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Laminar Wash™ AUTO 1000 System User Manual

Laminar Wash™ AUTO 1000 System User Manual

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This user manual has been written for the purpose of providing technical, installation, operating and troubleshooting information to the operators of the Laminar Wash™ AUTO1000 System. The content of this manual includes:

- How to set up and operate the AUTO1000
- The principle of operation and modes of function for the AUTO1000
- Safety features of the AUTO1000 and precautions to ensure safe operation
- Troubleshooting procedures and maintenance

Introduction to the Laminar Wash AUTO1000 System

The Laminar Wash AUTO1000 System is a fully automated system for surface staining and intracellular staining protocols. It comes pre-programmed with modifiable protocols that are easy for the user to immediately use with no programming needed. The platform is designed to produce the most quantitative and reproducible results for flow cytometry users. The Laminar Wash AUTO1000 System reduces user variability and day-to-day variation prevalent in flow cytometry. Unlike custom automation or centrifugation-based systems, the AUTO 1000 provides easy, turnkey automation and exceptional flexibility. In addition, the AUTO 1000 is much more compact, affordable, and lower maintenance than automation systems built around centrifugation.

Technical Specifications

Description	*Specification*
Physical	
Dimensions Length: Width: Height (door closed): Height (door open):	1359 mm (53.5 in.) 709 mm (27.9 in.) 889 mm (35.0 in.) 1300 mm (51.2 in.)
Weight	112 kg (250 lbs)
Electrical	
Input Power (Primary) Universal Supply: Output Power (Secondary) Power: Wattage:	100 - 240 VAC, 50-60 Hz, 5A +42 VDC +/-5% 600 Watts maximum
Environmental	
Operating Temperature: Relative Humidity: Altitude:	15° - 28°C (59° to 82°F) 30% to 85% R.H. non-condensing 0 – 2000 m above sea level
Storage Temperature:	-20°C - 70°C
Relative Humidity:	10% to 90% R.H. non-condensing
Operation	
Plate Type	Laminar Wash Plate in 96-well format
Liquid level detection Independent Channels:	Pressure-based liquid level detection(pLLD) Capacitive liquid level detection (cLLD)
Communication Type:	Ethernet

Laminar Wash HT2000/HT2100 Performance		
Flow rate at nozzle	2-20µl/s	
Volume capacity	80µl per nozzle	
Wash sequence	96 wells simultaneous washing	
Dilution factor per cycle	Approx. 3.5 times	
Well Residual Volume	Average	26 ± 3µL
	Maximum	< 30 µL
	Minimum	> 20 µL

Safety

User Attention Notifications

Several user attention phrases are used throughout this manual. Each phrase should draw the following level of attention from the user:

NOTE	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
CAUTION	Cautions users regarding potentially hazardous situations in regard to user injury or damage to the instrument if the information is not heeded.
!WARNING!	Warn users that serious physical injury can result if warning precautions are not heeded.

Chemical Compatibility

Refer to the following product specific user manual for the chemical compatibility table.

- Laminar Wash™ HT2000/HT2100 System User Manual
- AUTO1000 Pipetting Channels (see below)

The tables for chemical compatibility are based on information from different manufacturers. The results refer to laboratory tests with raw materials. The results with these materials are often associated with effects that cannot be observed under laboratory conditions (e.g. temperature, pressure, tension, chemical influences of substances, design features, etc.). The results listed may be considered only as a guideline. In case of doubt we recommend significant tests. The chemical resistance is not sufficient for an evaluation of a particular material for a product. Particular regulations (e.g. explosion prevention in the case of flammable liquids) have to be taken into account.

SST	Stainless steel	PE	Polyethylene
Z	Z	PEEK	Polyetheretherketone
AA 5083 0	Aluminum	PMMA	Polymethyl-metharcylate
EPDM	Ethylene-propylene-elastomer	POM	Polyoxymethylene
FPM	Fluoroelastomer	PP	Polypropylene

FFKM	Kalrez	**PTFE**	Polytetrafluorethylene
FFPM	Per-Fluor-elastomer	**PVC**	Polyvinylchloride
FKM	Viton	**PVDF**	Polyvinylidenefluoride
NBR	Acrylnitril-butadiene-rubber	**SI**	Silicone

	Materials												
Chemical	420 SST	304 SST	303 SST	316L SST	PE	PP	PTFE	PEEK	FKM	FFKM	EPT	ZrO2	CO-RE
Acetic acid, 20%	2	1	1	1	1	1	1	1	2	1	1	0	1
Acetic acid, glacial	2	1	1	1	1	1	1	1	4	1	1	0	1
Acetone	1	1	1	1	2	1	1	1	4	1	1	0	1
Acetonitrile	1	1	1	1	1	3	1	0	2	0	3	0	3
[Ammonium hydroxide, 5%	1	1	1	1	1	1	1	1	1	1	1	0	1
(Chloroform	1	1	1	1	3	3	1	1	1	1	4	0	4
[Deionized water	1	1	1	1	1	1	1	1	1	1	1	0	1
Dimethyl formamide	1	1	1	1	1	1	1	1	3	1	1	0	1
Dimethy| sulforde	1	1	1	1	1	1	1	0	0	0	1	0	1
Ethyl acetate	1	1	1	1	2	1	1	1	4	1	1	0	1
Hexane	1	1	1	1	3	2	1	1	1	1	4	0	4
Hydrochloric acid, 5%	4L	2L	3L	2L	1	1	1	1	1	1	1	1	1
Hydrochionic acd, 20%	4L	3L	3L	2L	1	1	1	1	1	1	1	1	1
Hydrogen peroxide, 10%	1	1	1	1	2	2	1	1	2	2	2	1	2
Isopropy! Alcohol	1	1	1	1	1	1	1	1	1	1	1	0	1
Methanol	1	1	1	1	1	1	1	1	2	1	1	0	1
Methylene chionde	1	1	1	1	1	1	1	1	2	1	1	0	4
Nitric acid, 6-10%	1	1	1	1	1	1	1	1	1	1	1	1	3
Nive acid, 70%	1	1	1	1	1	1	1	1	2	1	1	1	3
Phosphate buffer	1	1	1	1	1	1	1	1	1	1	1	0	1
Phosphoric acid, 85%	3	2	3	2	1	1	1	0	1	1	1	1	3
Potassium hydroxide conc.	3	1	2	1	1	1	1	1	3	1	1	1	2
Sodium acetate	1	1	1	1	1	1	1	0	4	1	1	0	1
Sodium borate	1	1	1	1	1	1	1	0	1	1	1	0	1
Sulfuric acid, 1-75%.	4	2	3	2	1	1	1	2	1	1	1	1	3
Urine	1	1	1	1	1	1	1	1	1	1	1	0	1
Triethylamine	1	1	1	1	0	4	1	0	4	0	4	0	4
Toluene	1	1	1	1	3	3	1	1	1	1	4	0	4
Sodium hydroxide 5%	1	1	1	1	1	1	1	1	1	1	1	1	1
Formic acid 5%	3	1	2	1	1	1	1	1	2	1	1	0	2
Sodium hypochloride 10%	3L	2L	2L	1L	1	1	1	0	1	1	1	0	1
Ethanol	1	1	1	1	1	1	1	1	1	1	1	0	1

Effects (Key to codes in the table above)	
1	No effect, little or no noticeable change
2	Slight corrosion or discoloration
3	Moderate corrosion or other changes in physical properties or dimensions; not recommended for continuous contact.

4	Severe corrosion or physical change; prolonged contact not recommended.
0	No data
Assigned Materials	
CO-RE Head consists of 303 SST, EPDM, PEEK, ZrO_2 and PTFE.	
98 Head consists of 303 SST, EPDM, PEEK, ZrO_2 and PTFE.	
384 Head consists of 316L SST, Viton and ZrO_2.	

Chemical Hazards

WARNING! CHEMICAL HAZARD

Some chemicals used can be potentially hazardous and can cause injury or illness.

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or clothing). For additional safety guidelines consult the SDS.
- Do not leave chemical containers open.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's clean-up procedures as recommended on the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazards

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Minimize contact with chemical waste. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or clothing).
- Use precaution when emptying the waste bottle.
- Dispose of waste bottle contents in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Safety Data Sheets

Some chemicals used with the AUTO 1000 may be listed as hazardous. Warnings are displayed on the labels of all chemicals when hazards exist.

SDSs provide users with safety information needed to store, handle, transport and dispose of the chemicals safely, as well as emergency treatment information in the event of unintentional personnel exposure. Curiox recommends updating laboratory SDS records periodically.

AUTO 1000 Safety Symbols and Markings

The “Biohazard” symbol alerts the operator to situations where special care is required to remain protected from chemical or biochemical hazards.

The “UV Light” symbol indicates the presence of artificial UV light which could result in significant personal exposure.

WARNING: The “Warning!” symbol contains information that must be followed to prevent personal injury to those operating the equipment.

IMPORTANT: The “Important” symbol gives instructions that must be followed to prevent damage to equipment or loss of data.

NOTE: The “Note” symbol provides useful information to improve system performance or directs you to supplemental information to improve your understanding of overall operations.

The “Procedure” symbol is followed by a set of installation or operational steps.

Pinch point hazard. Keep hands clear.

Separate collection for electrical and electronic equipment.

General Precautions

- Do not use any other plate except the specified Laminar Wash 96-well Plate in the LW plate position. Do not use 96-well plates other than the recommended brands and volumes for sample input/output (Costar) and reagent plate (2mL Axygen).

  Use of these plates is strongly recommended. Other brands of plates have different dimensions and may result in transport failure unless the system is modified by a Curiox service engineer to accommodate them.

- Use only the supplied power adapter cord for electrical supply to the unit.
- Do not allow particles larger than 70µm to enter any of the liquid tubes. Ensure that the inlet filter in connected to the buffer inlet port on HT2000/HT2100.
- Be careful not to spill liquid onto the interior of the AUTO 1000.
- Always perform a cleaning cycle with an appropriate cleaning solution at the end of an experiment.
- Keep the HT2000/HT2100 Fluidic Head original packaging material in case the unit should ever need to be serviced.
- Do not attempt to open or remove the instrument casing or motor parts. Doing so will void the calibration and warranty and may cause permanent damage to the instrument.
- Only Curiox personnel are qualified for servicing the AUTO1000.

Important Safety Precautions.

Save these precautions.

Installation.

- *IMPORTANT.* Examine the packaging for signs of damage. If the crate or instrument has been damaged,
- *IMPORTANT.* Verify the contents of the package with shipping lists.
- *WARNING.* Never move a fully assembled instrument by yourself. Four or more people are required to move the instrument, which weighs between 220 and 300 lbs, depending on its configuration. Always remove labware and pedestals prior to moving.

Operation.

