

▶ Usage history of the instrument

▶ Instrument status information on the status bar from the NovoExpress software. If there is a warning or error message, please also provide that information.

▶ Experiment information conducted on the instrument, if not confidential.

▶ Details of recent instrument QC test.

For support within the US, please call 866-308-2232.

For support within China, please call 400-600-1063.

For users in other countries or regions, contact your local ACEA Biosciences representatives or distributors, which may be found at www.aceabio.com.

🔍 *When encountering an issue during operation of NovoCyte flow cytometer, NovoSampler or NovoSampler Pro instrument, it is highly recommended to submit a Technical Support Request by clicking **Home → Technical Support Request** in the NovoExpress main window. The Technical Support Request Creation Wizard automatically collects NovoCyte configurations, NovoExpress system logs, current screenshot, current experiment file and other information that helps in the diagnosis and troubleshooting of NovoCyte instrument. User can also attach any other files using this function. Please refer to the NovoExpress® Software Guide for detailed information on this function.*

1. Introduction

NovoSampler Pro is an optional accessory to the NovoCyte system (Figure 1-1). The NovoSampler Pro is used with the NovoCyte Flow Cytometer to achieve automatic collection of sample in compatible sample plates or tubes. NovoSampler Pro uses an integrated orbital shaker to achieve efficient mixing of the sample in each well and achieve high uniformity across the entire multi-well plate for volumetric absolute counting.
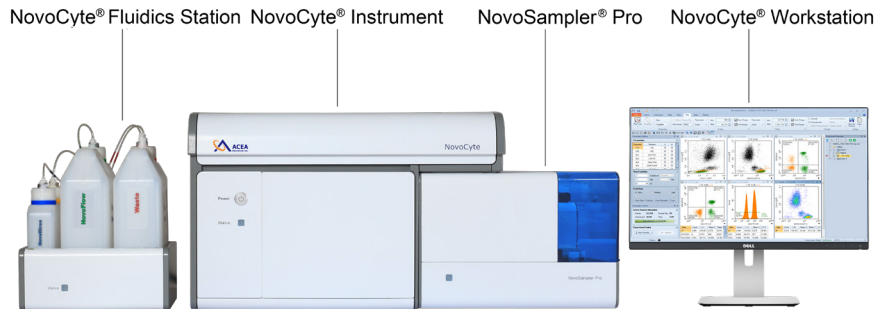
NovoCyte® Fluidics Station   NovoCyte® Instrument   NovoSampler® Pro   NovoCyte® Workstation

Figure 1-1 NovoCyte and NovoSampler Pro System Configuration

This chapter will describe the hardware and installation of the NovoSampler Pro.

1.1 Hardware Overview

The NovoSampler Pro has a built-in orbital shaker to hold the multi-well plate and mix the sample, a cover, and status indication LED, as shown in Figure 1-2.
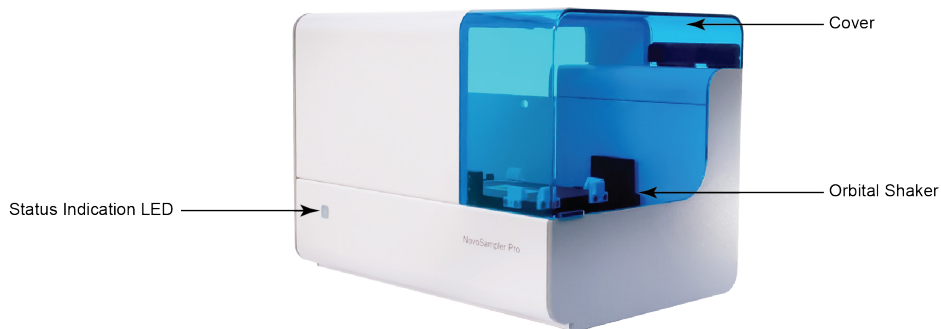
Cover

Orbital Shaker

Status Indication LED

Figure 1-2 NovoSampler Pro

⚠️ *The NovoSampler Pro contains moving parts which may cause injury. To prevent accidents, ensure that the cover to the NovoSampler Pro is closed during sample acquisition. The cover should only be opened when loading samples or when required for maintenance. If the cover is opened during sample acquisition, the NovoSampler Pro will stop running and an error message will appear on the NovoExpress Software.*

The orbital shaker in NovoSampler Pro is shown in Figure 1-3. The shaker is able to hold compatible multi-well plates and the NovoSampler 24 tube rack.
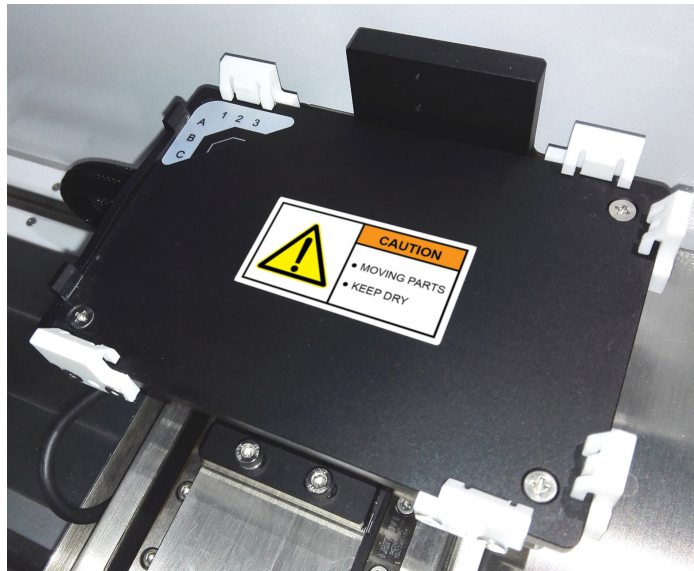
Figure1-3 Orbital Shaker in NovoSampler Pro

⚠️ *Before loading multi-well plates and NovoSampler 24 tube rack, identify the correct orientation for loading into the orbital shaker. Make sure the sample plate is positioned properly on the orbital shaker and the clips hold the sample plate tightly. The sample plate should be inserted down to completely contact the surface of the orbital shaker without any tilting. Incorrect placement may cause misalignment between the sample wells and sample injection probe, resulting in sampling from incorrect wells or damage to the sample injection probe or the orbital shaker.*

☣️ *The inner surface of the NovoSampler Pro may come in contact with residual biohazardous material. When working with NovoSampler Pro, use appropriate protective equipment, including gloves, clothing, and eyewear.*

NovoSampler 24 tube rack compatible with 12 × 75 mm tubes is included with NovoSampler Pro.

⚠️ *Only NovoSampler 24 tube rack could be used with the NovoSampler Pro. Use the other type of tube rack may cause NovoSampler Pro malfunction and damage the instrument.*

The NovoSampler Pro status indication LED indicates the status of the NovoSampler Pro. The color of the status indication LED and the corresponding NovoSampler Pro status are listed in Table 1-1.

LED Color	Status of the NovoSampler Pro
Green	Normal.
Orange	NovoSampler Pro warning. Click the status bar in the NovoExpress Software to review the warning.
Red	NovoSampler Pro error. Click on the status bar in the NovoExpress Software to review the error.

Table 1-1 The Status Indication LED Color and Corresponding NovoSampler Pro Status

🔍 *For additional information regarding warnings and errors, refer to Section 5 Troubleshooting in NovoCyte® Flow Cytometer Operator's Guide.*

Compatibility

The NovoSampler Pro is compatible with the following plates and tubes:

► 12 × 75 mm tubes with NovoSampler 24 tube rack

► 96-well plates (V-bottom)

► 96-well plates (U-bottom)

► 96-well plates (flat-bottom)

► 48-well plates

► 24-well plates

The minimum sample volume requirement for each plate and tube type is listed in Table 1-2.

Sample Plate Type	Minimum Sample Volume	Dead Volume
12 × 75 mm tube with NovoSampler 24 tube rack	90 µL	< 50 µL
96-well plates (V-bottom)	90 µL	< 50 µL
96-well plates (U-bottom)	90 µL	< 50 µL
96-well plates (flat-bottom)	90 µL	< 50 µL
48-well plates	210 µL	< 170 µL
24-well plates	310 µL	< 270 µL

Table 1-2 The Minimum Sample Volume Requirement for Each Plate and Tube Type

Throughput

Table 1-3 summarizes the working conditions and the throughput for NovoSampler Pro.

Working Condition	Standard Mode	High-Throughput
Sample flow rate (µL / min)	66	120
Sampling volume (µL)	10	10
Mix (cycles)	1	1
Rinse (cycles)	1	0
Mixing Speed (rpm)	24-well plate: 2500	24-well plate: 2500
	48-well plate: 1500	48-well plate: 1500
	96-well plate: 1500	96-well plate: 1500
	NovoSampler 24 tube rack: 1500	NovoSampler 24 tube rack: 1500
Mixing Duration (s)	10	10
Acceleration (s)	0	0

►►►

Working Condition	Standard Mode	High-Throughput
Throughput (min)	96-well plate : < 75	96-well plate : < 45
	48-well plate: < 40	48-well plate: < 24
	24-well plate: < 20	24-well plate: < 12
	NovoSampler 24 tube rack: < 20	NovoSampler 24 tube rack: < 12

Table 1-3 Throughput of NovoSampler Pro

Sample Mixing

NovoSampler Pro has a mixing function to help keep the sample in suspension. This function achieves effective mixing with all compatible plates. Users can set the appropriate mixing conditions for their specific sample, as the tendency of sample to settle depends on the characteristics of the sample (sample density, liquid viscosity, etc.).

For setting the mixing conditions, see Section 3.2 Experiment Setup in this guid for more information.

Carryover

NovoSampler Pro can automatically rinse the sample injection probe and fluidic tubing to reduce cross-contamination between samples. When this function is used, the NovoSampler has a carryover rate of less than 0.1%.

For setting sampling conditions, see Section 3.2 Experiment Setup in this guid for more information.

1.2   Installation

This section describes the process of installing the NovoSampler Pro.

⚠️ *Before installing the NovoSampler Pro, NovoCyte instrument must be turned off.*

🛑 *Before installing the NovoSampler Pro, the tube holder for manual sample acquisition must be removed.*

1 To remove the tube holder, loosen the screws as shown in Figure 1-4 and take the tube holder off from NovoCyte.

Figure 1-4 Remove the Tube Holder

2 Position the NovoSampler Pro to NovoCyte instrument as shown in Figure 1-5. Align the left and back side of the sampler with the NovoCyte and gently push the sampler against the NovoCyte. Make sure the NovoSampler Pro is closely in contact with the NovoCyte. Make sure there is no gap between the NovoSampler Pro and NovoCyte on both left and back sides.
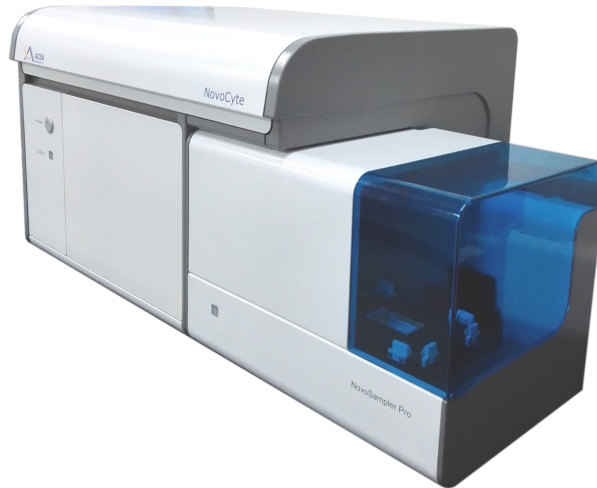

Figure 1-5 Properly Positioning the NovoSampler Pro

⚠️ *Ensure that the NovoSampler Pro is in close contact with NovoCyte Flow Cytometer on the left and back sides during installation is very important for the proper operation of the NovoSampler Pro. Failure to do so would cause calibration failure or the collision of the sample injection probe to the sample plate.*

3 Open the cover, find two screw holes. Align the two holes of the NovoSampler Pro to the two holes on the NovoCyte instrument (Figure 1-6). Tighten the screws (Figure 1-7) to install the NovoSampler Pro.

Figure 1-6 Align the Two Holes of the NovoSampler Pro

► ► ►

Figure 1-7 Tighten the Screws

⚠️ *Use appropriate force (i.e., finger tight) when tightening the screws. Excessive force may result in damage. Ensure that the connections are tight and that the sampler is stable during sample acquisition.*

4 Connect the cable between the NovoSampler Pro and the NovoCyte Flow Cytometer as shown in Figure 1-8.

Figure 1-8 NovoSampler Pro and NovoCyte Flow Cytometer Cable Connection

▶ ▶ ▶

5 Power on the NovoCyte Flow Cytometer by pressing the power button ⏻ on the instrument. Start the NovoExpress Software by double-clicking the icon 𝒩 from the workstation desktop. The software will display a dialog box requesting to calibrate the NovoSampler Pro, as shown in Figure 1-9. Click *Calibrate* to start the calibration of the NovoSampler Pro. Calibration can be postponed, but the software will not allow for samples to be collected until a calibration is performed successfully. To manually start the NovoSampler Pro calibration, click the *Instrument → NovoSampler Pro → Calibrate* on the NovoExpress software.
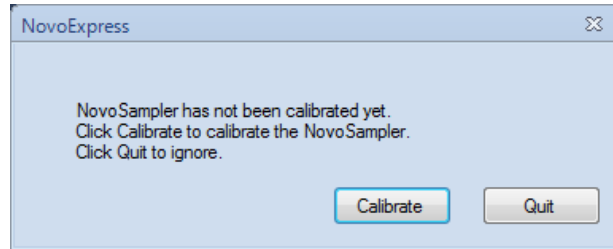

Figure 1-9 Dialog Box to Indicate NovoSampler Pro Calibration

🔍 *If the NovoCyte is already powered on, ensure NovoExpress is launched, click **Instrument → NovoSampler® Pro → Power Up** in NovoExpress software main window to power on the NovoSampler Pro (Figure 1-10). The software will prompt a dialog box requesting to restart the NovoExpress software, as shown in Figure 1-11. Click Yes to restart the software. After log in the software, software will prompt to calibrate the NovoSampler Pro as shown in Figure 1-9. User can click Calibrate to calibrate the NovoSampler Pro.*
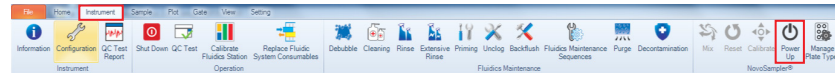

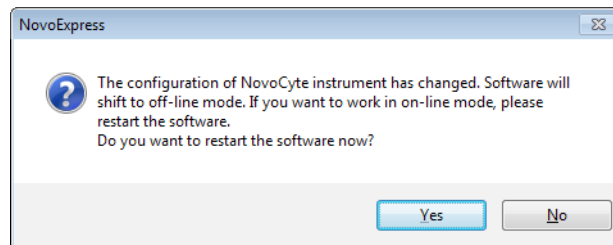**Figure 1-10 Power on NovoSampler Pro**

Figure 1-11 Dialog Box to Restart NovoExpress Software

🔍 *For additional information on starting up the NovoCyte Flow Cytometer, refer to the Section 2.1 Instrument Start-up in NovoCyte® Flow Cytometer Operator's Guide.*

⚠️ *Before calibrating the NovoSampler Pro remove any plates in the orbital shaker. Plates or other items in the orbital shaker may result in a collision and damage to the instrument.*

2. Software Overview

The NovoExpress Software controls the functions of NovoSampler Pro, including calibration, reset, sample mixing, and sample acquisition. The NovoExpress Software will automatically detect if the NovoSampler Pro is installed and connected to NovoCyte instrument.