- *WARNING.* When using AUTO 1000, Good Laboratory Practices (GLP) must be observed. Suitable protective clothing, safety glasses and protective gloves must be worn.
- *BIOHAZARD WARNING.* If working with biohazardous samples, observe and carry out the maintenance procedures, paying particular attention to cleaning and decontamination. Wear gloves when handling the pipetting arm, the pedestals, containers, and the tips. Avoid touching tips discarded into the waste bin. Any surfaces on which liquid is spilled must be decontaminated.
- *WARNING.* Empty the AUTO 1000 tip waste bin as soon as it is full, but only after a run is complete or the instrument is paused.
- *BIOHAZARD WARNING.* Waste may contain biohazardous or chemical contaminated material.

Prior to System Operation

Ensure that all users of the AUTO 1000 have:

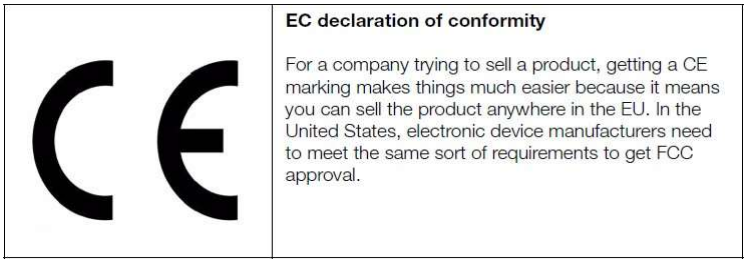
- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.
- Received instruction on handling of biohazards if biohazardous materials are to be used on the system.
- Read and understand all SDS related to your application.

CAUTION

Avoid using the AUTO 1000 in a manner not specified by Curiox. While the system has been designed to protect the user, this protection may be impaired if the instrument is used improperly.

CE Mark

CE Compliance Marked



EC declaration of conformity

For a company trying to sell a product, getting a CE marking makes things much easier because it means you can sell the product anywhere in the EU. In the United States, electronic device manufacturers need to meet the same sort of requirements to get FCC approval.

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2. Functional Description

Chapter Overview

Communication and Control Panel

Movement Indicator and Power Button

Machine Door

High Density Deck (4 X 5)

Carrier System

Core Grippers

1000 µL Channel, 300 µL Disposable Tips

Integrated Device – CPAC Peltier

Optional Reagent Cooling CPAC

Direct Reading Grid for AUTO1000

- *Usage Guidelines*
- *Instruction for Use*

This chapter gives a detailed overview of the parts of the AUTO 1000 and the phases which outline a typical washing process.

The main components of the AUTO 1000 are the HT2000/HT2100, deck, gantry, and four independent channels, as shown below.

Figure 2-1 *: Front view of the AUTO1000 with main components labelled.*

Communication and Control Panel

The communication and control panel of the AUTO 1000 are shown below. Ethernet cable is connected from the PC to the communications port (first from right).

Figure 2-2*: Communication and Control Panel.*

Movement Indicator and Power Button

The movement indicator provides visual, at-a-glance, cues to the operational status. Blue light follows the movement of gantry.

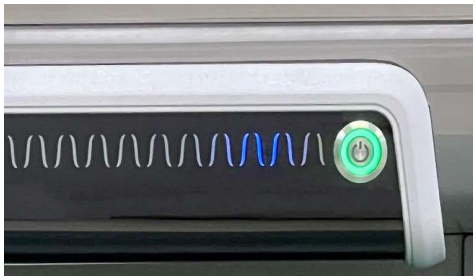


Figure 2-3: *Movement Indicator and Power Button*

Machine Door

To open, the user pulls the handle towards them and lifts the door up.

The door should be closed and locked at all times except when cleaning or loading the deck:

Fixture 2-4: *(Left) User pulls handle to open door.*
(Right) Door is opened for cleaning or deck loading purposes.

High Density Deck (4 X 5)

The AUTO 1000 is equipped with high-density deck, which allows for tip and plate stacking as well as integration with a shaker and coolers.

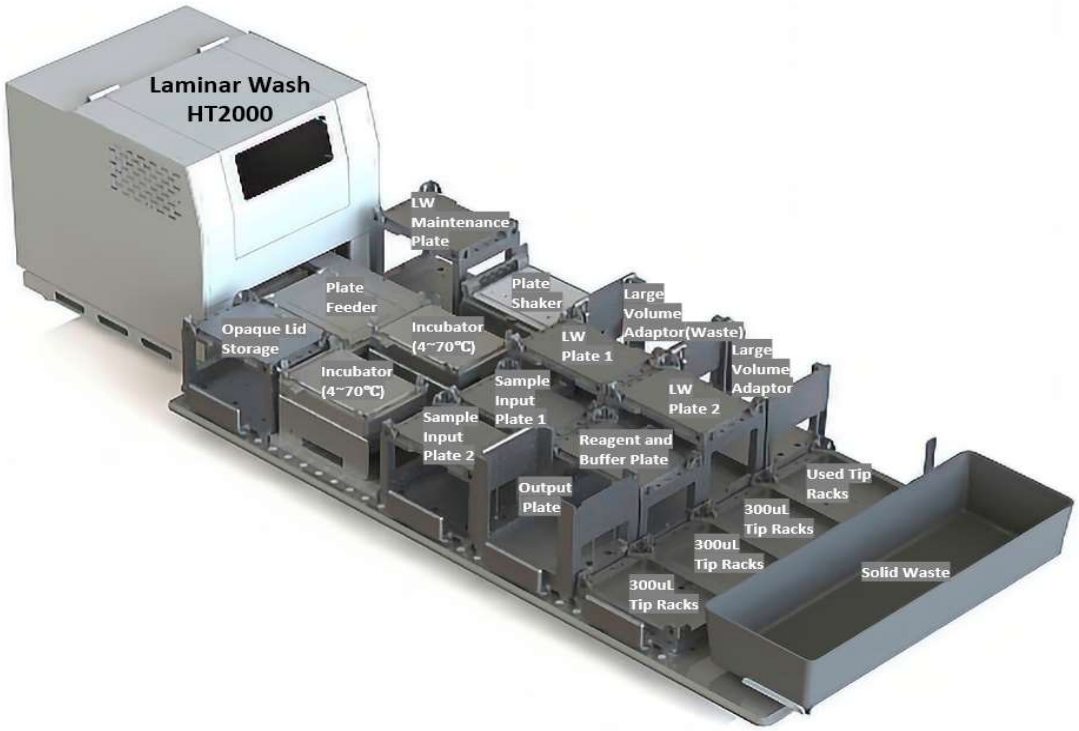


Figure 2-5: High Density Deck (4 X 5)

The basic deck layout for the AUTO 1000 system includes:

Column	Item	Description
A	Laminar wash maintenance plate	Storage space for HT2000/HT2100 priming and calibration plate.
	Plate feeder	Deck space to allow for extension of the plate feeder from Laminar Wash HT2000/HT2100 plate.
	Opaque lid storage	This can hold one lid for use when incubating the LW plate. Users may use either an opaque lid or a transparent lid.
B	Plate shaker	Plate vortexer specified for use with LW plate; specifications are 1650rpm, 1.0mm span. This deck is also able to be used for incubation at ambient temperature, but is currently only used for vortexing.
	Incubator CPAC (Cold Plate Air Cooled Heater/Cooler) Peltier (2 decks)	Only the first Peltier Incubator is used for incubation step, unless User is using 2 sample input plates, in which case both Peltier decks will be used. These decks are commonly used as pipetting decks. The temperatures can range from 4°C to 70° C(Accuracy at 37°C: ±0.3 K, Uniformity at 37°C: ±0.5 K)
C	Large Volume Adaptor (LVA) Waste	Waste deck for used Large volume adaptor.
	Sample Input Plate	Place Sample Input Plate containing samples in this deck use

	(2 decks)	if AUTO1000 transfer of samples from Sample Input Plate to LW plate is desired.
	LW plate (2 decks)	Decks for LW plate. Users may pre-load samples directly to the LW plates to reduce sample loss due to transfer and dead volumes from Sample Input plates to LW plates (Manual, see section on **Operation**), or configure AUTO 1000 to transfer samples from Sample Input Plate to LW (Auto).
D	Large Volume Adaptor (LVA)	LVA is used for incubation at large volumes on LW plate between 70 and 150µL. The GUI will prompt user to place the required number of LVA according to the protocol configured.
	Reagent and Buffer Plate	Deck for 96 deep-well plate containing reagents and panels for assay run. See section on **Reagent Plate**.
	Output plate or Direct Reading Grid (DRG)	Deck for microtiter U-bottom plate where the final sample is transferred to as indicated on the final step of protocol. If User prefers to add a Direct Reading Grid to LW plate for direct acquisition, this deck will hold a DRG adaptor and DRGs.
E	300µL tip racks (3 decks)	Tip racks must be stacked 4x for the pipetting channels to be able to reach them. Empty tip racks may be stacked.
	Used tip racks	Deck where empty tip racks may be transferred to.
F	Solid waste	Trough for used pipettes. Users are required to empty solid waste trough before beginning each run.

Carrier System

The carrier system functions in the manner specified below.

Column A: Carrier 1 can slide out when the HT2000/HT2100 feeder is retracted into the HT2000/HT2100.

Column B: Cannot slide out, CPAC(Cold Plate Air Cooled Heater/Cooler) Peltier(s) and shaker are in fixed positions.

Column C: Carrier 2 can slide out.

Column D: Carrier 3 can slide out. but if the optional reagent cooling CPAC is installed, this can't slide out.

Column E: Carrier 4 is for three full stacks of tips (four racks per stack).

Figure 2-6: Carrier System

System Modules

The system modules are identified in the manner specified below.

Differing heights of standoffs exist for different labware:

Short standoff for Deep Well Plate (DWP)

Tall standoffs for conventional 96-well labware.

Stacker - for storing Large Volume Adaptors (LVA) and/or 96-well plates.

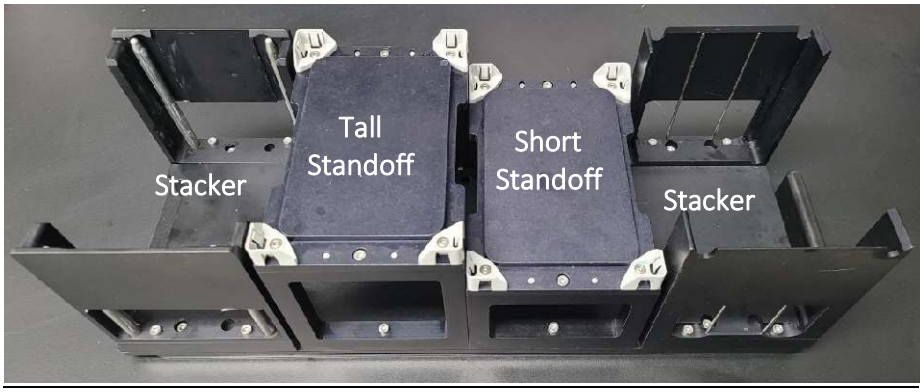


Figure 2-7: System Modules

Core Grippers

Using two pipetting channels in parallel, the machine can transport plates or tips across the deck without the need for a dedicated labware gripper. All channels are programmed to equip the

grippers, and the channels used depend on the location of targeted labware on the deck. Grippers are parked at a magnetic holder when not in use.

Figure 2-8: (left) Core Grippers, (right) Parked Grippers

1000µL Channel, 300 µL Disposable Tips

The AUTO 1000 utilizes 1000 µL channels and 300 µL tips. Pipetting specifications are shown in the table below. For pipetting volumes of less than 10 µL, the use of 10/50 µL volume disposable tips is recommended. Should 10/50 µL tips be required, please contact Curiox to have your software updated accordingly.

Disposable Tip Size	Volume	Accuracy	Precision
50 µl	1ul	5.0%	5.0%
	5ul	2.5%	2.0%
	50ul	2.0%	1.0%
300 µl	10µl	5.0%	2.5%
	50µl	2.0%	1.0%
	300µl	1.0%	1.0%
1000 µl	10µl	7.5%	3.5%
	100µl	2.0%	1.0%
	1000µl	1.0%	1.0%

Table. Pipetting specifications for disposable tips

Integrated Device – CPAC Peltier

The AUTO 1000 is integrated with a CPAC Peltier system with temperature ranging for 4 to 70°C.

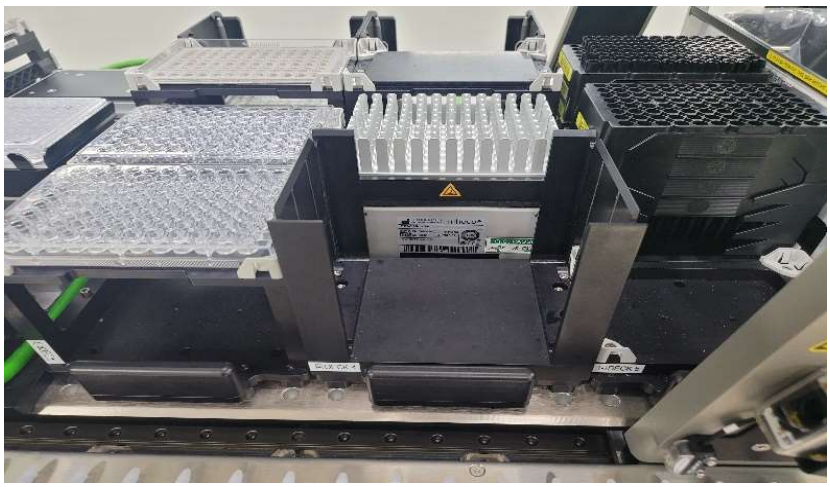
- Turn on/off the Power Button (located at the back of the Inheco controller) at the start/end of each day.
- Ensure the laptop is connected to the controller for communication and control. LED screen illumination will indicate that the controller is ON.
- AUTO 1000 Program will adjust the temperature according to the value set by user in the GUI. The Inheco controller cables are color-coded based on its specific function, as defined below.
- Green cable(s) connects to Peltier device(s)
- Beige cable connects to laptop
- Black cable connects to AC voltage source

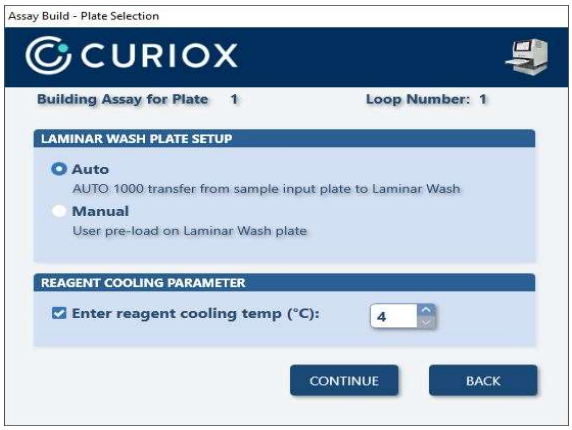
Figure 2-9: (left) Inheco controller front-view and (right) back-view

Optional Reagent Cooling CPAC

Reagent Cooling CPAC – Optional Item

If user has added the Reagent Cooling CPAC option, CPAC is installed underneath the Reagent Plate as shown the fixture below.





After installing the reagent cooling CPAC, there have been changes to the GUI, it's now possible to adjust the Reagent cooling temperature setting.

Figure 2-10: Installed Reagent Cooling CPAC

When the Reagent Cooling CPAC is installed, user can set the temperature through the GUI, and the system controls the temperature according to the configured setting.

Direct Reading Grid for AUTO 1000

Usage Guidelines

The DRG is intended for use as an alternative to the Output Plate on the AUTO 1000. It is fitted directly onto the Laminar Wash Plate after the Final Washing step (Refer to ***Figure 2-11***). Please ensure that there is a DRG adaptor properly fitted on the DRG deck if the user intends to conduct direct acquisition using the DRG (Refer to ***Figure 2-12***). If the user wishes to transfer the sample to an Output Plate, please remove the DRG adaptor and place only 1 or 2 Output plates on the same deck.

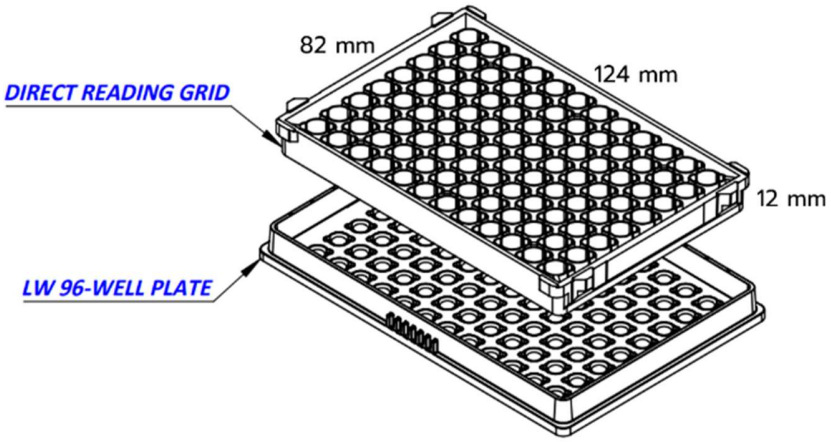


Figure 2-11. DRG and Laminar Wash Plate Diagram

The DRG is not validated with buffers containing surfactants, detergents, and protein concentrations of >10%. Please ensure buffers are free of precipitates and contaminants. Old and contaminated buffers can possibly cause failure.

Instructions for Use

Automation workflow (with DRG, GR-LW-96-A) is as follow:

- Ensure GUI version is v3.2.13.A and after. (Recommend using latest version)
- Place DRG On the associated deck. Make sure that there are 2x DRGs placed on the associated deck if user intends to run 2x sample plates. The default DRG deck will be Deck 4 position 4 (Please refer to the AUTO1000 GUI for a labelled diagram).
- Make sure A1 Position of the DRG is located at the Top/Left hand side of the deck.
- Set up the remainder of the plates for the AUTO1000 run. Once the Run parameters have been inputted, run the selected protocol.
- The DRG will be transferred to the Laminar Wash Plate(s) after the final Laminar Wash and topped up with desired volume.

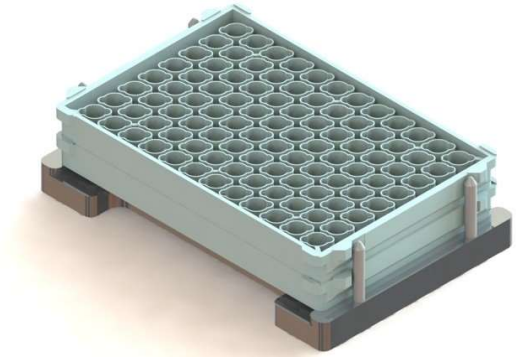


Figure 2-12. DRG Adaptor with fitted DRG

The DRG is intended for one-time use.

3. Setup

Chapter Overview

Installation

Buffer Inlet Bottle Cap Installation

Opening for HT2000/HT2100 Cables and Tubing

Installation

Prior to the scheduled installation, ensure that five boxes have arrived on site:

- Laminar Wash HT2000/HT2100 washing station
- Laminar Wash Buffer Exchanger
- AUTO Consumables
- NIMBUS
- NIMBUS Accessories

Should any of these be missing, please contact your Curiox representative.

Buffer Inlet Bottle Cap Installation

NOTE: The buffer inlet bottle cap must not be used on the waste bottle.

1. Eight sets of buffer inlet bottle caps are packed with the machine. Each bottle cap comes with pre- installed tubing, as shown below.

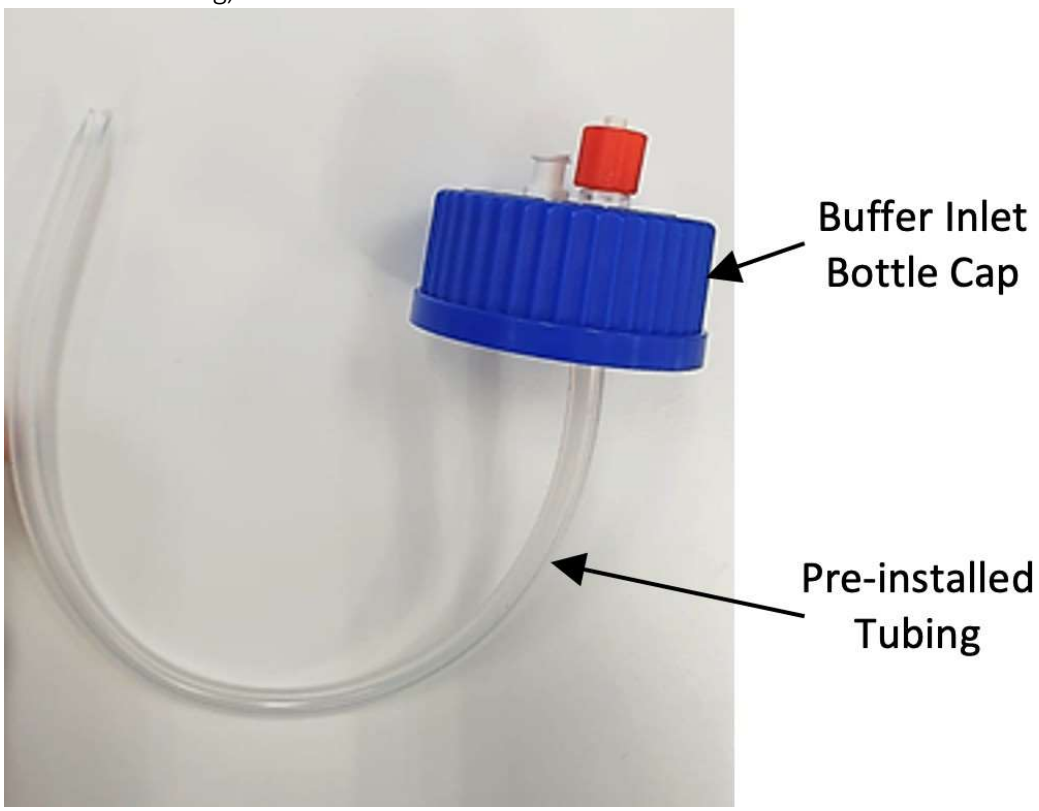


Figure 3-1: *Buffer inlet bottle cap accessories*

2. Connect a buffer inlet bottle cap onto a 2L glass bottle containing the cleaning solution or wash buffer of choice, as shown below. Install a clean filter onto one end of the inlet tubing and insert into the red port on the BX10. Insert the other end into wash buffer bottle via the buffer inlet bottle cap.