2.1 Software User Interface

The software interface when NovoSampler Pro is connected is shown in Figure 2-1. Compared to the software interface with NovoCyte alone, differences are observed in the *Menu Bar, Cytometer Control* panel*, Experiment Manager* panel*,* and *Work List*. The *Plate Manager* panel is only visible when NovoSampler Pro is installed.
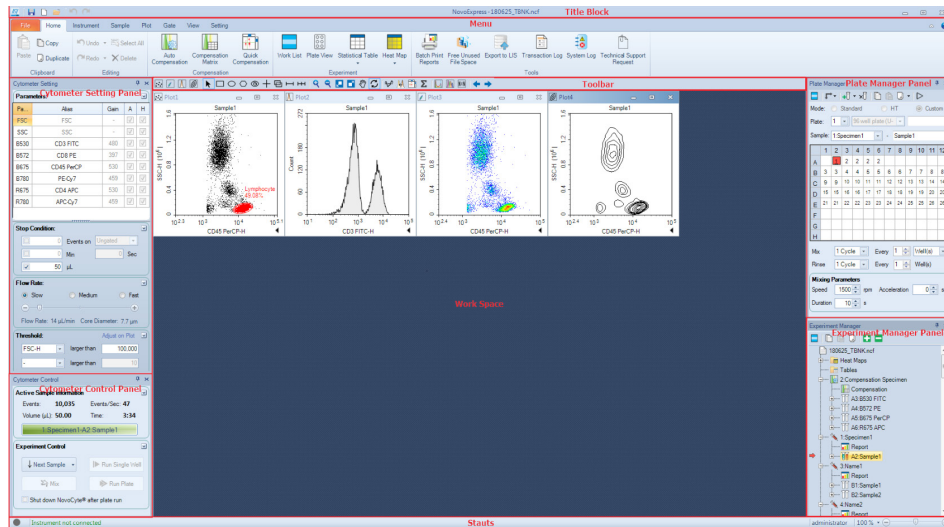

Figure 2-1 NovoExpress Software Interface with NovoSampler Pro Installed

When analyzing NovoCyte data offline on a computer not connected to a NovoCyte Flow Cytometer, the NovoExpress software can be set to NovoSampler Pro not installed. To view the NovoSampler Pro related software interface and functions in the offline mode, open the instrument configuration window by clicking *Instrument → Instrument → Configuration* to change the setting (Figure 2-2). Check the *NovoSampler installed* checkbox, choose *NovoSampler Pro* in the prompted dialog box (Figure 2-3), and click *OK*. The software will prompt a dialog box, asking to restart software. After restarting, the *Cytometer Control* panel, the *Experiment Manager* panel and the *Work List* are all updated, and the *Plate Manager* panel will be visible.
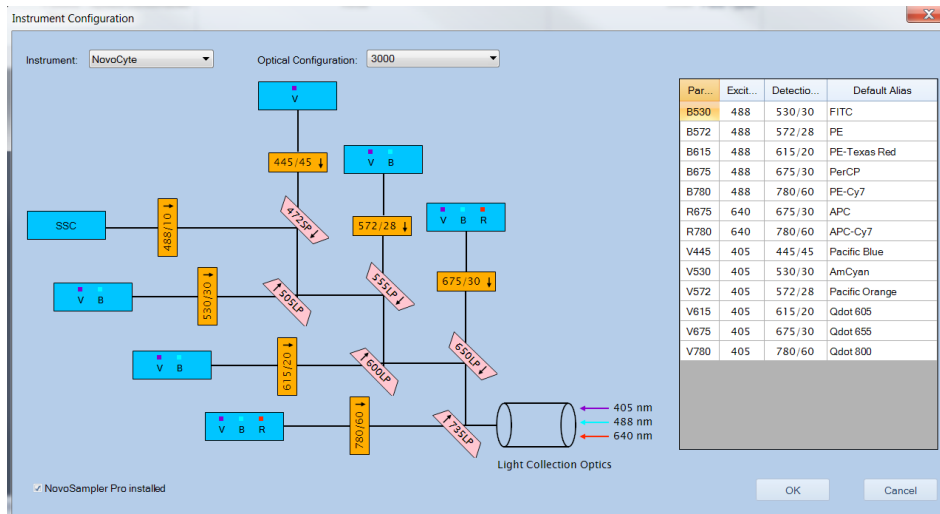
Figure 2-2 The Instrument Configuration in NovoExpress Software Offline Mode
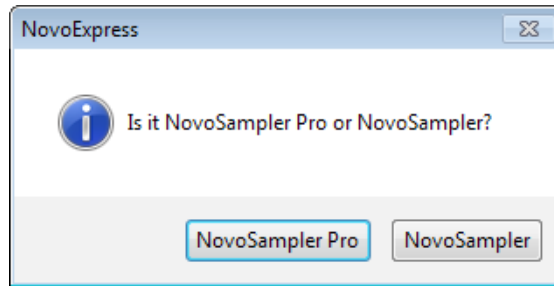

**Figure 2-3 Dialog Box to Choose NovoSampler Pro or NovoSampler
in NovoExpress Software Offline Mode**

2.2 Menu Bar

In the *Instrument* tab, the functions of the NovoSampler Pro are available when the NovoSampler Pro is connected. These functions include *Mix*, *Reset*, *Calibrate/Stop Calibration, Power Down/Power Up,* and *Manage Plate Types*. The *Menu Bar* is shown in Figure 2-4.
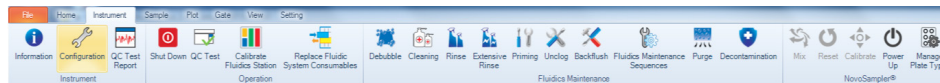

Figure 2-4 The Instrument Tab of Menu Bar

Mix

To use the *Mix* function, click the *Mix* button from *Instrument → NovoSampler Pro → Mix*. This function will immediately begin to mix the sample. The *Mix* button from the *Cytometer Control* panel has the same function.

Reset

To use the *Reset* function, click the *Reset* button from *Instrument → NovoSampler Pro → Reset*. The reset function will move the orbital shaker to the home position. This will allow a reset of the NovoSampler Pro in case of misalignment or error.

Calibrate

To use the *Calibrate* function, click the *Calibrate* button from *Instrument → NovoSampler Pro → Calibrate*. This function automatically aligns the NovoSampler Pro with the NovoCyte Flow Cytometer.

Stop Calibration

Once calibration is initiated, the *Calibrate* button in the *Instrument → NovoSampler Pro* menu will automatically become *Stop Calibration* button. Click *Stop Calibration* will stop the NovoSampler Pro calibration.

Power Up

When clicked (and the NovoSampler Pro is properly installed), the NovoCyte will power on and allow the operation of the NovoSampler Pro. After execution, the status indication LED on NovoSampler Pro will light up in green color. The NovoExpress will prompt a message to restart the software. After the software restart, a dialogue box requesting to calibrate the NovoSampler Pro will be shown. Click *Calibrate* to start the automatic NovoSampler Pro calibration procedure. The software features for control and use of the NovoSampler Pro will be available after restart of the NovoExpress software.

This feature is only visible when logged into NovoExpress Software with the account with **Power Down/Up NovoSampler** *privilege.*

Power Down

When clicked (and the NovoSampler Pro is properly installed), the NovoCyte will power off the NovoSampler Pro. After execution, the status indication LED on NovoSampler Pro will be off. The NovoExpress will prompt a message to restart the software. After the software restart, only the NovoCyte stand-alone features will be available.

This feature is only visible when logged into NovoExpress Software with the account with **Power Down/Up NovoSampler** *privilege.*

NovoSampler Pro should only be uninstalled when powered off. Uninstalling the NovoSampler Pro when it is still powered up may cause damage of the NovoSamplerPro.

Manage Plate Types

User can add, remove, calibrate, and modify plate settings in the *Manage Plate Types* window. To use this function, click the *Manage Plate Types* button from *Instrument → NovoSampler Pro → Manage Plate Types*. The *Manage Plate Types* dialog is shown in Figure 2-5.
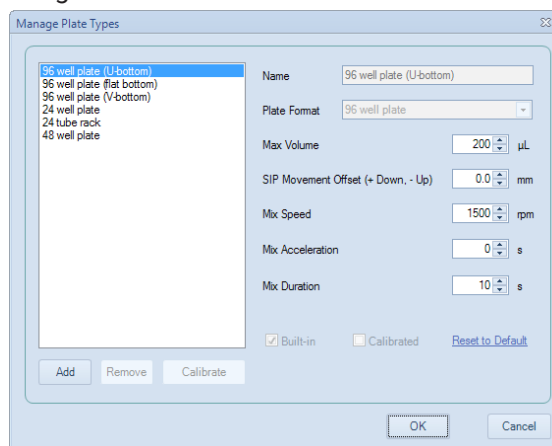

Figure 2-5 The Manage Plate Types Dialog Box

A new plate type must be calibrated before it could be used on NovoSampler Pro. Once calibrated, the same type of plate can be used for an experiment without any calibration needed.

Plate Type is defined by the format of the plate (24/48/96-well plate or 24 tube rack) and the geometry of the well. For example, two U-bottom 96-well plates are considered as different types if they have different height of the well.

Specify the plate name to be calibrated in details to help easy recognition of different plate type, such as Nunc™ 96-Well Polypropylene Plates, Corning® Carbo-BIND™ 96-well plates, etc.

All the default plate types are listed in the left box in the *Manage Plate Types* window. NovoExpress software has six built-in plate types including *96-well plate (U-bottom)*, *96- well plate (flat bottom)*, *96-well plate (V-bottom)*, *24-well plate, 24 tube rack* and *48-well plate*.

► *Name*: The name of the selected plate type. Edit it to rename the customer plate other than the built-in plate types. The name of the built-in plate type cannot be changed.

► *Plate Format*: The plate format of the selected plate type. Supporting format includes *"96-well plate"*, *"48-well plate"*, *"24-well plate"*, *"24 tube rack"*. This property cannot be changed.

► *Max Volume*: Set the limit for the maximum sampling volume user can input in the *Stop Condition* of *Cytometer Setting* panel for an experiment. Max Volume can be set in a range between 10 μL to 5000 μL.

Input an appropriate Max Volume to avoid running out of sample for a well during an experiment.

► *SIP Movement Offset (+ Down, - Up)*: The sample injection probe height offset relative to the default height when aspirating the sample from each well or tube. Positive value means the height is decreased. Negative value means the height is increased. The *SIP Movement Offset* can be set in a range between -10 mm to 5 mm.

Set SIP Movement Offset to be Positive can help to reduce the dead volume from each well.

⚠ *Be cautious to set the* **SIP Movement Offset**. *Incorrect setting would result in collision of SIP to the bottom of the well. During plate type calibration, NovoSampler Pro automatically calibrates the default SIP height. Normally, there is no need to adjust the* **SIP Movement Offset**.

► *Mix Speed*: The mix speed of the orbital shaker. The *Mix Speed* for 96, 48 and 24-well plate can be set in a range between 60 rpm to 3000 rpm. The *Mix Speed* for 24 tube rack can be set in a range between 60 rpm to 1800 rpm.

► *Mix Acceleration*: The acceleration time for the orbital shaker to reach the specified *Mix Speed*. *Mix Acceleration* can be set from 0 second to 10 seconds.

► *Mix Duration*: The mixing time for the orbital shaker to mix the sample. *Mix Duration* can be set from 1 second to 120 seconds.

► *Built-in*: Checked if the selected plate is the built-in plate type. Built-in plate types cannot be renamed or deleted. The built-in information is read only.

► *Calibrated*: Indicate if the selected plate type is calibrated. Only calibrated plate type can be used in experiments.

► *Reset to Default*: Click this button to reset the *Max Volume* and *SIP Movement Offset* of the selected plate type to default. Only built-in plate types can be reset to default.

The built-in plate type has been tested with biological samples, including human peripheral blood sample and cultured cell lines, to determine the default mixing settings. At the default mixing settings, the biological samples can be effectively mixed and uniformly suspended inside each well. The table below lists the default mixing settings and the maximum values allowed to be used for six built-in plate types.