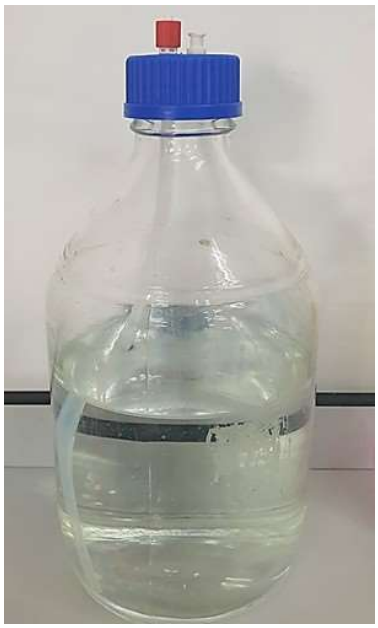


Figure 3-2: *Buffer inlet bottle cap connected to 2L bottle containing wash buffer of choice.*

Opening for HT2000/HT2100 Cables and Tubing

HT2000/HT2100 tubing can be connected to wash buffer bottles and cables routed through an opening on the left side panel of the AUTO 1000.

Figure 3-3: *Opening for HT2000/HT2100 cables and tubing*

4. Operation

Chapter Overview

Before Operating the AUTO1000

Operational Safety

Operation Mode

StartUp Maintenance

- *Nimbus Independent Channel Daily Maintenance*
- *Laminar Wash HT2000/HT2100 StartUp Maintenance*

Running a method

Simulation Mode

Run a saved Protocol

Building a Protocol on AUTO1000 GUI

Setting up the Reagent Plate

Shutdown Maintenance

Example Protocols

- *Surface Staining protocol with viability and unstained controls*
- *Surface and Intracellular staining protocol*

Before Operating the AUTO 1000

The AUTO1000 should be placed on a level surface and in an environment with temperature and humidity bounds as stipulated in “Technical Specifications” on page 5.

Operational Safety

The AUTO 1000 is equipped with the following built-in safety features which prevent the machine from operating under unsafe conditions:

Carrier Sensor

The carrier sensor indicator lights green only when any carrier is not properly inserted. The system prompts an error upon method activation, and operation is halted until the fault has been resolved. The indicator lights green and orange when all carriers are fully inserted.

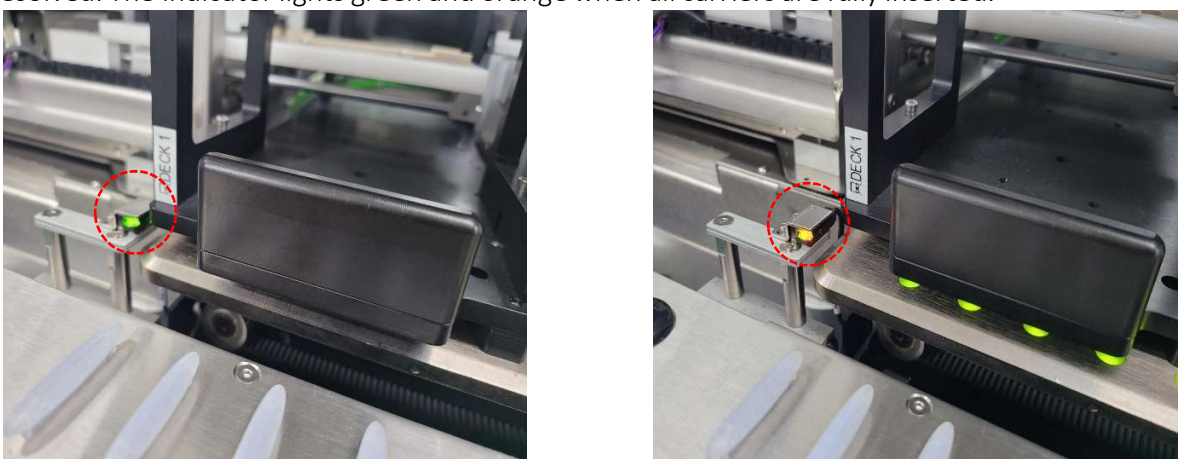


Figure 4-1 *(Left) Indicator is green when the carrier is not inserted properly and blocking the sensor.*

(Right) Indicator is green and orange when the carrier is inserted properly and not blocking the sensor.

Door Lock

The door automatically locks when the system is running – this is for user safety as the gantry moves with considerable speed and force. The door lock is not activated by shutting the door, but rather controlled by the running program. The locks make an audible click when activating/deactivating.

Emergency Stop

Should emergency stopping of the program be necessary, the AUTO1000 power button on the front of the enclosure will immediately halt gantry movement.

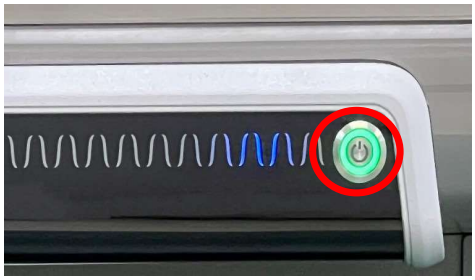


Figure 4-2. Power switch used for emergency stopping purposes

Safety Precautions

In addition to the built-in safety features of the AUTO 1000, below are some precautions operators are advised to take while using the AUTO 1000 to ensure their safety and to preserve the accuracy of the experiments.

- Keep the area around the power supply free from liquid.
- Follow all biohazard safety protocols
- Follow all maintenance instructions as laid out in the maintenance schedule (pg ??)
- If an unexpected error occurs, contact your Curiox representative

Operational Mode

Quick start guide on preparing AUTO1000 for sample preparation

Below is a flow chart depicting the protocol in setting up AUTO1000 for a sample preparation run. The following instructions will guide the user on performing daily start up and maintenance, building a protocol on the GUI, followed by daily shutdown and maintenance.

NOTE: The following user guide will use the following GUI version:
CurioxLWAuto1000SamplePrep_v3.3.2_generalNTR_20230920.pkg

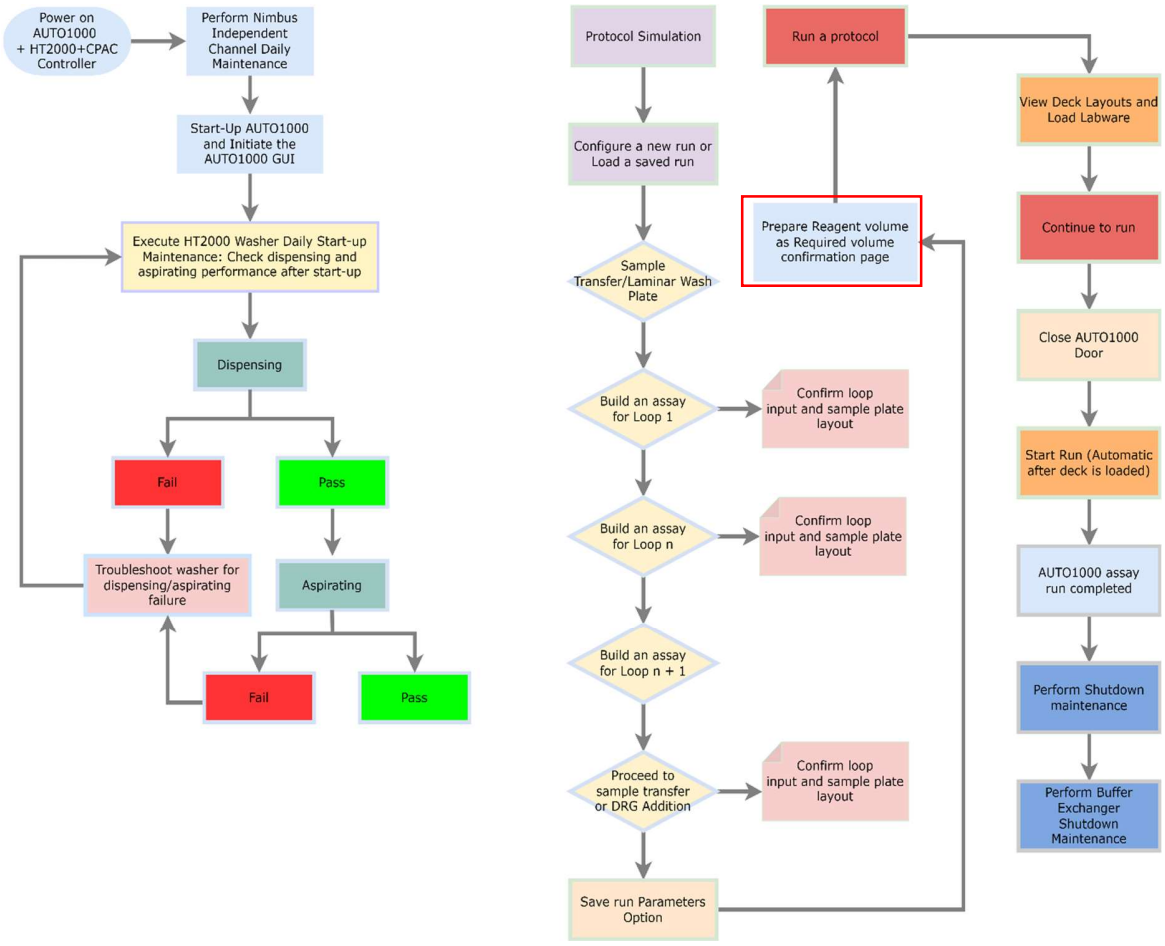


Figure 4-3. Flow chart depicting sequence of start-up, assay setup, assay run and shutdown on AUTO1000.

Start Up Maintenance

NIMBUS Independent Channel Daily Maintenance.

Begin Start-up by performing the AUTO1000 Independent Channel Daily Maintenance. (Calibration shortcut, maintenance, clean deck, etc) Prior to performing the first run on the AUTO 1000, the **Daily start-up maintenance must be completed.**

1. Switch on the AUTO 1000 by individually switching on the following instruments in no particular order:

- AUTO1000 gantry
- HT2000/HT2100
- CPAC controller
- Laptop

2. Before starting the calibration, remove the **BAR** located inside the NIMBUS. It is magnetically secured and can be easily removed by hand.

Figure.4-4. Remove BAR before calibration

3. To execute the daily maintenance/ calibration, User must first open the **Calibration** application on the laptop.

Figure.4-5. Screenshot of the Calibration application on User's desktop

4. The *Nimbus Independent Channel Calibration* window will pop up. Select **Run Maintenance**.

NOTE: Prior to running maintenance, please ensure the Tip Eject Plate is removed and the teaching needles and CORE Grippers are in place.

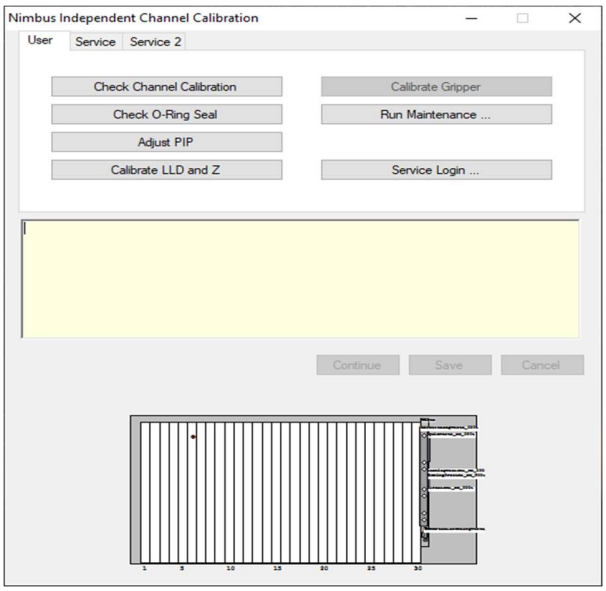


Figure 4-6. *Screenshot of the first page of* ***Nimbus Independent Channel Calibration window.***

5. In the new window *Nimbus Channel Maintenance*, user must indicate the **Operator** and **Instrument Serial Number** for performing the maintenance steps. Please note that these options will be automatically filled for future maintenance runs.

User must then select the **MicroLab Nimbus 8 Channel** instrument. And select **Daily** as the *Maintenance Type*. Initiate **Run Maintenance**.

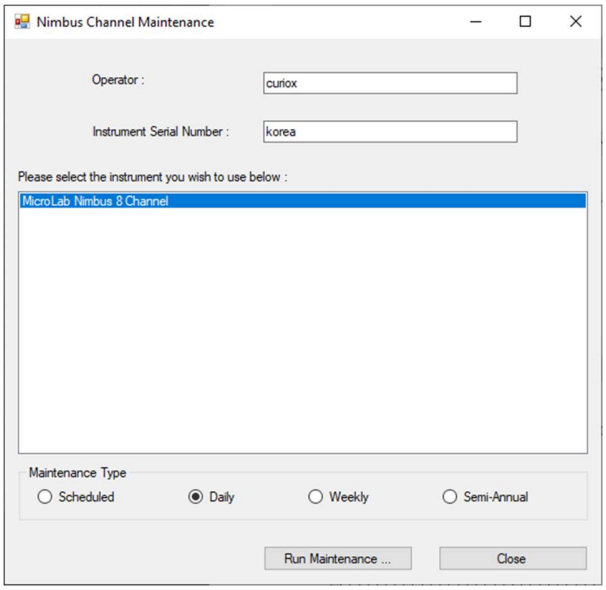


Figure 4-7. *Screenshot showing the* ***Nimbus Channel Maintenance window.***

6. The *Hamilton Four Probe Daily Maintenance* window guides User through the necessary maintenance tasks.

The first task requires User to inspect that no objects are misplaced on the carriers. If necessary, clean the deck with a low lint wipe and alcohol.

Check **Inspect the deck and carriers for cleanliness** once this is completed.

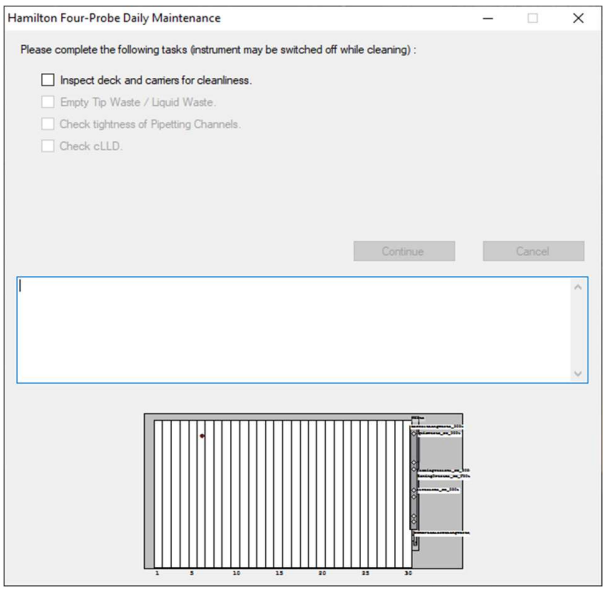


Figure 4-8. *Screenshot showing prompt for User to* ***Inspect the deck and carriers for cleanliness.***

7. The second check mark prompts user to **Empty Tip Waste/Liquid Waste.**

User should empty waste basket before returning it to the correct position.

Once this is completed, check as marked and continue.

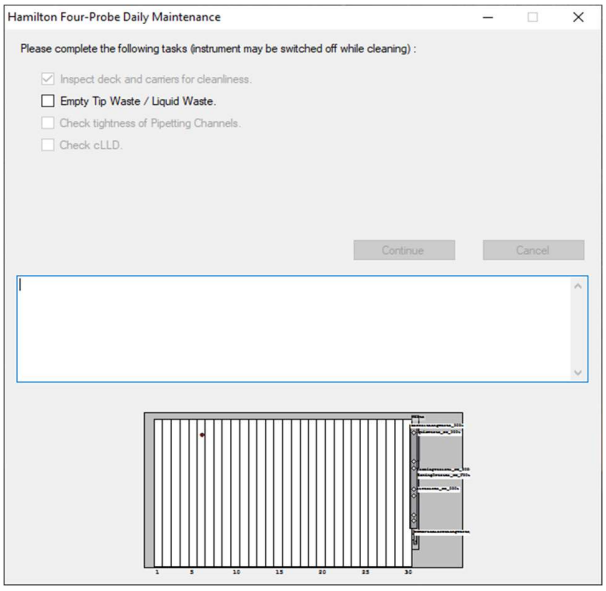


Figure 4-9. *Screenshot prompting User to* ***Empty Tip Waste / Liquid Waste.***

8. The third check mark prompts User to **Check tightness of Pipetting Channels**.

User should double check that the Tip Eject Plate is removed and the teaching needles are in place prior to checking the box.

Select **Continue** and the AUTO1000 will then proceed to perform the check.

NOTE: Failure to do so will lead to impact of the Pipetting Channels with the Tip Eject Plate.

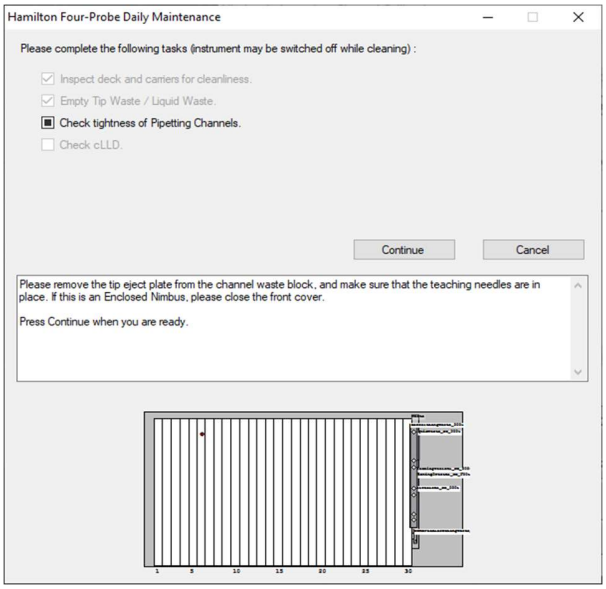


*Figure 4-10. Screenshot prompting User to **Check tightness of Pipetting Channels**.*

9. The final task will prompt user to **Check cLLD**

User should double check that the Tip Eject Plate is removed and the teaching needles are in place prior to checking the box.

Select **Continue** and the AUTO1000 will then proceed to perform the check.

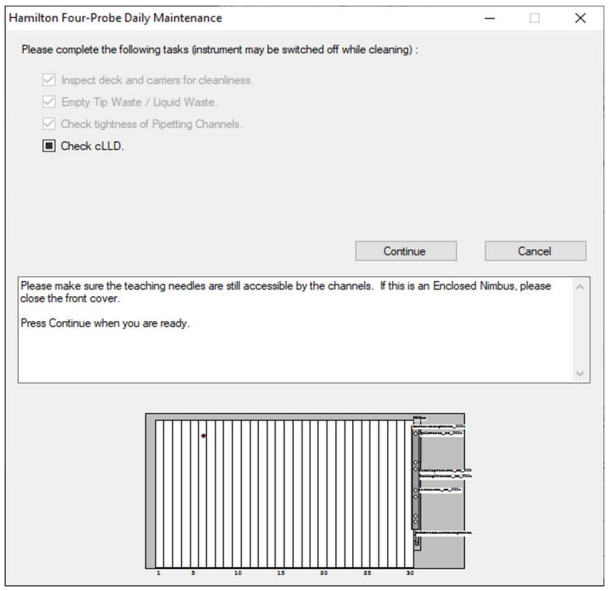


*Figure 4-11. Screenshot prompting User to **Check cLLD***

10. The result of the cLLD check will be displayed after the check.

Maintenance completion will be prompted on the screen. After this is complete, User should select **OK**.

Figure 4-12. Results of daily maintenance and calibration checks are displayed on the final window.

11. After the calibration is complete, reinsert the BAR. Simply align the hole on the BAR with the designated position.

Figure 4-13. Insert the BAR back in plate

Laminar Wash HT2000/HT2100 Start Up Maintenance

It is recommended to perform priming of the HT2000/HT2100 with 1% Tween-20 in 70% Ethanol followed by 1% Tween-20 in DI water prior to the day's operation. Follow the steps below to prepare the machine for operation.

1. Select □ **Check dispensing and aspirating performance after start-up** to ensure that all wells are evenly dispensed with liquid. After selecting this, click

Figure 4-14. Options for HT2000/HT2100 daily start-up and shut-down maintenance.

2. After selecting **Continue**, user will be prompted to load the washer maintenance plate onto the deck highlighted by flashing yellow and select **Load Washer Maintenance Plate**. After placing this plate on the deck, ensure that the AUTO1000 door is closed and select **Start**.

Figure 4-15. *Screenshot prompting User to load AUTO1000 deck with Washer Maintenance plate.*

3. After the maintenance plate has been used, user will be prompted to **Place a "Calibration" Laminar Wash plate on the washer tray**. After the Calibration plate is placed on the washer tray, select **Continue**.

Figure 4-16. *Prompt to remind User to place Calibration plate on washer tray following priming.*

4. Machine dispenses 80µL of primed buffer, prompting the user to verify that all wells are evenly dispensed. If all wells appears to be of a uniform volume, select **Pass** and **Continue** to point 4. If volumes do not appear uniform, select **Fail** and refer to point 5.