🔍 *Determine the proper mixing speed and volume before running the experiment can help to avoid any potential spills during the experiment running.*

🔍 *When NovoSampler Pro is selected to work in Standard and High-Throughput mode, the software will automatically apply the default settings for mixing cycle, rinse cycle, mixing speed, acceleration, mixing duration, sampling volume and flow rate.*

Plate Type	Default Value				Max. Value			
	Mix Speed (rpm)	Mix Duration (s)	Accel-eration (s)	SIP Offset (mm)	Mix Duration (s)	Accel-eration (s)	Mix Speed (rpm)	Max Volume (µL)
24 tube rack	1500	10	0	0			1800	5000
96-well plate (V-bottom)	1500	10	0	0			3000	100
96-well plate (U-bottom)	1500	10	0	0	120	10	3000	200
96-well plate (flat-bottom)	1500	10	0	0			3000	200
48-well plate	1500	10	0	0			3000	1000
24-well plate	2500	10	0	0			3000	2000

Table 2-1 Default and Maximum Allowed Mixing Settings for the Built-in Plate Types of NovoSampler Pro

► *Remove*: Click this button to delete the selected plate type. Only non-built-in plate types can be removed.

► *Add*: Click this button to add a customer plate type. Input plate type name for new plate type in the prompted window. The *Copy From* function helps to quickly set up a customer plate type based on the format of an existing plate type. Choose a proper existing plate type from the *Copy From* drop-down list. The *Plate Format*, *Max Volume*, *SIP Movement Offset*, *Mix Speed*, *Mix Acceleration* and *Mix Duration* settings are copied from the *Copy From* plate type.
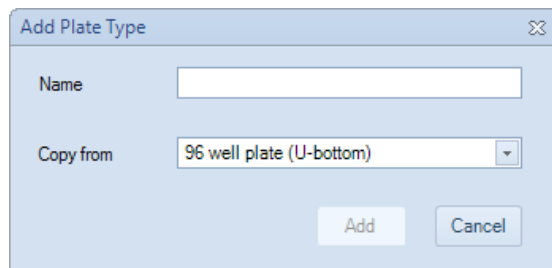

Figure 2-6 The Add Plate Type Dialog

► *Calibrate*: Click this button to calibrate the selected plate type. Only calibrated plate type can be used in experiments. The *Calibrated* checkbox indicates if the selected plate type is already calibrated. Figure 2-7 shows the dialog box prompted when the *Calibrate* button is clicked.
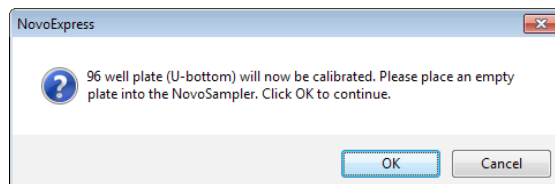

Figure 2-7 Dialog Box Prompted when Clicking Calibrate Button

Click *OK* after properly placing a plate of the selected plate type on the orbital shaker of the NovoSampler Pro. It will take several minutes to complete the plate type calibration process. If the plate is not placed properly, the calibration could fail and a dialog box shown in Figure 2-8 will be prompted. Reposition the plate properly and conduct the calibration again.
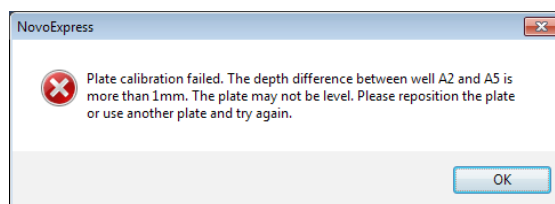

Figure 2-8 Dialog Box Prompted when the Plate Type Calibration Fails

⚠ *Before loading multi-well plates and NovoSampler 24 tube rack, identify the correct orientation to position the plate or the tube rack onto the orbital shaker. Make sure the sample plate is positioned properly on the orbital shaker and the clips hold the sample plate tightly. The sample plate should be inserted down to completely contact the surface of the orbital shaker without any tilting. Incorrect placement may cause misalignment between the sample wells and sample injection probe, resulting in damage to the sample injection probe or the orbital shaker.*

🔍 *Before calibrating the NovoSampler 24 tube rack, ensure a 12 X 75 mm tube is fully inserted in each position of the tube rack.*

🔍 *Once plate type calibration is initiated, the* **Calibrate** *button in the* **Manage Plate Types** *window will automatically become* **Stop Calibration** *button. Click* **Stop Calibration** *will stop the plate type calibration.*

2.3 Cytometer Control Panel

Figure 2-9 shows the *Cytometer Control* panel when NovoSampler Pro is connected. The *Active Sample Information* area contains the sample name and the sample well ID. In the *Experiment Control* area, there are two options to start the sample acquisition, including *Run Single Well* and *Run Plate*. Click *Run Single Well* to run a single well in the plate. Click *Run Plate* to run multiple wells.
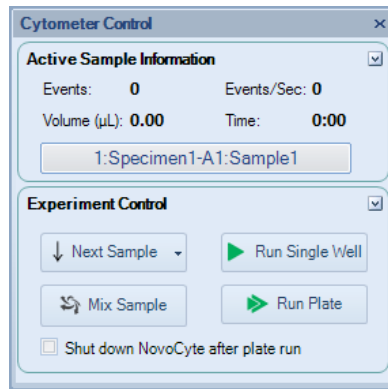
Figure 2-9 Cytometer Control Panel

When **Run Single Well** is selected, the NovoSampler Pro does not perform the mixing function before sample acquisition. To mix the sample, click the **Mix Sample** button in the **Cytometer Control** panel.

When **Run Single Well** is selected, NovoSampler Pro will automatically rinse the sample injection probe after sample acquisition. This SIP rinse function will minimize the carryover between samples.

Before acquiring a sample, ensure the sample is the Active Sample and the Well ID is correct. In the **Experiment Manager** panel, double-click a sample node to set the sample to be the Active Sample. The active sample will be indicated by a red arrow on the left of the sample in the **Experiment Manager** panel.

For additional information regarding the **Run Single Well** function, see Section *3.4 Single Sample Acquisition* in this guide. For additional information regarding the **Run Plate** function, see *Section 3.6 Automatic Sample Acquisition* in this guide.

NovoExpress can automatically shut down NovoCyte after plate run is completed. Check the *Shut down NovoCyte after plate run* checkbox in *Cytometer Control* panel to activate the automatically shut down function. When the plate run is completed, NovoExpress will prompt a 60 seconds count down dialog box to confirm whether or not to shut down the NovoCyte. The dialog box is shown in Figure 2-10. If no action taken after 60 seconds, NovoExpress will shut down the NovoCyte automatically.
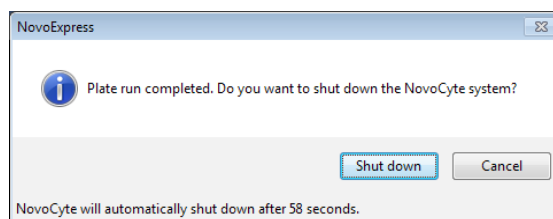

Figure 2-10 Dialog Box to Confirm whether or not to Shut down the NovoCyte

Automatic shutdown function is only effective for **Run Plate**. **Run Single Well** will not trigger the shut down process.

2.4   Experiment Manager Panel

Figure 2-11 shows the *Experiment Manager* panel when the NovoSampler Pro is connected. The samples listed in the *Experiment Manager* panel include the Well ID and the sample name, separated by a colon. Click the sample node to select the sample, right-click and select *Rename* to rename the sample. When renaming the sample, both the Well ID and the sample name can be modified. Specimen is

also associated with a Specimen ID. The Specimen ID is the number before the specimen name, separated by a colon. The Specimen ID is used to identify specimen and can be modified. For additional information regarding the Specimen ID, see *Section 2.5 Plate Manager Panel* in this guide.
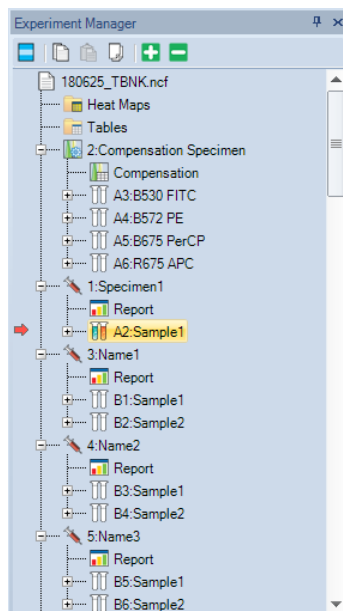

Figure 2-11 Experiment Manager Panel

For additional information regarding the **Experiment Manager** *panel, refer to the Section 6 Experiment Manager in NovoExpress® Software Guide.*

2.5   Plate Manager Panel

The *Plate Manager* panel (Figure 2-12) will only be available when the NovoSampler Pro is installed or the *NovoSampler installed* checkbox is checked in the *Instrument Configuration* window in the NovoExpress software offline mode. This management tool provides users with an intuitive and convenient way to manage samples on a multi-well plate or on NovoSampler 24 tube rack. The *Plate Manager* also allows user to set up the working mode, plate type, mixing and rinsing conditions.
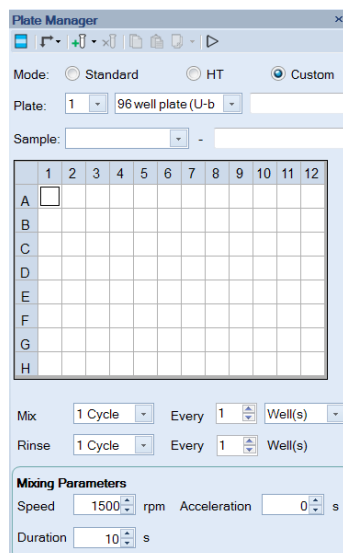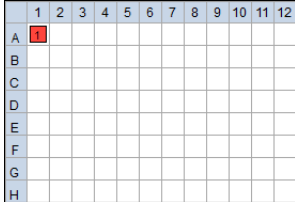

Figure 2-12 Plate Manager Panel
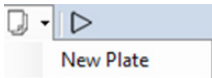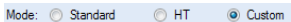
Table below provides details on all functions of the *Plate Manager* panel:

Icon	Description
	The *Plate Manager* panel displays an 8 x 12 grid indicating a 96-well plate map. In settings for a 24 tube-rack or a plate type other than a 96-well plate, invalid wells will be grayed out.

In one plate, multiple samples can be set with the same Well ID. In the grid, one well will show one sample at one time. The currently visible sample in a well is defined as selected sample of a well.

The state of each well can be:

► ☐ This icon indicates that this well is selected. Multiple wells can be selected at the same time.

► 1 The number is the Specimen ID and indicates to which specimen the selected sample belongs to.

► 1 An underlined number indicates that the selected sample has acquired data.

► 1 The arrow in the upper right corner indicates that the well has multiple samples. Context menu of the well will list all samples.

► *1* The italicized number indicates the acquisition sequence of the selected sample.

► 1 A red background indicates the well contains the Active Sample.

► 1 A green background indicates that the well contains sample in queue to be collected.

► 1 / 1 An alternating green and dark green background indicates that the well contains sample currently in acquisition.

► 1 / 1 An alternating green and red background indicates that the well contains sample currently in acquisition and also is the Active Sample.

The following operations can be operated on the plate grid:

► Click: Click the header of a row or a column to select the entire row or column. Click the top left corner cell selects all wells in the plate.

► Right-click: Displays context menu.

► Double-click: Set selected sample to be the Active Sample.

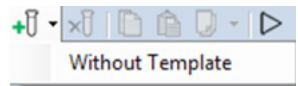► Drag: Drag to select multiple wells in a rectangular range.

► Ctrl + Click: Hold Ctrl to select multiple individual wells.

► Shift + Click: Hold Shift to select consecutive wells between two wells.

Icon	Description
	Work List: View and edit *Work List*.

► ► ►
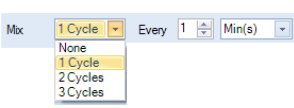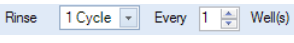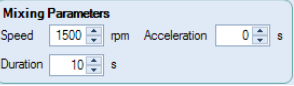
Icon	Description
	Samples Created Order: Selects whether to automatically proceed horizontally across the plate or vertically across the plate when creating new samples.
	New Sample(s) on Selected Well(s): Creates new samples on the selected wells. The created samples will have the same template as the Active Sample with the same compensation matrix, report, data analysis, and *Cytometer Setting*. The samples will be created in the specimen with name specified in *Specimen Name* box. If specified specimen name is empty or does not exist, a new specimen will be created. The created sample name will use the name specified in the Sample Name box as a prefix. If the sample name box is empty, the default sample name prefix (sample 1, sample 2, …) will be used.
	Without Template: Creates new samples on the selected wells without template applied. The compensation matrix, report, and data analysis template will be empty. But the cytometer setting will be the same as the current cytometer setting in the *Cytometer Setting* panel.
	Delete Sample(s) on Selected Well(s): Deletes the selected samples from the selected wells. If one well has multiple samples defined, only the selected sample will be deleted.
	Copy Sample(s) on Selected Well(s): Copies templates of selected samples from the selected wells. If no sample has defined in the selected wells, this function is disabled. If part of selected wells has samples defined, only the wells which have sample defined will be copied.
	Paste Sample(s) on Selected Well(s): Pastes sample template or sample events to the selected wells according to copied content. ► If all the selected wells have samples defined, then it applies templates of the copied samples to the selected samples of selected wells one by one repeatedly. ► If all the selected wells have no samples defined, then it creates samples with the same templates as copied samples on the selected wells. The samples will be created in the specimen with the name specified in *Specimen Name* box. ► If part of the selected wells have samples defined and the rest part have no samples defined, or the selected samples of the selected wells are in different specimen, this tool will be disabled. ► If *Copy Events* is selected from context menu of a well, this tool will be available when only one well is selected and the selected sample of the selected well contains no events.