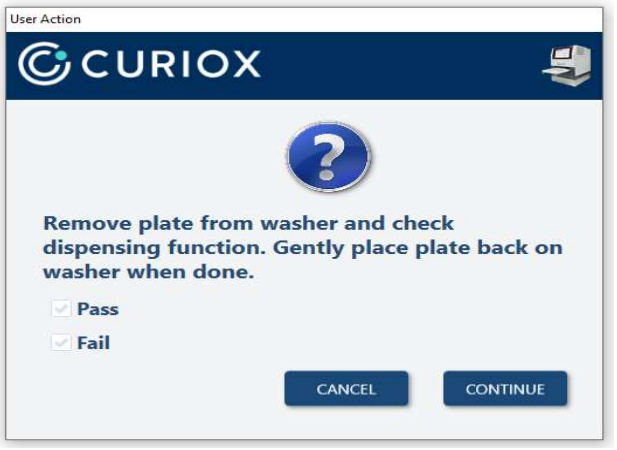


Figure 4-17. *Dispensing function verification.*

5. Return the Calibration Plate to the washer tray. The HT2000/HT2100 will then perform an aspirate function, reducing the volume in each well to approximately 25µL. If the wells appear uniform after the aspirating function is complete, please select **Pass**. Make sure to remove the Laminar Wash Plate from the Washer tray before selecting **Continue** as prompted. The GUI returns to the screen in Step 1 to enable protocol run.

If volumes do not appear uniform, select **Fail** and refer to point 6.

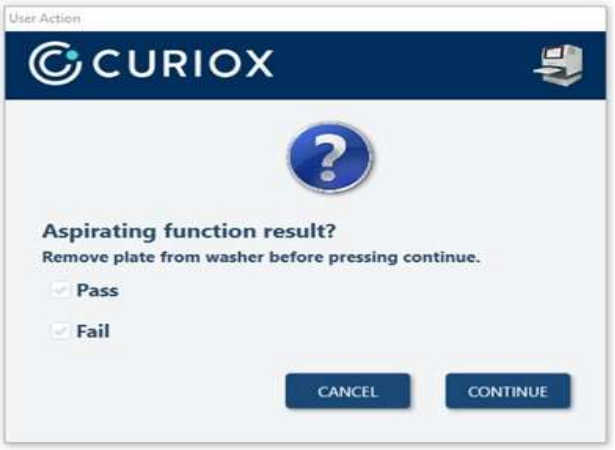


Figure 4-18. Aspirating function verification.

6. If any of the wells failed the dispensing verification test, perform troubleshooting as per the steps outlined in "Section 5: Troubleshooting, Laminar Wash HT2000/HT2100 System User Manual".

Figure 4-19. Prompt to perform troubleshooting if dispensing failure was reported on Step 4.

7. If any of the wells failed the aspirating verification test, perform troubleshooting as per the steps outlined in "Section 5: Troubleshooting, Laminar Wash HT2000/HT2100 System User Manual".

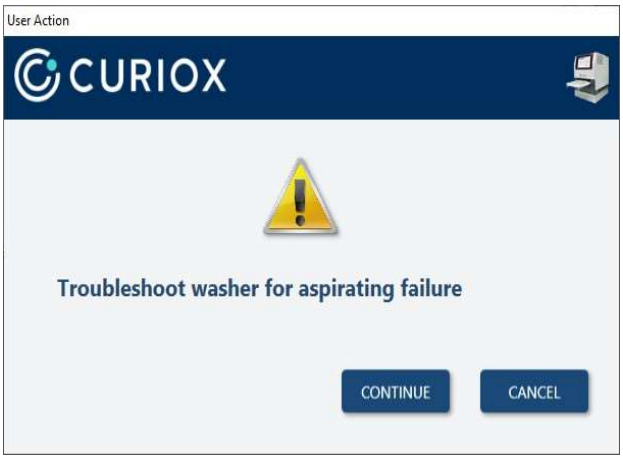


Figure 4-20. Prompt to perform troubleshooting if aspirating failure was reported on Step 5.

Running a Method

Select and open GUI using the following instructions.

1. Select the program *Hamilton Run Control* on the laptop Desktop

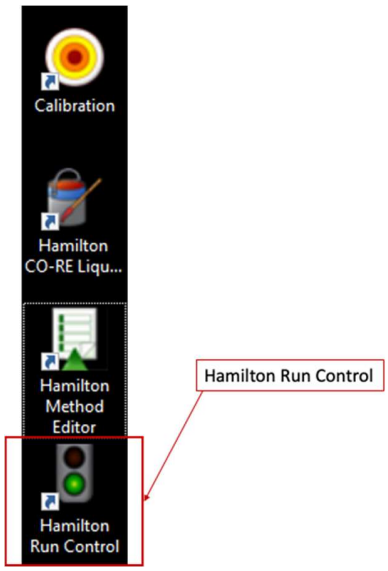


Figure 4-21. *Select Hamilton Run Control from Desktop.*

2. Under *File > Open*, select the directory *C:\Program Files (x86)\Hamilton\Curiox* And select the desired GUI method file.

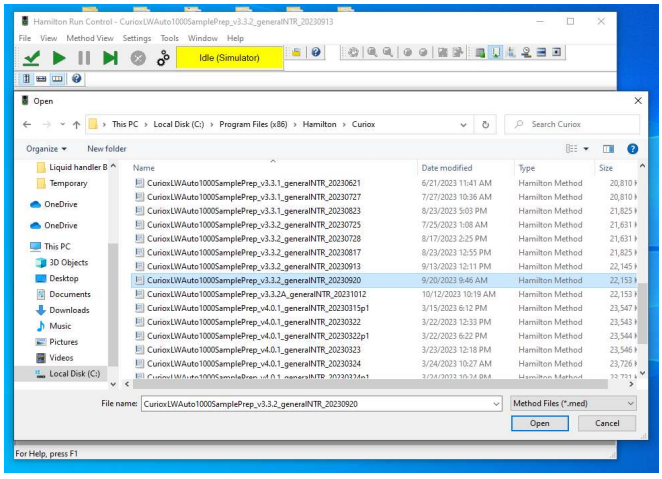


Figure 4-22. *Select the appropriate AUTO1000 GUI method file.*

3. When initialization of Hamilton Run Control is complete, hit the **Start** button in the tool bar to begin AUTO1000 GUI.

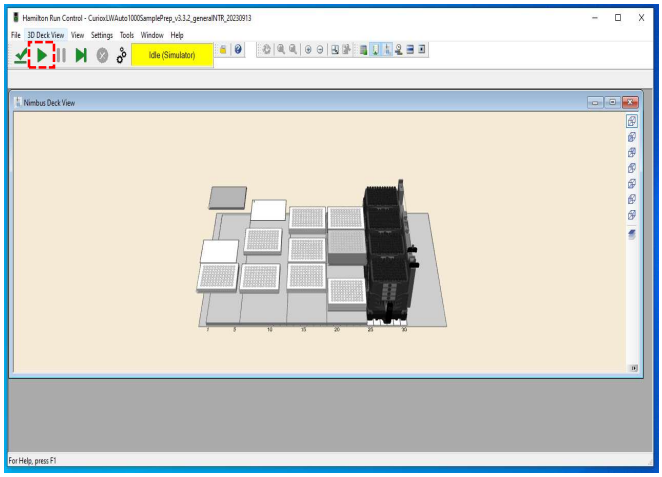


Figure 4-23. *Select Play button to start AUTO1000 GUI.*

4. Curiox AUTO1000 GUI appears, proceed to start up the HT2000/HT2100 washer or set up assay protocol.

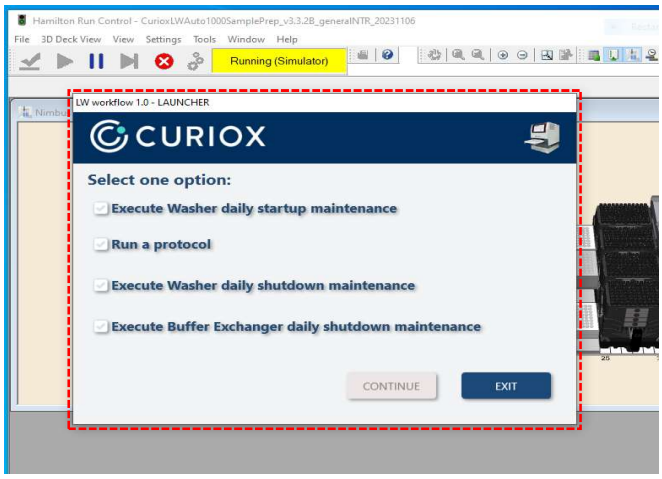


Figure 4-24. *Proceed to start up the HT2000/HT2100 washer or set up assay protocol using AUTO1000 GUI.*

Simulation Mode

Simulation mode allows the user to operate the GUI without being connected to the AUTO1000 or peripherals. It is useful for planning experiments, precoding protocols, and calculating the volumes each protocol will be withdrawing from the reagent plate during an actual run. Simulation mode is denoted by the yellow status window at the top of the screen.

To enter simulation mode, open Hamilton Run Control and push the “Simulation Mode” button under the Settings tab. To deactivate simulation mode, ensure you are connected to the AUTO with the power on, then push the “Simulation Mode” button again.

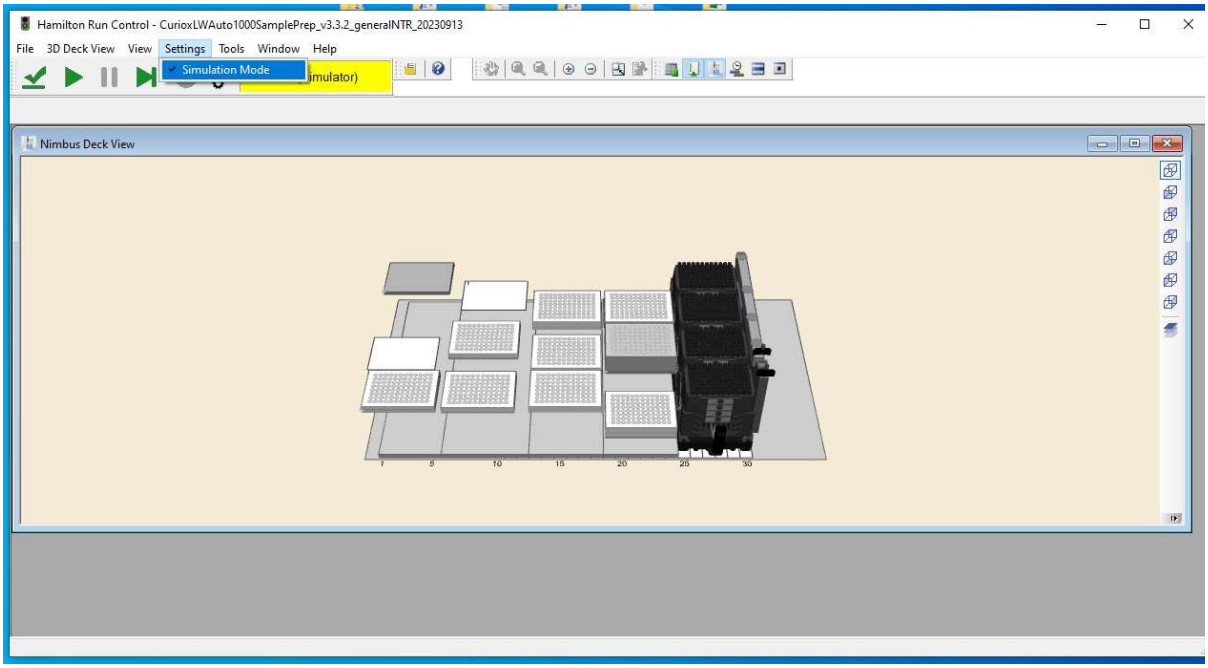


Figure 4-25. Simulation Mode indicator and activation button.

Simulation mode can run the method at three different speeds: Slow (true to life); Fast (operations are simulated at highly increased speeds); and No Delay (operations simulated as fast as processing power allows).

To switch simulation speeds, select “System Configuration Editor” under the Tools tab. Navigate to “Microlab Nimbus 8 Channel” over on the left hand menu, then change “Simulation Speed” over on the right to your desired speed.

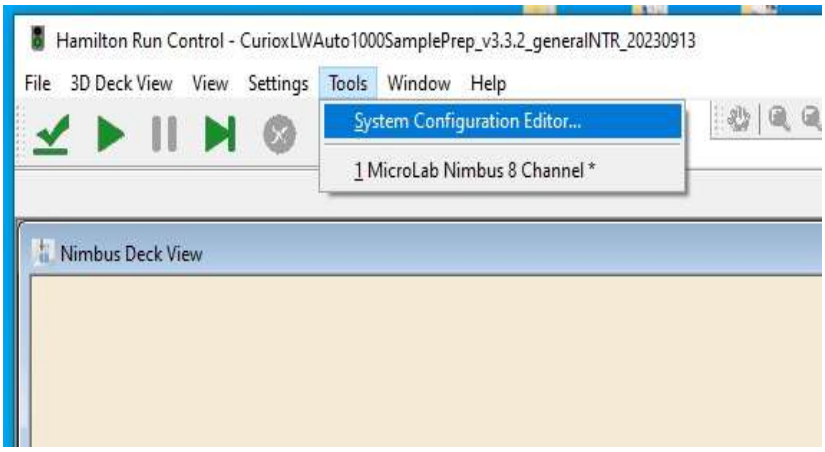


Figure 4-26. Opening the System Configuration Editor.

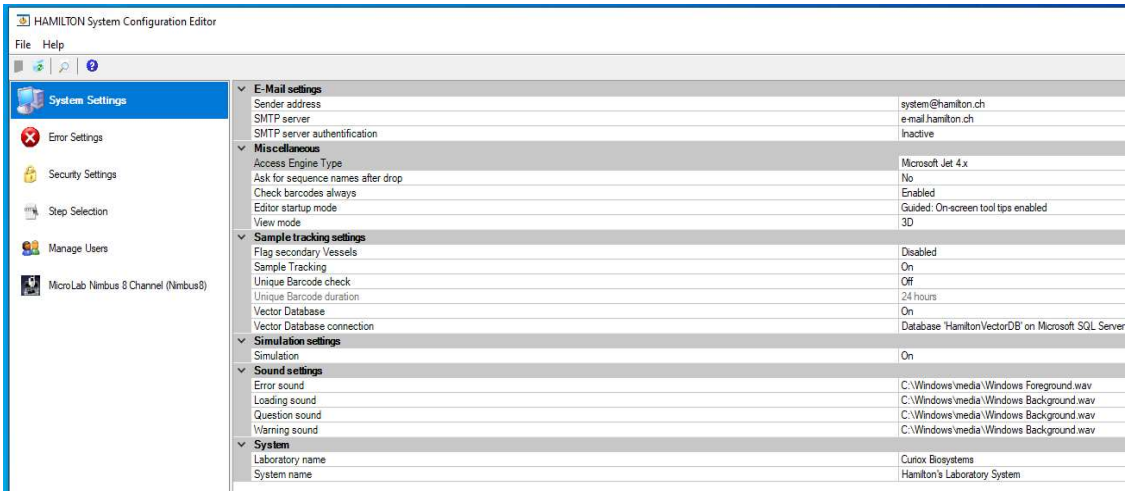


Figure 4-27. Changing the simulation speed.

The GUI can then be run by the normal launching process: opening the most up-to-date method in the "File" tab, then hitting the triangular "Start" button.

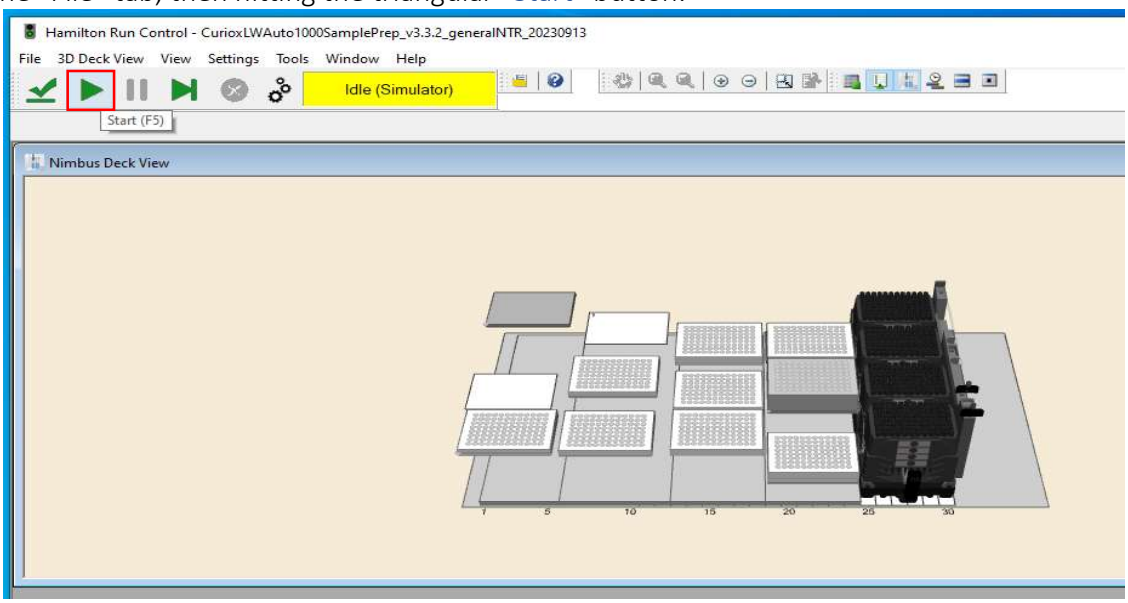


Figure 4-28. Activating the simulation

As they run, simulations generate Trace files and Mapping files as though they were a normal run.

Run a Saved Protocol

To run an existing saved protocol, follow the steps below.

1. Select **Run a protocol**

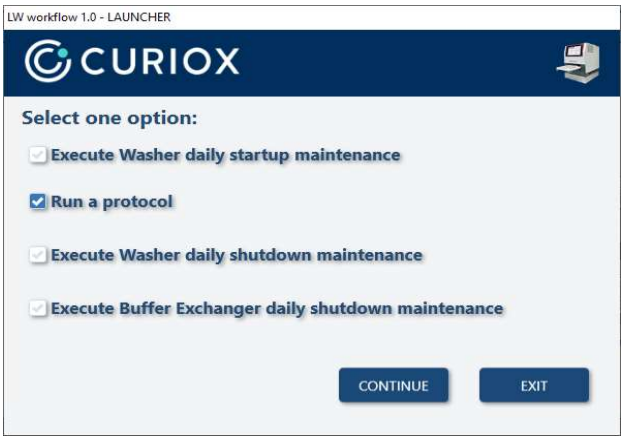


Figure 4-29. Run a protocol

2. Select Option to:

□ **Configure new run** – see next section Build a Protocol

□ **Load saved run** – retrieve saved assay parameters

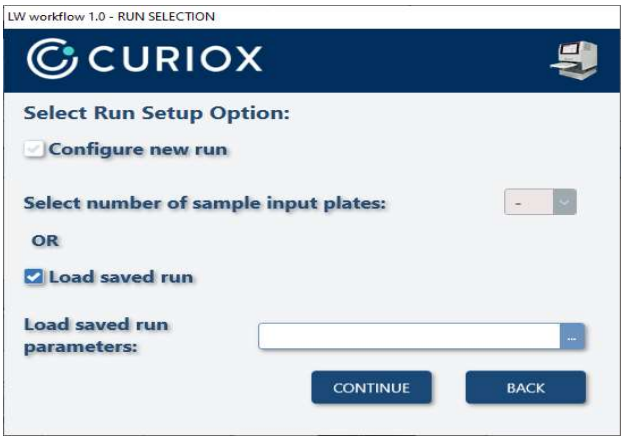


Figure 4-30. Selecting a pre-saved protocol and file path.

Building a protocol on AUTO1000 GUI

To build a protocol in the AUTO1000 GUI, user must refer to the Reagent Plate Layout shown below (*Figure 4-31.*). This figure represents a generic reagent plate for a two Sample Input Plate protocol. Each panel/reagent can be distributed across wells as per the user's configuration, up to the maximum number of samples. Additionally, each plate can have 2-4 output buffer panels based on the required volume. After setting up a protocol on AUTO, refer to the section on **Setting up the Reagent Plate** for information on volumes and positions to input each reagent.

Reagent Plate Layout

Figure 4-31*. Reagnet Plate layout for two sample input plate (left) accommodating 10 panels / reagents each, or reagent plate layout for one sample input plate (right), accommodating 22 panels / reagents in Total.*

The GUI considers a typical protocol setup as loops, consisting of the following:

- Loop 1: sample input and set up of sample plate. This may be proceeded by incubation and wash on HT2000/HT2100.
- Loop 2 and subsequent loops: Addition of reagents from Reagent plate. This may be proceeded by incubation and wash on HT2000/HT2100.
- Finish: Instructions to prepare samples for acquisition on flow cytometer by transfer to output plate or placement of DRG for direct acquisition.