► ► ►

Icon	Description
New Plate	*Duplicate Sample(s) on Selected Well(s)*: Creates new samples on empty wells, duplicating the template of the selected samples of the selected wells. If part of the selected wells have samples defined and the rest part have no samples defined, or the selected samples of the selected wells are in different specimen, this tool will be disabled. If the specimen contains more than one sample, and all the samples are selected, clicking this icon will create duplicated samples under a newly created specimen. *New Plate*: Creates new plate and specimen, and creates new samples in the new specimen, duplicating the template of the selected samples of the selected wells. The new samples have the same well ID with the selected wells.
▷	*Show Run Order*: When selected, a blue outline shows on the icon ▷ . The sample acquisition sequence will be shown in the plate view.
Mode: ○ Standard ○ HT ● Custom	*Mode*: User is allowed to select the working mode (Standard, High- Throughput and Custom) of the NovoSampler Pro in this area. Default mixing parameters (i.e. Mixing Speed, Acceleration, Duration, Mix and Rinse pattern), Stop condition and Flow Rate setting will be automatically applied when *Standard* or *HT* mode is selected. User can only customize these settings when *Custom* mode is selected. 🔍 *Selecting the working mode of NovoSampler Pro is only available before the sample is created.*
Plate: 1 ▾ 96 well plate (U ▾)	This area is designed to input plate information. The left drop-down box is the *Plate ID*. The *Plate ID* is a positive integer and can be modified by highlighting the area and entering a new number. The middle drop-down box is the *Plate Type*. The drop-down box lists all the available plate type defined in the *Manage Plate Types* window from the *Instrument → NovoSampler Pro* menu. Pull down the list and select the corresponding plate type for the experiment. The right box is the *Plate Name*. Input plate name in this box to help identify the plate. 🔍 *The plate type cannot be modified once the sample acquisition has begun.*
Sample: 1:Specimen1 ▾ - Sample1	This area is designed to input the specimen and sample information. The left drop-down box is the *Specimen ID* and *Specimen Name*, separated by a colon. The right box is the *Sample Name*. When creating new samples, the samples will be created in a specimen with specimen name specified in the *Specimen Name* box. If the *Specimen Name* box is empty or does not exist, a new specimen will be created. The default new specimen name is *Specimen1, Specimen2,…* The default sample name is *Sample1, Sample2,…*

► ► ►

2

Icon	Description
	Mix: Sets the mixing conditions. *Mix* can be set for *None* (no mixing), *1 Cycle, 2 Cycles, 3 Cycles* for every defined number of wells or every defined number of minutes.
	🔍 *Default mixing conditions (i.e. Mix 1 Cycle Every 1 Well) will be automatically applied and cannot be modified by user when Standard or HT mode is selected.*
	Rinse: Sets the rinse cycle to clean the sample injection probe. *Rinse* can be set for *None* (no rinse), *1 Cycle, 2 Cycles, 3 Cycles* for every defined number of wells.
	🔍 *Default rinse conditions (i.e. Rinse 1 Cycle Every 1 Well when Standard mode is selected, or None when HT mode is selected) will be automatically applied and cannot be modified by user when Standard or HT mode is selected.*
	🔍 *There is always one rinse cycle at the end of each plate run even the **Rinse** setting is set to **None**.*
	Mixing Parameters: Sets the mixing parameters for the orbital shaker. When NovoSampler Pro works in Custom mode, these mixing parameters for each plate type are defined in the Manage Plate Type window from the *Instrument → NovoSampler Pro* menu, and can be modified in this area for the current experiment. When NovoSampler Pro works in Standard or HT mode, default mixing parameters will be automatically applied and cannot be modified by user. Please refer to Table 2.1 in this guide for more details.
	Speed: The mix speed of the orbital shaker.
	Acceleration: The acceleration time for the orbital shaker to reach the specified *Mix Speed*.
	Duration: The mixing time for the orbital shaker to mix the sample in one cycle.

In addition to the *Tools* menu, the *Plate Management* panel also provides a rich set of context menu actions.

Context menu when a single well is selected

If the selected well has sample already defined, the right-click menu displays a list of the samples defined in the selected well and the software functions available to be used. This context menu is the same as the context menu by right-clicking a sample node in the *Experiment Manager* panel. From this context menu, you can quickly select the defined sample, open, close, delete, and create plots, copy and paste templates, copy and delete events, delete, rename and duplicate the sample, import template or the data from a FCS file, or export as a template or the data in FCS and CSV formats, move the sample to the selected specimen. For additional information regarding these functions, refer to the *Section 6.2.2 Right-Click Menu* in *NovoExpress® Software Guide*.

If the selected well has no sample defined, the right-click menu has two items available, *New* and *Paste*. *New* will create new sample without a template. *Paste* will create a new sample with the same template as the copied sample.
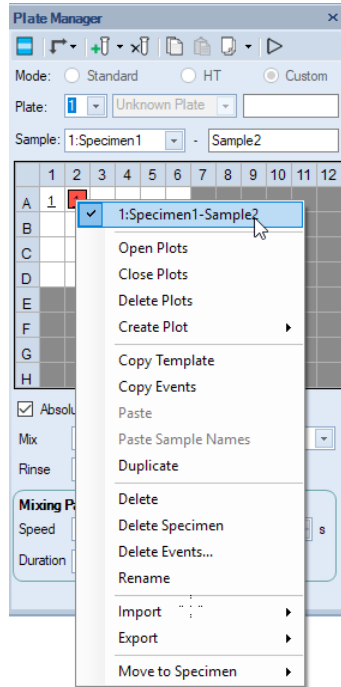
Figure 2-13 Context Menu when a Single Well which has Sample Defined is Selected

If the selected well has no sample defined, the right-click menu (Figure 2-14) generally has two items available, *New* and *Batch Creation of New Specimens*. *New* will create new sample without a template. *Batch Creation of New Specimens* allows user to create new specimen(s) with customized specimen and sample organization. Refer to *Context menu when multiple wells are selected* for more details on this function.

The Paste Sample Names will be available if user copied the sample name in previous operation.
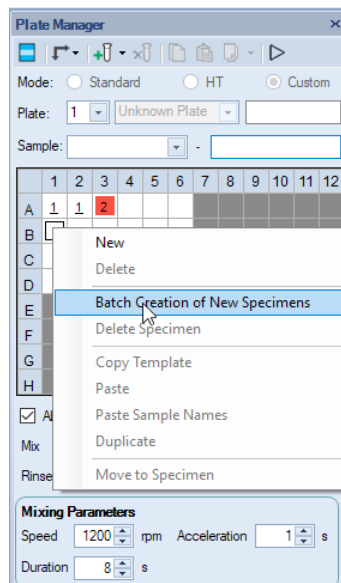
Figure 2-14 Context Menu when a Single Well which has no Sample Defined is Selected

Context menu when multiple wells are selected
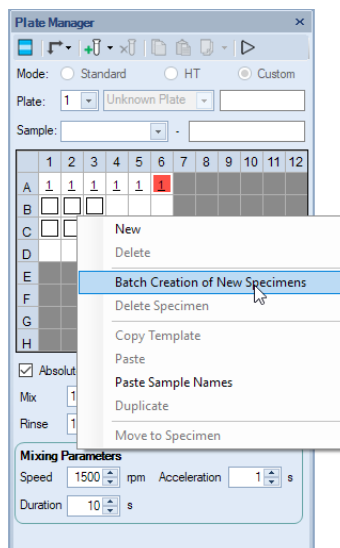
Figure 2-15 Context Menu when Multiple Wells are Selected

► *New*: Same as the *New Sample(s) on Selected Well(s)* tool in the toolbar.

► *Delete*: Same as the *Delete Sample(s) on Selected Well(s)* tool in the toolbar.

► *Batch Creation of New Specimens*: Create new specimen(s) with customized specimen and sample organization. When clicked, software will prompt following window (Figure 2-16). User need define item(s) in this window, click *OK* to complete or cancel to go back to the previous window.
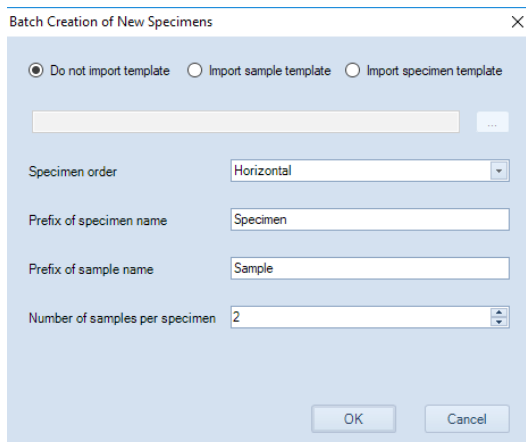
Figure 2-16 Dialog Window for Batch Creation of New Specimens
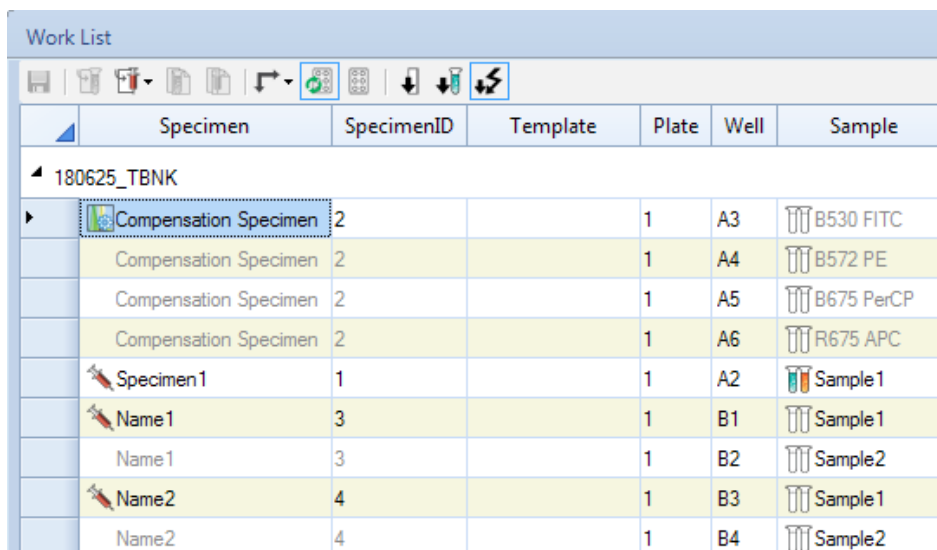
• *Do not import template*: Select this option if no templates need to be imported

• *Import sample template*: Select this option if sample template needs to be imported for newly created samples. Click ··· to load the template file (.nct file).

• *Import specimen template*: Select this option if specimen template needs to be imported for newly created specimens. Click ··· to load the template file (.nct file).

• *Specimen order*: direction of creating the specimens for selected samples. For example, when A1, A2, B1, B2 are selected, and Horizontal is selected as the specimen order, and 2 samples per specimen is selected, software will automatically create two new specimens and new samples in these four wells. Sample A1 and A2 will be placed under the first newly created specimen. Sam-

ple B1 and B2 will be placed under the second newly created specimen.

- *Prefix of specimen name*: Set the prefix for the name of the newly created specimen(s).

- *Prefix of sample name*: Set the prefix for the name of the newly created sample(s).

- *Number of samples per specimen*: Set the number of newly created samples per each newly created specimen.

▶ *Delete Specimen*: Delete the selected specimen and associated samples on selected wells.

▶ *Copy Template*: Same as the *Copy Sample(s) on Selected Well(s)* tool in the toolbar.

▶ *Paste*: Same as the *Paste Sample(s) on Selected Well(s)* tool in the toolbar.

▶ *Paste Sample Names*: Rename samples of the selected wells with the names copied from a spreadsheet program like Microsoft Excel, or create new samples with copied name on selected wells if there is no sample defined. If part of selected wells have samples defined and the rest part have no samples defined, this menu will be disabled.

*If you have an Excel spreadsheet defining the sample names in a plate format, use **Paste Sample Names** function from the right-click context menu to copy the sample name from the spreadsheet and quickly create new samples for a plate in NovoExpress Software.*

▶ *Duplicate*: Same as the *Duplicate Sample(s) on Selected Well(s)* tool in the toolbar.

▶ *Move to Specimen*: Move the selected sample(s) to the selected specimen or new specimen.

2.6   Work List

The *Work List* can be used to set up the sample for a plate. The *Work List* allows users to create new samples, import specimens or samples from a template, duplicate specimens or samples, and copy and paste sample information. To open the *Work List* window, click the *Work List* button from *Home → Experiment → Work List,* or alternatively, click the *Work List* icon , from *Experiment Manager* panel or *Plate Manager* panel. The *Work List* window is shown in Figure 2-17.

Figure 2-17 Work List Window

Compared to the *Work List* in NovoExpress Software with no NovoSampler Pro installed, there are three additional icons shown on the menu bar. The created *Work List* with NovoSampler Pro installed will also include the *Plate ID* and *Well ID* columns. The other functions remain the same. Refer to the *Section 4.2 Work List* in *NovoExpress® Software Guide* for the detailed information and functions to create a *Work List*. The table below describes the functions of the three additional icons.

Icon	Description
	Well ID Setting Direction When new samples are created in the *Work List*, the *Well ID* will be assigned sequentially. Use the *Well ID Setting Direction* button in the *Work List* window to set whether new samples are created in a row-by-row sequence or a column-by-column sequence for a plate.
	Automatic Setting Well ID When selected, a blue outline will show on the icon. When *Well ID* of a sample is modified manually, the NovoExpress Software will automatically assign new *Well ID* for blank samples below that sample in an order set in the *Well ID Setting Direction* based on the plate type information.

► ► ►
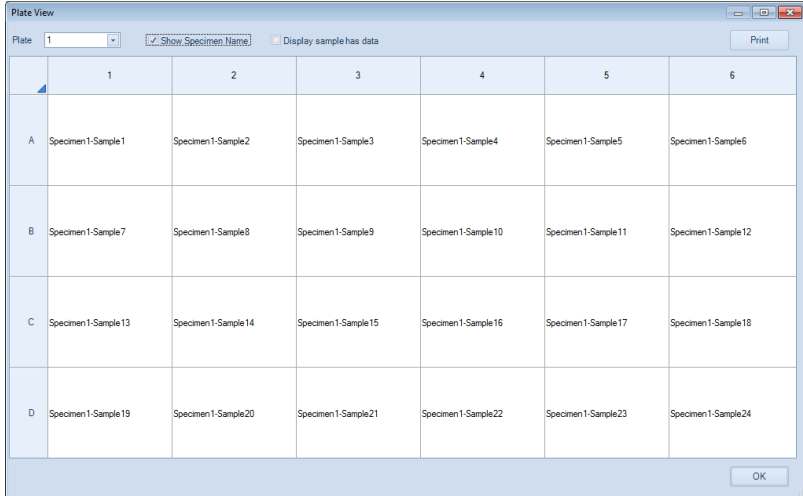
Icon	Description
	Plate View Click this icon opens the *Plate View* window. *Plate View* window is read only and is used to check the information of the blank samples on a plate view. The *Plate* drop-down menu lists the *Plate ID* for all the created plates. Select a *Plate ID* from the drop-down menu and view the sample information for this plate. Check the *Show Specimen Name* checkbox to enable the specimen name associated with each well to be shown. Check the *Display sample has data* checkbox to also show samples which have collected data. **Figure 2-18 Plate View Window**

> The **Plate ID** and **Well ID** in the **Work List** can be modified to assign Plate ID and Well ID for the samples.

> When collecting repeats from a sample well is desired, multiple samples can be assigned to the same Well ID.

2.7   Statistical Tables

The statistical tables provide a summary for multiple samples, gates, and parameters, enabling batch analysis and data comparison. For data generated using NovoSampler Pro, two additional columns *Plate ID* and *Well* ID are available.

To show or hide *Plate ID* or *Well ID* column in statistical table, click the *Show Columns* button ⬆, and select or deselect the column from the drop-down menu. The drop-down menu is shown in Figure 2-19.
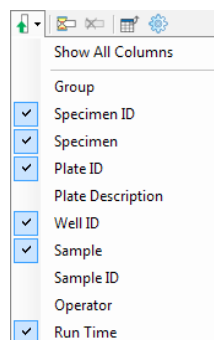
Figure 2-19 Drop-down Menu of the Show Columns Button

> For additional information regarding the **Statistical Tables**, refer to the *Section 5.6 Statistical Tables* in *NovoExpress® Software Guide*.

2.8   Options

Additional settings for NovoSampler Pro operation can be set by clicking *Settings → Options → Experiment* from the menu. The window is shown in Figure 2-20.
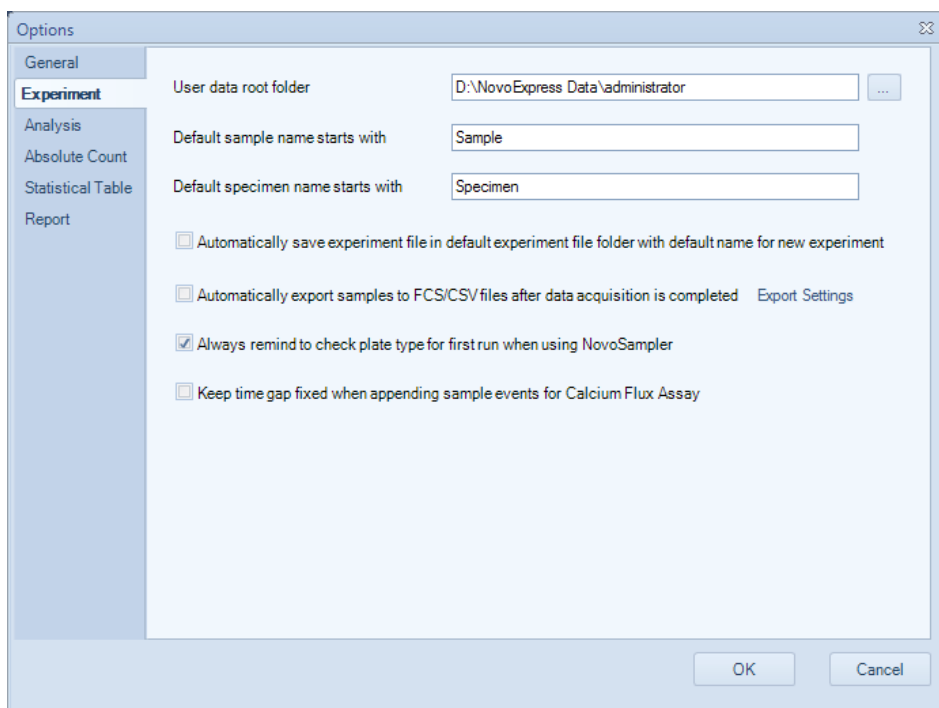
Figure 2-20 Define Default Experiment Settings using Options Window

The following settings are specific to the operation of the NovoSampler Pro:

► *Always remind to check plate type for first run when using NovoSampler*: With this setting checked, a window will be prompted to remind user to check if the correct plate type is selected before starting the experiment.

> It is highly recommended to enable this setting to remind user to check and make sure the correct plate type is selected for the experiment to avoid collision of the sample injection probe.

3.   Sample Acquisition

This chapter describes the sample acquisition process using NovoSampler Pro, including the following sections:

► Preparation

► Experiment Setup

► Instrument QC Test

► Single Sample Acquisition

► Edit Work List

► Automatic Sample Acquisition

► Analysis of Data

► Shutdown

In this chapter, a 96-well plate will be used for sample acquisition. Firstly, a QC Test using ACEA Flow Cytometer QC Particles will be performed in well A1. The ACEA QuadriTest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC Reagent (Cat # 8730008) will be used to prepare a single sample of human peripheral blood in well A2. The sample will be used to determine the gate for the lymphocyte sub-population. Well A3, A4, A5, and A6 are compensation beads labeled respectively with FITC, PE, PerCP, and APC conjugated antibodies for automatic fluorescence compensation using NovoExpress software and generating the compensation matrix. In well B1 to E12, 48 samples will be created and analyzed for 24 specimens with 2 samples in each specimen. For each specimen, one sample will be stained with ACEA QuadriTest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC Reagent (Cat # 8730008) and the second sample will be stained with ACEA QuadriTest CD3 FITC/CD16+CD56 PE/CD45 PerCP/CD19 APC Reagent (Cat # 8730009).

3.1   Preparation

Before using the NovoSampler Pro for sample acquisition, please be familiar with the following operation and information:

► NovoCyte Flow Cytometer startup and shutdown procedures

► QC testing procedure

► NovoExpress Software's *Cytometer Settings*, *Work List*, and *Experiment Manager* panels

► NovoSampler Pro sample volume requirements

For additional information regarding the operation of the NovoCyte Flow Cytometer, refer to the Section 2 Instrument Operation in NovoCyte® Flow Cytometer Operator's Guide.

For additional information regarding using the NovoExpress Software, refer to the NovoExpress® Software Guide.

Minimum Sample Volume Requirements for the NovoSampler Pro

► For samples in a 12 × 75 mm tube, the minimum sample volume is 90 µL.

► For samples in a 96-well plate, the minimum sample volume in a well is 90 μL.

► For samples in a 48-well plate, the minimum sample volume in a well is 210 μL.

► For samples in a 24-well plate, the minimum sample volume in a well is 310 μL.

► The recommended sample concentration range is 1×10^6 to 5×10^6 cells/mL.

Confirm Sufficient Sheath Fluid Volume

During sample acquisition, the NovoCyte Flow Cytometer consumes about 0.4 liters of sheath fluid per hour. A warning will appear if the sheath fluid in the sheath fluid container is less than 0.9 liters. To avoid the termination of the plate run due to the lack of sheath fluid or a full waste container, before starting an automatic sample acquisition with NovoSampler Pro, always make sure there is a sufficient volume of sheath fluid in the sheath fluid container and the waste container has enough capacity.

3.2   Experiment Setup

Start the NovoCyte Flow Cytometer and NovoExpress Software according to the process described in the *NovoCyte® Flow Cytometer Operator's Guide*. As the flow cytometer powers up, the NovoSampler Pro will also start and be ready to use.

Before sample acquisition using the NovoSampler Pro, select the plate type from the *Plate Manager* panel (Figure 3-1). If the *Plate Manager* panel is not visible, check the box from *View → Show → Plate Manager* to open it. The plate type can then be selected from the *Plate Type* drop-down menu. In this example, the *96-well plate (U-bottom)* is selected. For other compatible plate types, refer to *Section 1.1 Hardware Overview* in this guide.
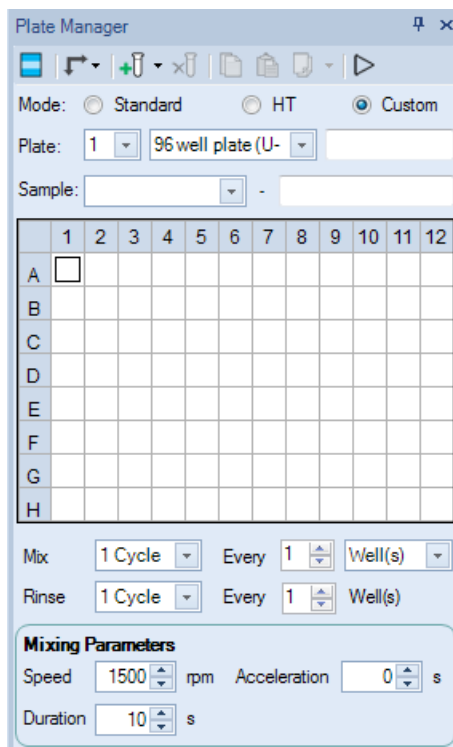
Figure 3-1 Plate Manager Panel

⚠️ *Before starting sample acquisition, ensure that the correct plate type is select-ed. An incorrectly selected plate type may result in the collision of the sample injection probe with the plate and could cause damage to the instrument.*

3.3   Instrument QC Test

Quality Control (QC) test is used to monitor the performance of NovoCyte Flow Cytometer. Use the ACEA Flow Cytometer QC Particles daily after startup to QC the NovoCyte instrument and make sure it functions properly.

3.3.1   Prepare QC Particles

To prepare the ACEA Flow Cytometer QC Particles for the QC test:

1 Label a 12 × 75 mm tube for the QC test. Add 1 mL dilution buffer (0.8 mL PBS and 0.2 mL NovoRinse solution) into the tube.

2 Take one bottle of QC Particles from storage. Invert the bottle a few times by hand to mix the particles thoroughly.

3 Add two drops of QC Particles (Cat. # 8000004) into the test tube.

4 Vortex the test tube to mix the QC Particles thoroughly.

5 Transfer 150 µL of the QC Particles sample from the test tube to the A1 well of a 96-well plate and run the QC Test immediately. If the prepared QC Particles sample is not intended to be run immediately, store the sample in a 2-6°C refrigerator away from light. The prepared QC Particles sample should be used within four hours after preparation.

■

3.3.2   Run the QC Test

To load the sample plate and run the QC test:

1 Open the cover of the NovoSampler Pro and place the sample plate on the orbital shaker.

⚠️ *Make sure the plate is positioned with the correct orientation onto the orbital shaker with the clips of the orbital shaker holding the plate tightly.*

▶ ▶ ▶

② In the NovoExpress Software, open the QC Test window by clicking *Instrument →
Operation → QC Test* button. The *QC Test* window is shown in Figure 3-2.

Figure 3-2 QC Test Step 1

③ In the window, enter the name of the operator, select the *QC Particles Lot ID*, and
select the Well ID of the QC Particles sample. In this example, the Well ID is A1. Click
Next and follow the software prompt to run the QC test and view the test reports. For
more information regarding the *QC Test* function, refer to the *Section 2.3 Instrument
Quality Control* in *NovoCyte® Flow Cytometer Operator's Guide*.

3.4   Single Sample Acquisition

This section describes the collection of a single sample of human peripheral
blood prepared using the ACEA QuadriTest CD3 FITC/CD8 PE/CD45 PerCP/CD4
APC Reagent. The sample will be used to set up the gate for the lymphocyte
subpopulation. Based on this single sample, settings can be optimized for other
human peripheral blood samples in the example described in this chapter, includ-
ing setting up a proper acquisition threshold and determining the gates for the
lymphocyte subpopulation.

To begin the collection of the single sample in well A2, follow the procedure:

① After the completion of the QC Test, the orbital shaker will return to the home posi-
tion. After the orbital shaker moves to home position, open the NovoSampler Pro
cover and remove the sample plate.

② Add 150 μL of the sample of human peripheral blood stained with ACEA QuadriTest
CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC Reagent to well A2 of the sample plate.

③ Place the sample plate onto the orbital shaker of the NovoSampler Pro and close
the cover.

④ In NovoExpress Software, create a new experiment file. Click the *Next Sample* button
on the *Cytometer Control* panel to create a new sample (named *A1: Sample1* by de-
fault) and specimen (named *Specimen1* by default). Save the file to a specific folder
on NovoCyte workstation by clicking *File → Save*.

▶▶▶

5 Setting conditions for sample acquisition is described in the *Section 3 Running Samples* in *NovoCyte® Flow Cytometer Operator's Guide*. In this experiment, the parameters should be set to collect the Height parameter from FSC, SSC, B530, B72, B675, and R675. The aliases for *B530, B572, B675,* and *R675* could be modified to *CD3 FITC, CD8 PE, CD45 PerCP,* and *CD4 APC*, respectively. Set the stop condition to *50 µL*. Set the sample flow rate to *Low*. Set the threshold to collect samples with *FSC-H greater than 100,000*.

The volume stop condition must be set when using NovoSampler Pro.

6 Change the sample information. Set the Well ID as *A2* using one of the two following methods :

► Click *Sample1* in the *Experiment Manager* panel to select the node (Figure 3-3). Right-click and select *Rename*. The Well ID can now be modified to *A2*.
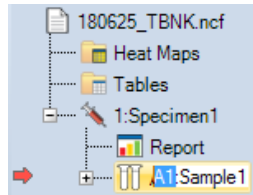
Figure 3-3 Modify Well ID in the Experiment Manager Panel

► In the *Active Sample Information* window of the *Cytometer Control* panel (Figure 3-4), click the sample name and modify the Well ID to *A2*.
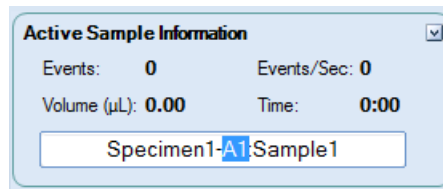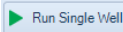
Figure 3-4 Modify Well ID in the Cytometer Control Panel

*The well can be named from a range depending on the plate type selected from the **Plate Manager** panel. If a 96-well plate is selected, the wells can range from A1 to H12. If a 24-well plate or the 24-well tube rack is selected, the wells can range from A1 to D6.*

7 In the *Cytometer Control* panel, click the *Run Single Well* button ▶ Run Single Well to start sample acquisition from well A2.

8 In the Workspace, create a density plot by clicking the *Density Plot* button from the toolbar. Change the x-axis parameter to *CD45 PerCP-H* and the y-axis parameter to *SSC-H* (Figure 3-5). Use the *Zoom In* tool , to display the appropriate coordinate range for the sample. Draw an *Elliptical Gate* to gate the lymphocyte population on the plot, and rename the gate as *Lymphocyte*.
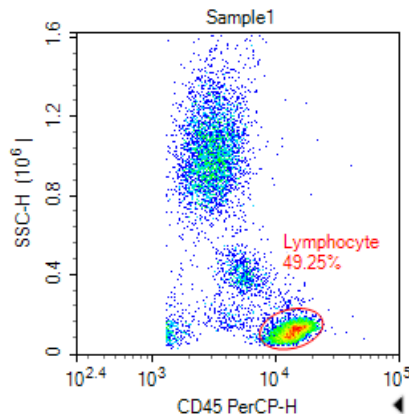
Figure 3-5 Create Density Plot and Draw Lymphocyte Gate

3.5   Edit Work List

In this section, the *Work List* will be used to create auto compensation samples and experiment samples.

3.5.1   Creating Compensation Control Samples

To create the auto compensation samples, click *Home → Compensation → Auto Compensation* to open the *New Auto Compensation* window. In the window, select the four parameters used in the experiment, *FITC (B530), PE (B572), PerCP (B675),* and *APC (R675)*. The software will then create four single-stained samples to be used to calculate fluorescence compensation, and the software will automatically assign the samples to wells A3 to A6. The four created samples will be added to the *Compensation Specimen* node in the *Experiment Manager* panel as shown in Figure 3-6.

For detailed information about the auto compensation, refer to Section 3.5.1 Automatic Fluorescence Compensation in NovoCyte® Flow Cytometer Operator's Guide.
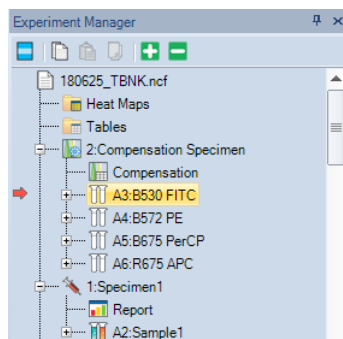
Figure 3-6 Creating Compensation Control Samples

3.5.2   Creating Experiment Samples

New samples could be created through the *Work List* window or through the *Plate Manager* panel.

Create experiment samples through the Work List window

To open the *Work List*, click the *Work List* button from *Home → Experiment → Work List*. Alternatively, the *Work List* window can be opened by clicking the *Work List* button , from the *Experiment Manager* panel or *Plate Manager* panel.

After opening the *Work List* window:

1 Set the *Well ID Setting Direction* to *Horizontal,* and enable *Automatic Setting Well ID* (a blue outline shows on the icon).

►►►
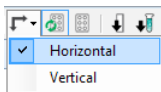
2 Right-click the header in the row for *Specimen1 Sample1* and select *Copy*. Move the cursor to the last empty row below *Specimen1 Sample 1*, right-click the header and select *Insert Copied Rows as Specimen*. The software will create a new specimen, named *Specimen2*, and the specimen will contain a sample, named *Sample1*, which will contain the *Lymphocyte* gate from *Specimen1 Sample1*.

3 Double-click the *Specimen Name* cell of the *Specimen2 Sample1* row to modify the specimen name. Rename the specimen as *Name1*. Double-click the *Stop Condition* cell and set the stop condition to 5,000 events in the *Lymphocyte* gate.

4 Click the header of the *Name1 Sample1* row to select the entire row. From the toolbar of the *Work List* window, click the *Duplicate as Sample* button. A new sample will be created within the *Name1* specimen and named *Sample2*. In the new sample *Name1 Sample2,* modify the alias under the *B572* and *R675* parameters. Rename the alias for *B572* as *CD16 + CD56 PE* and the alias for *R675* as *CD19 APC*.

5 Select both *Name1 Sample1* and *Name1 Sample2* rows. Click the *Duplicate as Specimen* button from the toolbar 23 times to create the remaining 23 specimens and 46 samples. Rename each specimen as *Name2*, *Name3*, etc.

6 In the *Name1 Sample1* row, rename the *Well ID* to *B1*. The remaining sample wells will automatically update. The complete *Work List* is shown in Figure 3-7.

Work List

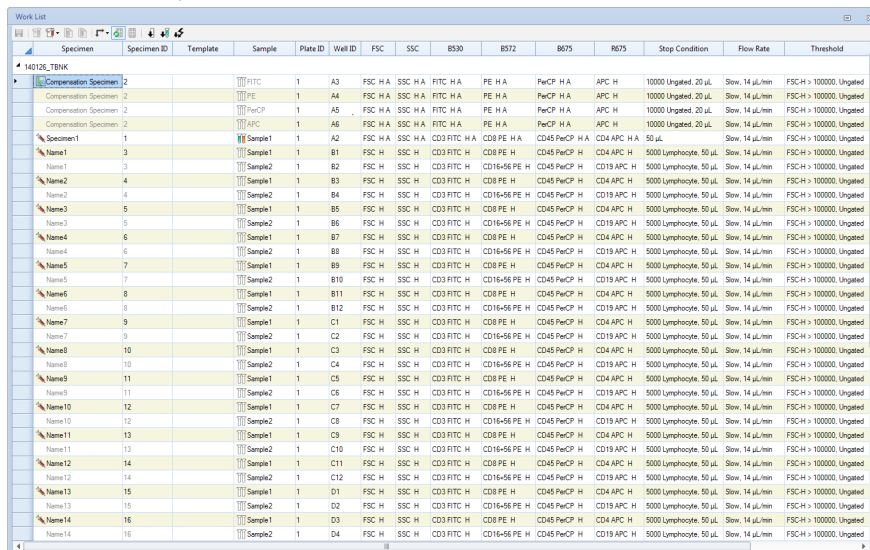| Specimen	Specimen ID	Template	Sample	Plate ID	Well ID	FSC	SSC	B530	B572	B675	R675	Stop Condition	Flow Rate	Threshold
140126_TBNK														
Compensation Specimen	2		FITC	1	A3	FSC H A	SSC H A	FITC H A	PE H A	PerCP H A	APC H	10000 Ungated, 20 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Compensation Specimen	2		PE	1	A4	FSC H A	SSC H A	FITC H A	PE H A	PerCP H A	APC H	10000 Ungated, 20 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Compensation Specimen	2		PerCP	1	A5	FSC H A	SSC H A	FITC H A	PE H A	PerCP H A	APC H	10000 Ungated, 20 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Compensation Specimen	2		APC	1	A6	FSC H A	SSC H A	FITC H A	PE H A	PerCP H A	APC H	10000 Ungated, 20 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Specimen1	1		Sample1	1	A2	FSC H A	SSC H A	CD3 FITC H A	CD8 PE H A	CD45 PerCP H A	CD4 APC H A	50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name1	3		Sample1	1	B1	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name1	3		Sample2	1	B2	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name2	4		Sample1	1	B3	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name2	4		Sample2	1	B4	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name3	5		Sample1	1	B5	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name3	5		Sample2	1	B6	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name4	6		Sample1	1	B7	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name4	6		Sample2	1	B8	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name5	7		Sample1	1	B9	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name5	7		Sample2	1	B10	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name6	8		Sample1	1	B11	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name6	8		Sample2	1	B12	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name7	9		Sample1	1	C1	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name7	9		Sample2	1	C2	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name8	10		Sample1	1	C3	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name8	10		Sample2	1	C4	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name9	11		Sample1	1	C5	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name9	11		Sample2	1	C6	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name10	12		Sample1	1	C7	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name10	12		Sample2	1	C8	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name11	13		Sample1	1	C9	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name11	13		Sample2	1	C10	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name12	14		Sample1	1	C11	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name12	14		Sample2	1	C12	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name13	15		Sample1	1	D1	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name13	15		Sample2	1	D2	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name14	16		Sample1	1	D3	FSC H	SSC H	CD3 FITC H	CD8 PE H	CD45 PerCP H	CD4 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated
Name14	16		Sample2	1	D4	FSC H	SSC H	CD3 FITC H	CD16+56 PE H	CD45 PerCP H	CD19 APC H	5000 Lymphocyte, 50 µL	Slow, 14 µL/min	FSC-H > 100000, Ungated

Figure 3-7 Work List Window after Experiment Samples Created

7 Check the *Work List* to ensure the settings are correct, and click the *Apply Modifications* button from the toolbar to save the changes made to the *Work List*. You will see the new samples automatically showing up in the *Experiment Manager* panel (Figure 3-9).

Create experiment samples from the Plate Manager panel

1 Open the *Plate Manager* panel by checking the *View → Show → Plate Manager* box if it is not visible.

2 Double click well A2 to set sample *Specimen1 Sample1* as the Active Sample. Click well B1, input *Name1* in the specimen name box, input *Sample1* in the sample name box, and click the *New Sample(s) on Selected Well(s)* button in the *Plate Manager* panel toolbar to create a new sample with the same template as sample *Specimen1 Sample1* in well B1.

3 In the *Cytometer Setting* panel, change the event number stop condition to 5000 events on gate *Lymphocyte*.

►►►

④ Set the *Samples Created Order* tool 🡒 in the *Plate Manager* panel toolbar to *Horizontal*.

⑤ Click the *Duplicate Sample(s) on Selected Well(s)* button 🗍 in the *Plate Manager* panel toolbar, rename the created sample as *Sample2*, modify alias of B572 as *CD16+56 PE* and modify alias of R675 as *CD19 APC*.

⑥ Select both well B1 and B2, click the *Duplicate Sample(s) on Selected Well(s)* button 🗍, in the *Plate Manager* panel toolbar 23 times to create the remaining 23 specimens and 46 samples. Sequentially rename each of the newly created specimens as *Name2*, *Name3*, etc. The created samples in the *Plate Manager* panel are shown in Figure 3-8.

⑦ Set the Mixing parameters as shown in Figure 3-8 as well.
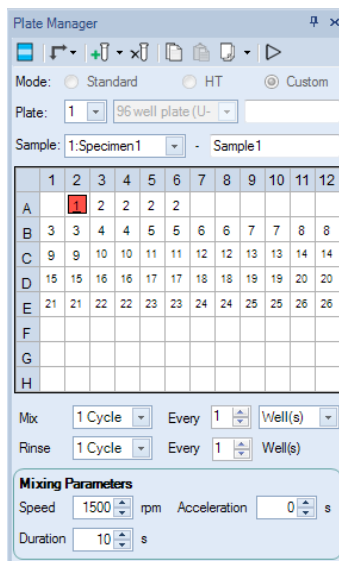

Figure 3-8 Plate Manager Panel after Experiment Samples Created

The *Experiment Manager* panel will be updated with the newly created samples. The *Experiment Manager* panel is shown in Figure 3-9.
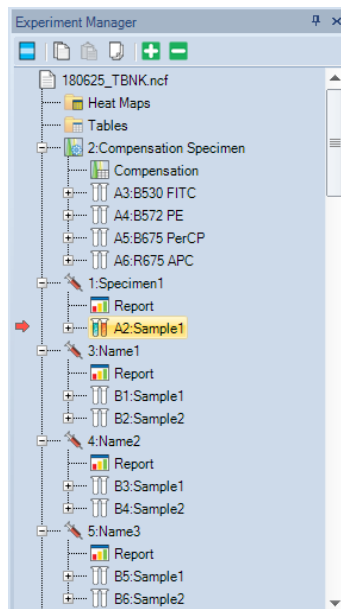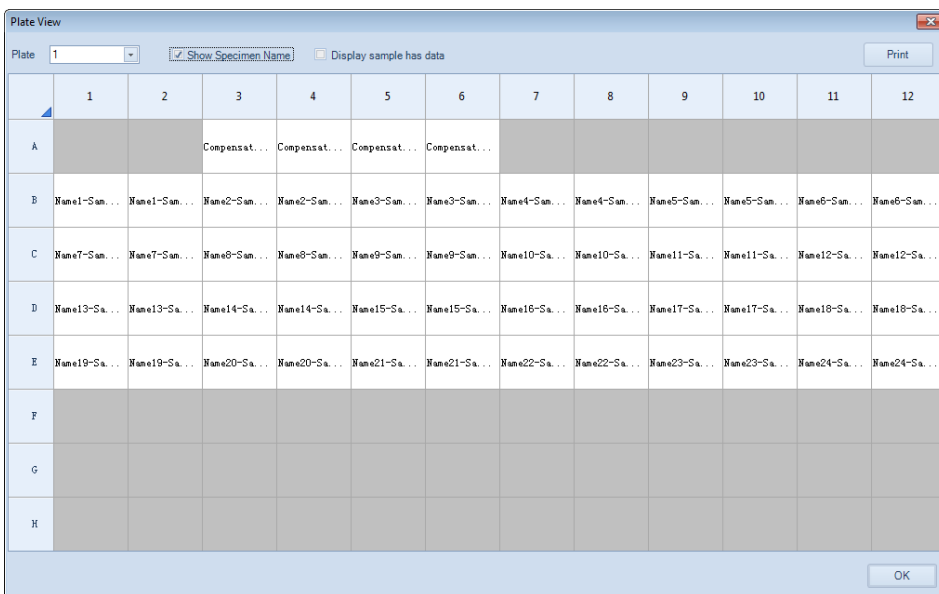
Figure 3-9 Experiment Manager Panel after Experiment Samples Created

🔍 *In the **Experiment Manager** panel, a variety of templates can be used to eas-ily perform fluorescence compensation and data analysis. Please refer to the Section 6 Experiment Manager in NovoExpress® Software Guide.*

🔍 *If the sample acquisition conditions are different between samples, the **Work List** allows to individual collection conditions to modify individual samples.*

🔍 *For additional information regarding the **Work List**, refer to the Section 4.2 Work List in NovoExpress® Software Guide.*

3.5.3   Plate View

Plate view provides an intuitive view of the sample wells. To open the plate view, click the *Plate View* button from *Home → Experiment → Plate View*. In plate view, if the experiment contains multiple plates, the displayed plate can be switched using the drop-down menu. The plate view is shown in Figure 3-10.

Figure 3-10 Plate View Window

🔍 *If a stop condition has not been set for a sample, the sample well will not be set and the sample will not be displayed in the plate view.*

3.6   Automatic Sample Acquisition

This section will describe the automatic sample acquisition procedure.

3

1 Remove the sample plate from the NovoSampler Pro, and load the plate according to the layout set for the experiment. In this experiment, the four single-stained samples for fluorescence compensation are in wells A3 to A6. The 48 samples from 24 different specimens are in wells B1 to E12. For each specimen, the first sample is stained with ACEA QuadriTest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC reagen and the second sample is stained with ACEA QuadriTest CD3 FITC/CD16+CD56 PE/CD45 PerCP/CD19 APC Reagent. After preparing the sample plate, load the plate onto the orbital shaker of the NovoSampler Pro and close the cover.

2 In the *Cytometer Control* panel, click the *Run Plate* button [▶ Run Plate] . The *Select well(s) to run* window will open as shown in Figure 3-11. In the window, select the wells to be collected. Wells selected for collection will be displayed in blue, unselected wells will be displayed in white, and wells that are not available for collection will be displayed in gray. In this experiment, select all of the samples from A3 to E12. Click the *Run* button to begin sample acquisition. The selected samples will be collected in the order listed in the *Work List*.
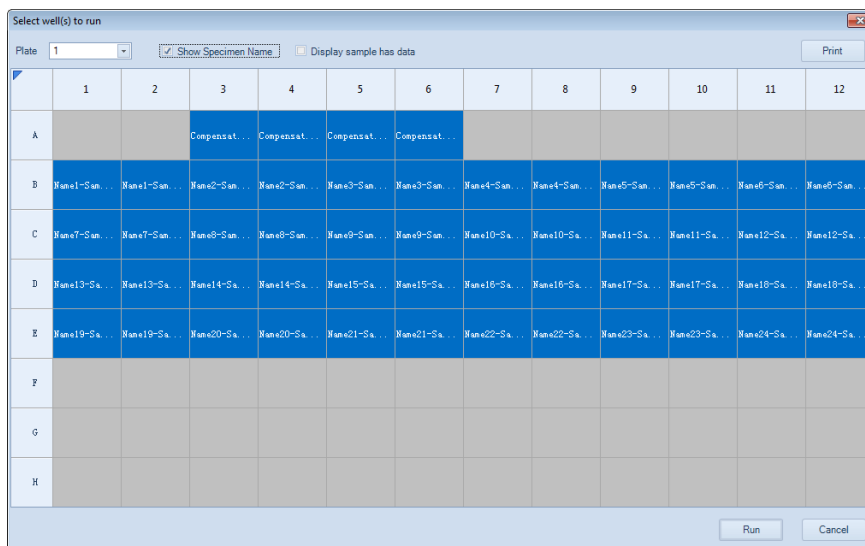
Figure 3-11 Window for Selecting Wells to Run

Click the header row or header column to select all of the available wells in the row or column. Click the top-right corner cell of the grid [▨] , to select all available wells on the plate.

3 The automatic Sample acquisition workflow with NovoSampler Pro is shown in Figure 3-12. The process will begin with mixing the sample. The orbital shaker with the sample plate will then move to align the sample injection probe with the first sample well and it will be followed by sample acquisition. After sample acquisition is completed, the instrument will rinse the sample injection probe. The NovoSampler Pro will then mix the sample, move the orbital shaker with the sample plate to align the sample injection probe with the next sample well, and begin the sample acquisition. The process will continue until all of the selected samples are acquired. The frequency of mixing and rinsing will depend on the settings in the *Plate Manager* panel, which is opened from *View → Show → Plate Manager*.

► ► ►
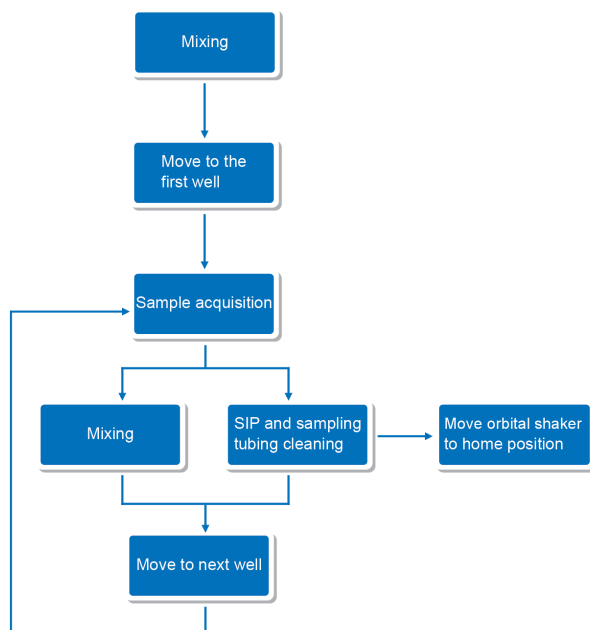
Figure 3-12 Work Flow of Automated Sample Acquisition Procedure with NovoSampler Pro

4 During automated sample acquisition, when starting acquiring a new sample, a window shown in Figure 3-13 will be prompted. In the prompted window, user can determine if the new sample will become the Active Sample. Active Sample is the sample shown in the Workspace for data analysis and indicated by a red arrow ➡ on the left of a sample in the *Experiment Manager* panel. Click *Yes* to make the sample to be acquired as the Active Sample so that you can observe the data for each new sample when it is acquired. Click *No* to keep the sample currently in analysis as the Active Sample so that you can continue working on the data analysis, in the meanwhile the rest of the samples are been acquired. If there is no user interference within 10 seconds after this window appears, NovoExpress Software will automatically make the new sample to be acquired as the Active Sample. Check the *Do not show this again in current run* checkbox and click *Yes* or *No* to make your selection as default for the rest of the plate run.
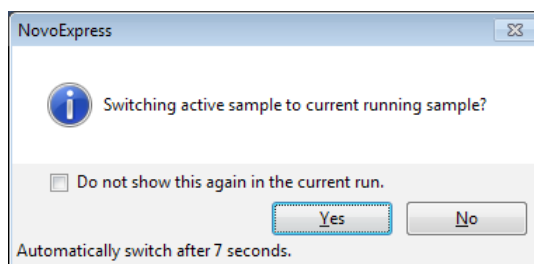
Figure 3-13 Window for Options on Determining the Active Sample during a Plate Run

■

*To pause automated sample acquisition, click **Pause** button (Figure 3-14) in the **Experiment Control** panel. The **Pause** button then changes to a **Resume** button. The automated sample acquisition will be paused after current sample acquisition is completed. To stop current sample acquisition immediately, click **Stop Single Well** button in the **Experiment Control** panel. To resume automated sample acquisition, click **Resume** button. In this case, the **Select well(s) to run** window will not prompt. The software will automatically continue to run the rest wells in the experiment. To stop automated sample acquisition, click **Stop** button.*
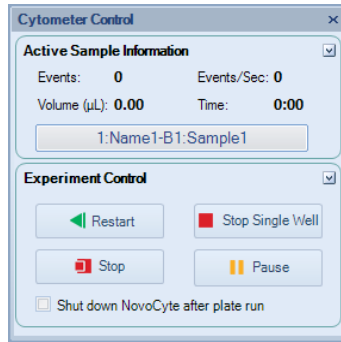
Figure 3-14 The Cytometer Control Panel after Automated Sample Acquisition Started

3.7   Analysis of Data

After the sample is collected, data analysis can be performed with NovoExpress Software. NovoExpress software provides tools for batch data analysis of multiple samples generated with NovoSampler Pro, such as analysis template, batch report, Statistical Table, etc. The data analysis process is summarized in Figure 3-15.
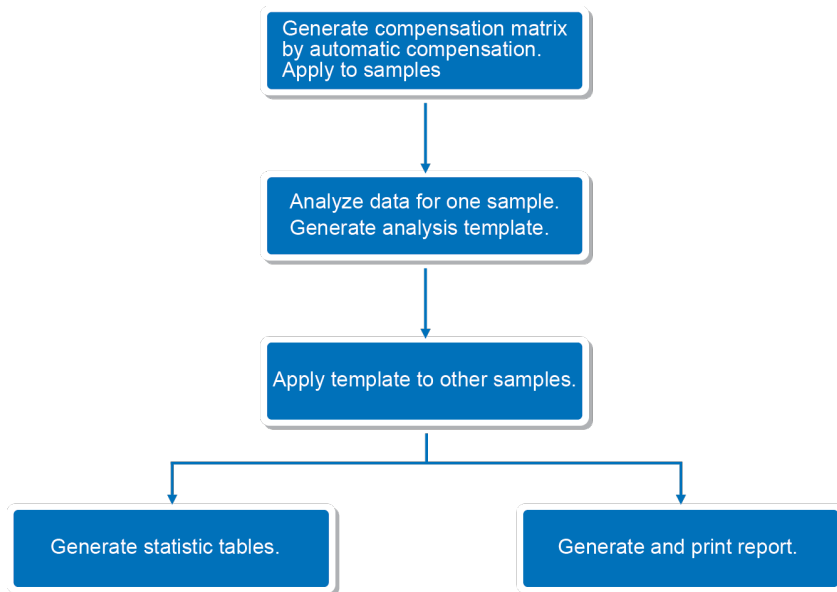
Figure 3-15 Data Analysis Procedure

The Well ID and Plate ID information is included in the exported FCS files. Third party software could read this information for data analysis after importing the FCS files.

For additional information regarding data analysis, please refer to the NovoCyte® Flow Cytometer Operator's Guide or the NovoExpress® Software Guide.

3.8　　Shutdown

When all experiments are completed for the day, shut down the system using the following procedure:

1 Shut down the NovoCyte Flow Cytometer

Press the power button on the NovoCyte Flow Cytometer, or click on the *Shut Down* button ⏻ , in the *Instrument* tab of the NovoExpress Software. The light in the power button will start flashing, and the instrument will automatically start the shutdown cleaning process. After the shutdown cleaning process is completed, the instrument will be turned off automatically.

The shutdown cleaning process will automatically clean the tubing inside the system without the need for user intervention.

*Check the **Shut down NovoCyte after plate run** checkbox in **Cytometer Control** panel to automatically activate the shutdown function after all the defined samples have been acquired. Refer to Section 2.3 Cytometer Control Panel in this guide for more information.*

2 Close NovoExpress software.

3 Shut down the NovoCyte workstation. Empty the waste container.

For detailed information on emptying the waste container, please refer to the Section 2.1.3 Empty Waste in NovoCyte® Flow Cytometer Operator's Guide.

■

4.   Cleaning NovoSampler Pro

The NovoSampler Pro frequently comes into contact with the experimental sample. To prevent corrosion of the instrument, periodically clean the outer and inner surfaces of the NovoSampler Pro.

Materials

► NovoRinse solution

► DI water

► Clean soft cloth

Procedure

1 Turn off NovoCyte Flow Cytometer and unplug the power cable.

⚠️⚡ *To prevent possible injury and damage to the instrument, turn off the power of the NovoCyte Flow Cytometer and unplug the power cable before cleaning or moving the instrument.*

2 Prepare a damp soft cloth with NovoRinse solution. Wipe the top surface of the orbital shaker and accessible outer and inner surfaces of the NovoSampler Pro.

3 Prepare a damp soft cloth with DI water. Wipe the surfaces again.

4 Wipe the surfaces one more time with a dry soft cloth.

■

⚠️☣️ *The NovoSampler Pro and NovoCyte instrument may come into contact with biohazardous material. During the cleaning procedure, use appropriate protective equipment, including gloves, clothing, and eyewear.*

⚠️ *Do not wipe the NovoSampler Pro using isopropyl alcohol or ethanol.*

5. Troubleshooting

This chapter will describe errors and possible solutions related to the NovoSampler Pro. For additional support, please contact your local ACEA technical support.

For technical support contact information, refer to the Technical Support section in the Prologue of this guide.

For errors related to NovoCyte Flow Cytometer, refer to the *NovoCyte® Flow Cytometer Operator's Guide*. For errors related to the software, refer to the *NovoExpress® Software Guide*.

Table 5-1 provides guidance on troubleshooting the NovoSampler Pro. Table 5-2 lists the message IDs and the associated software prompted messages.

Please contact ACEA Technical Support if issues are not resolved after performing the recommended solutions or if there are any issues which are not listed in these two tables.

Investigate possible causes and perform the recommended solutions in the given order.

Observation	Possible Causes	Recommended Solutions
Sample injection probe is not properly aligned with the wells or tubes	Plate or tube rack is not loaded correctly.	Load the plate or tube rack with the correct orientation. Make sure the plate or tube rack is seated onto the sample tray properly.
	NovoSampler Pro is not calibrated.	Click *Instrument → NovoSampler Pro → Calibrate* in the NovoExpress Software to re-calibrate the NovoSampler Pro.

Table 5-1 Troubleshooting Guide for NovoSampler Pro

Message IDs	Software Messages	Possible Causes	Recommended Solutions
0x0001	*Collision of SIP*	The movement of the SIP is blocked by some obstacles	Locate and clear the obstacles. Click the OK button in the prompted dialog box or wait 10 seconds for automatic error handling. The instrument will move the SIP to the home position. This procedure will take about 3 minutes.
		Incorrect plate selected in plate manager	Select the correct plate in the plate manager window.
		Incorrect positioning of plate in NovoSampler Pro	► Position the plate on the shaker correctly. Ensure the plate is seated flat on the stage inside the clamps. ► Re-calibrate NovoSampler Pro.

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Message IDs	Software Messages	Possible Causes	Recommended Solutions
0x0001	*Collision of SIP*	Dirty SIP or SIP wash apparatus	Clean the SIP or SIP wash apparatus following the procedures described in *Section 1.1 Preventative Maintenance in NovoCyte® Flow Cytometer Maintenance Guide*.
0x0002	*Running out of NovoFlow*	NovoFlow solution is not sufficient to continue to run any samples	Refill the NovoFlow container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in *NovoCyte® Flow Cytometer Operator's Guide*.
		NovoCyte Fluidics Station is not working properly	Replace the NovoCyte Fluidics Station.
0x0003	*Running out of NovoRinse*	NovoRinse solution is not sufficient to continue to run any samples	Refill the NovoRinse container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in *NovoCyte® Flow Cytometer Operator's Guide*.
		NovoCyte Fluidics Station is not working properly	Replace the NovoCyte Fluidics Station.
0x0004	*Running out of NovoClean*	NovoClean solution is not sufficient to continue to run any samples	Refill the NovoClean container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in *NovoCyte® Flow Cytometer Operator's Guide*.
		NovoCyte Fluidics Station is not working properly	Replace the NovoCyte Fluidics Station.
0x0005	*Waste container is full*	Waste container is too full to continue to run any samples	Empty the waste container following the procedures described in *Section 2.1.3 Empty Waste* in *NovoCyte® Flow Cytometer Operator's Guide*.
		NovoCyte Fluidics Station is not working properly	Replace the NovoCyte Fluidics Station.
0x0006	*System voltage is out of range*	System Error	► Restart the NovoCyte instrument. ► Contact ACEA technical support if the error persists.
0x0007	*System electric current is out of range*	System Error	► Restart the NovoCyte instrument. ► Contact ACEA technical support if the error persists.
0x0008	*Firmware configuration error*	System Error	► Restart the NovoCyte instrument. ► Contact ACEA technical support if the error persists.
0x0009 *0x000A* *0x000B*	*...... laser self test error*	Specified laser is not functioning properly	The instrument will automatically reset the laser and run a laser self-test. It takes approximately 5 to 10 minutes.

► ► ►

Message IDs	Software Messages	Possible Causes	Recommended Solutions
0x000C 0x000D 0x000E	*….laser is not connected*	Specified laser is not detected	▶ Restart the NovoCyte instrument. ▶ Contact ACEA technical support if the error persists.
0x000F	*NovoSampler communica-tion lost*	The cable between the NovoSampler (Pro) and NovoCyte Flow Cytometer is not securely con-nected	Reconnect the cable between the NovoSampler (Pro) and NovoCyte Flow Cytometer.
		NovoSampler (Pro) is not communicat-ing with NovoCyte	▶ Restart the NovoCyte instrument. ▶ Contact ACEA technical support if the error persists.
0x0010	*NovoSampler (Pro) has not been calibrated*	NovoSampler (Pro) is newly installed or re-connected.	Follow the prompted instructions to calibrate the NovoSampler (Pro).
0x0011	*NovoSampler (Pro) calibra-tion failed……*	NovoSampler (Pro) is not installed properly	Re-install and calibrate the NovoSam-pler (Pro) following the procedures described in *Section 1.2 Installation* in *NovoSampler® Operator's Guide* or *No-voSampler® Pro Operator's Guide*.
		The NovoSampler (Pro) cover was opened during calibration.	Close the NovoSampler (Pro) cover and redo the calibration.
0x0012	*The movement of plate is out of range*	The movement of the orbital shaker is blocked.	▶ Check the path of the orbital shaker to make sure there are no objects blocking the movement. Clear the block if there is any. ▶ Click *Instrument → NovoSampler Pro → Calibrate* in NovoExpress Soft-ware to re-calibrate the NovoSam-pler (Pro).
		NovoSampler (Pro) is not installed properly	Re-install and calibrate the NovoSam-pler (Pro) following the procedures described in *Section 1.2 Installation* in *NovoSampler® Operator's Guide* or *No-voSampler® Pro Operator's Guide*.
0x0013	*Cover of NovoSampler (Pro) is opened during moving plate*	The cover of NovoSampler (Pro) is open during mov-ing plate	Close the NovoSampler (Pro) cover. No-voSampler (Pro) will automatically reset and be ready for operation.

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Message IDs	Software Messages	Possible Causes	Recommended Solutions
0x0014	*Pressure is out of limit*	Waste container is not correctly connected to the instrument	Check the quick coupling connectors to ensure the waste container is correctly connected to the instrument.
		Sheath in-line filter is clogged	Replace the sheath in-line filter following the procedures described in *Section 1.2 Replacing Fluidic System Consumables* in *NovoCyte® Flow Cytometer Maintenance Guide*.
		Flow cell is clogged	Conduct Unclog from NovoExpress.
0x0015	*NovoSampler firmware error*	NovoSampler or NovoSampler Pro firmware is not working properly	Re-install or upgrade the NovoSampler or NovoSampler Pro firmware.
0x0016 0x0017 0x0018	*...... laser does not emit*	Specified laser is not connected properly or laser is not working properly	► Reconnect the laser cable properly. ► Restart the instrument. ► Contact ACEA technical support if the error persists.
0x0019 0x001A 0x001B	*...... laser communication error*	Interference or bad connection or bad laser	► Reconnect the laser properly. ► Restart the instrument. ► Contact ACEA technical support if the error persists.
0x001C	*Sample injection probe reset failed*	Bad connection or optocoupler damaged	► Click the *OK* button in the prompted dialog box or wait 10 seconds for automatically error handling. ► Restart the instrument. ► Contact ACEA technical support if the error persists.
0x001D	*Sampling Pump reset failed*	Bad connection or optocoupler damaged	► Restart the instrument. ► Contact ACEA technical support if the error persists.
0x001E	*The SIP module needs to be upgraded to support NovoSampler Pro*	SIP module is not compatible with connected NovoSampler Pro	Contact ACEA technical support for service.
0x0020	*System initialization is paused*	Liquid level in the reagent containers is not within normal range when NovoCyte is powered up.	► Make sure that the instrument reagent containers are placed correctly and the liquid level in the containers is within the normal range. ► Click *OK* on the prompted dialog to continue system initialization.

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Message IDs	Software Messages	Possible Causes	Recommended Solutions
0x0021	*Sheath fluid pump reset failed*	Bad connection or optocoupler damaged	▶ Restart the instrument. ▶ Contact ACEA technical support if the error persists.
0x0022	*Resetting NovoSampler Pro to zero position failed*	NovoSampler Pro is not working properly.	Click *Reset*. Restart the NovoSampler Pro.
0x0023	*Resetting NovoSampler Pro to home position failed*	NovoSampler Pro is not working properly.	Click *Reset*. Restart the NovoSampler Pro.
0x0100	*Instrument cover opened*	Instrument cover is open or not tightly closed	Close the instrument cover.
0x0101	*NovoFlow running low*	NovoFlow solution is below the pre-set volume limit	Refill the NovoFlow container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in *Novo-Cyte® Flow Cytometer Operator's Guide*.
		NovoCyte Fluidics Station not working properly	Replace NovoCyte Fluidics Station.
0x0102	*NovoRinse running low*	NovoRinse solution is below the pre-set volume limit	Refill the NovoRinse container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in this guide.
		NovoCyte Fluidics Station not working properly	Replace NovoCyte Fluidics Station.
0x0103	*NovoClean running low*	NovoClean solution is below the pre-set volume limit	Refill the NovoClean container following the procedures described in *Section 2.1.2 Add Instrument Reagents* in *Novo-Cyte® Flow Cytometer Operator's Guide*.
		NovoCyte Fluidic Station is not working properly	Replace NovoCyte Fluidics Station.
0x0104	*Waste container is close to full*	Waste is above the pre-set volume limit	Empty the waste container following the procedures described in *Section 2.1.3 Empty Waste* in *NovoCyte® Flow Cytometer Operator's Guide*.
		NovoCyte Fluidics Station not working properly	Replace NovoCyte Fluidics Station.
0x0105	*Cover of NovoSampler (Pro) opened*	Cover of NovoSampler (Pro) is opened	Close the cover.

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Message IDs	Software Messages	Possible Causes	Recommended Solutions
0x0106	*NovoSampler (Pro) is disconnected when powered up*	The NovoSampler (Pro) is disconnected when powered up	► Shut down NovoCyte Flow Cytometer. ► Reconnect the cable of the NovoSampler (Pro) to NovoCyte flow cytometer. ► Turn on NovoCyte and follow the prompts to calibrate the NovoSampler (Pro). ► Contact ACEA technical support if the error persists.
0x0109	*NovoCyte Fluidics Station is not connected*	Cable between the NovoCyte Fluidics Station and the NovoCyte instrument is not properly connected	► Turn off the instrument. ► Reconnect the cable between the NovoCyte Fluidics Station and the NovoCyte instrument. ► Power up the instrument. ► Contact ACEA technical support if the error persists.
0x010A 0x010B 0x010C 0x010D	*… liquid level sensor failure*	Specified liquid level sensor in the NovoCyte Fluidics Station or NovoCyte Fluidics Cart is not working properly	► Reconnect the NovoCyte Fluidics Station or NovoCyte Fluidics Cart cable. ► Restart the NovoCyte instrument. ► Replace the NovoCyte Fluidics Station or NovoCyte Fluidics Cart. ► Contact ACEA technical support if the error persists.
0x010F	*Sheath filter is clogged. Please replace the sheath filter and run the Priming procedure*	Sheath in-line filter is clogged	Replace the sheath in-line filter following the procedures described in *Section 1.2 Replacing Fluidic System Consumables* in *NovoCyte® Flow Cytometer Maintenance Guide*.
0x0110	*Recovering collision error*	Plate stops at an incorrect position	No action is needed. Instrument will automatically recover the error.
0x0111	*Orbital shaker homing position reset failure*	Orbital shaker of NovoSampler Pro is not working properly	No action is needed. The NovoSampler Pro can be used normally.
0x7000	*NovoCyte Fluidics Cart firmware error*	NovoCyte Fluidics Cart firmware is not working properly	► Restart the instrument ► Replace the NovoCyte Fluidics Cart ► Contact ACEA technical support if the error persists.

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Message IDs	Software Messages	Possible Causes	Recommended Solutions
0x7001	*NovoFlow liquid level sensor is not connected*	NovoFlow liquid level sensor is not connected or does not work properly	► Reconnect the NovoFlow liquid level sensor ► Contact ACEA technical support if the error remains after reconnection. The sensor may be damaged and need to be replaced.
0x7002	*Waste liquid level sensor is not connected*	Waste liquid level sensor is not connected or does not work properly	► Reconnect the NovoFlow liquid level sensor ► Contact ACEA technical support if the error remains after reconnection. The sensor may be damaged and need to be replaced.
0x7100	*NovoCyte Fluidics Cart communication lost*	NovoCyte Fluidics Cart is not properly connected to the instrument	► Ensure the DVI cable between Novo-Cyte Fluidics Cart and the instrument is properly connected ► Restart the instrument ► Contact ACEA technical support if the error persists.
	Communication error (code: xx, xx). Please restart NovoCyte and NovoExpress.	USB cable between the NovoCyte instrument and workstation is not connected properly	► Reconnect the USB cable between the NovoCyte instrument and the workstation. ► Restart the instrument and NovoExpress software. ► Contact ACEA technical support if the error persists.

Table 5-2 NovoExpress Prompted Error Messages and Message IDs

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Appendix A   Accessories and Consumables

To order spare parts and consumables for NovoSampler Pro:

► Within US, call 1-866-308-2232 or visit www.aceabio.com.

► Within China, call 400-600-1063 or visit www.aceabio.com.cn.

► Users in other countries or regions, please contact your local ACEA Biosciences distributors.

For a complete list of distributors, please visit www.aceabio.com (USA), www.aceabio.com.cn (China).

Name	Product No.
NovoSampler Pro	2020070
NovoSampler 24 Tube Rack	2020079
NovoSampler Pro Connection Cable	2020078

Appendix B   Technical Specifications

Physical parameters	NovoSampler Pro Dimension (W × D × H) : 14.6 × 10.2 × 11.0 in (37 × 26 × 28 cm)
	NovoSampler Pro Weight: 19.8 lb (9 kg)
	NovoCyte Flow Cytometer with NovoSampler Pro installed: Work space requirements (W × D × H) : 186 × 80 × 80 cm
	Operating temperature: 15 °C ~ 32 °C
	Operating Humidity: < 80% and non-condensing
	Power requirements: 100 ~ 220 VAC, 47 ~ 63 Hz, ≤ 20 W
Sample Analysis	Compatibility: Compatible with standard 96-well plates (flat-bottom, U-bottom, V-bottom), standard 24-well, 48-well plates, and NovoSampler 24 tube rack for 12 × 75 mm tubes
	Carryover rate: less than 0.1% (with one rinse cycle per well)
	Mix mode: orbital shaker
	Rinse: automatically rinse the sample injection probe and fluidic tubing.
	Minimum sample volume: 90 µL for 96-well plates
	Dead volume: 50 µL for 96-well plates
Workstation	Operating System: Microsoft Windows®
	Software: NovoExpress

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