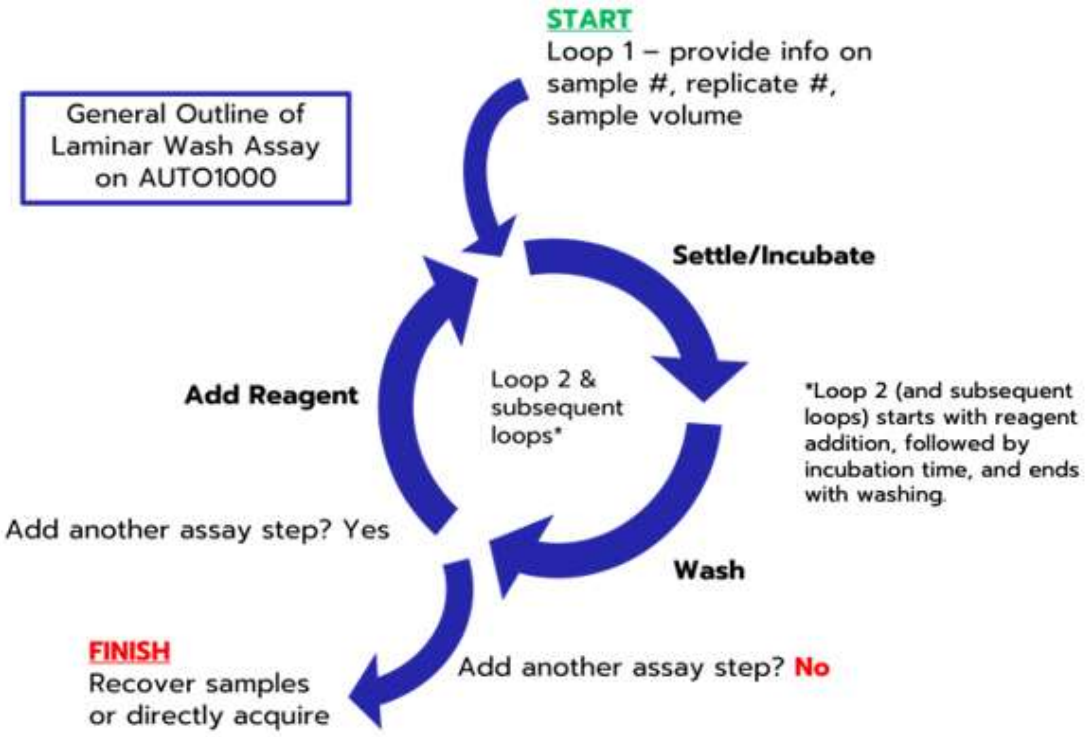


Figure 4-32. *Schematic of protocol information input in AUTO1000 with Loops.*

To build a new protocol, follow the steps below:

1. Select □ **Configure new run.**

A dropdown window for **Select number of sample input plates** allows selection to run only 1 plate or up to 2 plates in series.

NOTE: If the user wishes to run 2 sample plates, they will be given the option to pause and remove the first plate or continue with 2nd plate with no pause after the completion of the first plate. 22-Panel interface is applied when only 1 sample input plate is selected.

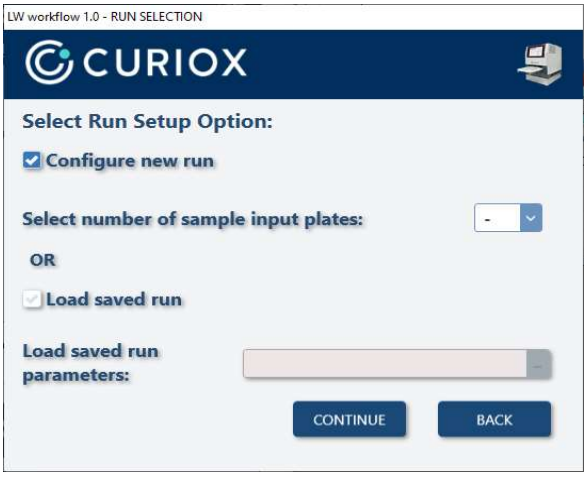


Figure 4-33. Select Configure new run.

2. If user has selected 2 input plates, they will have to **Select Run Setup Option** where they can either

□ **Pause and remove the 1st plate –** pause the run to manually remove the first plate from the deck after the first plate has been completed, or

□ **Continue to the 2nd plate with no pause** – the completed first plate will be moved to the peltier set to 4 ºC and covered with an opaque lid.

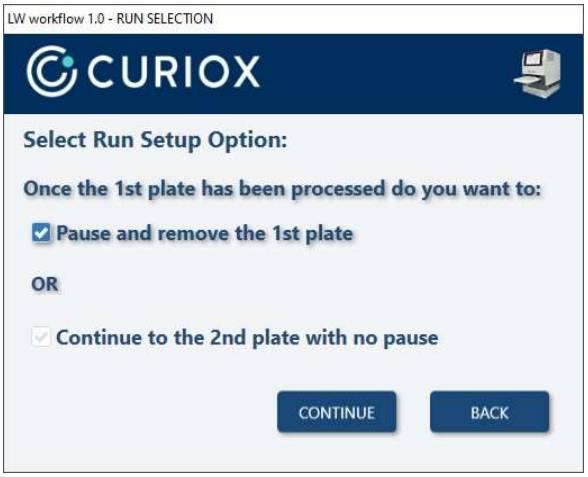


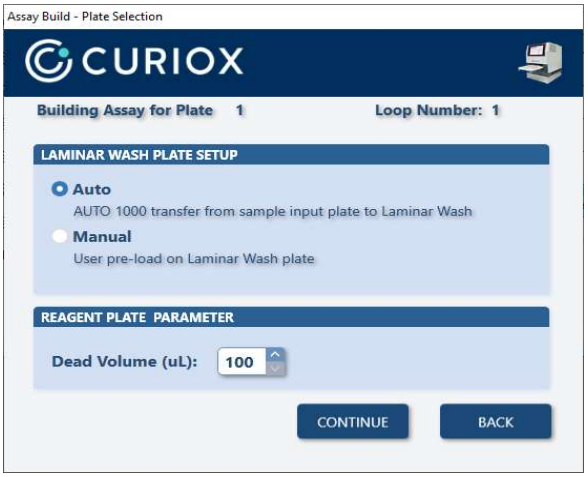
***Figure 4-34.** Prompt for User to select the Run Setup Option when using two Sample Input Plates.*

3. This screen gives the user the options to load their samples.

□ **Auto** – samples are loaded onto AUTO1000 deck in Sample Input plate. AUTO1000 transfers the samples to LW plate.

□ **Manual** – samples are loaded onto AUTO1000 deck in LW plate. AUTO1000 proceeds immediately to incubation or wash as input on the next screen.

Dead volume (ul) – The dead volume required when adding reagents to the plate



is set.

*Figure 4-35. Select source of cell input by **Auto** sample transfer from Sample Input plate or **Manual** pre-loading on LW plate.*

4. Assay Building Loops offer the options to define parameters for the transfer or incubation and wash of the samples.

There are two main sections in Loop 1:

GENERAL INPUT PARAMETERS – section to define samples and layout input on LW plate.

Number of input samples – define number of unique samples on sample input plate or Laminar Wash plate. This will be arrayed by rows.

Number of sample replicates – define number of technical replicates for each of the unique samples indicated above.

□ **Pipette mix source plate?** – define pipette mixing in samples from Sample Input Plate prior to transfer to LW plate.

Transfer volume (µL) – (if Auto transfer is selected) volume to transfer from Sample Input plate to LW plate. If Laminar Wash plate was previously indicated, input the volume on each well on LW plate here.

□ **Pipette mix following transfer to Laminar Wash plate** – select to pipette mix LW plate to distribute cells across the plate after completion of Sample Input transfer.

□ **Vortex Laminar Wash Plate** – select to vortex LW plate to distribute cells across the plate after completion of Sample Input transfer.

INCUBATION AND WASH PARAMETERS – section to define wash with HT2000/HT2100

□ **Enter Incubation time** (0 to 999 min) – define incubation time of LW for cell settling. Input '0' to skip incubation.

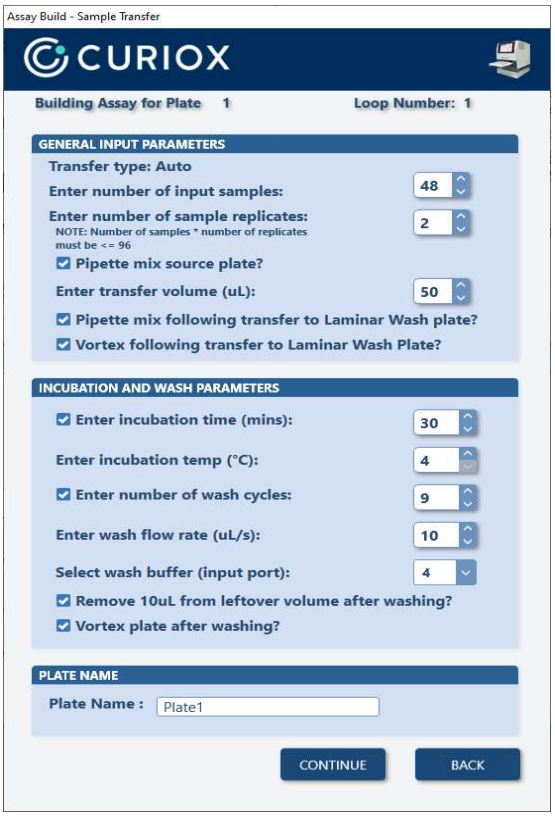


Figure 4-36. Build a Protocol - Loop 1

(Step 4 instructions contd.)

Wash buffer (input port) – select wash buffer primed through HT2000/HT2100 via BEX channel

□ **Remove 10µL from leftover volume after washing?** – enable aspiration of 10µL from residual volume after HT2000/HT2100 washing, to reduce final volume

□ **Vortex plate after washing?** Specify option to vortex plate after washing to lift cells off before next antibody addition

Plate name – Label plate with the desired name.

After selecting **Continue**, User will then view a

□ **Enter incubation temperature** (4 to 70ºC) – define incubation temperature of LW plate on CPAC deck. Incubation timers are not begun until temp within 5°C of target.

□ **Enter number of wash cycles** (0 to 50) – define number of washes on HT2000/HT2100

Wash flow rate (2 – 20µL/s) – flow rate of wash on HT2000/HT2100

5. Following confirmation of the input parameters, User can use this screen to review the plate layout the AUTO1000 will assume for the run.

Sample input begins from well A1. Unique samples are arrayed vertically, while technical replicates are arrayed horizontally.

To utilize all four pipette channels simultaneously, dispensing occurs vertically as opposed to horizontally which also allows for the same channel to dispense its given sample/reagent. Select **Continue** to proceed, or **Back** to return to parameter input.

6. User can select the option to include another assay step (referred to as Loop 2) by selecting **Yes** or to end assay protocol building and proceed to the sample output step by selecting **No**.

greyed- out version of parameter input screen to confirm the input parameters .

NOTE:

User has the option to disable any of the "INCUBATION AND WASH PARAMETERS" by removing the check mark on each step.

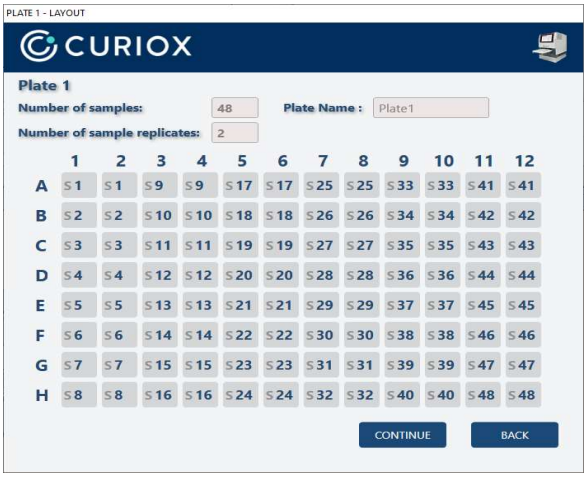


Figure 4-37. Confirming LW Plate Layout

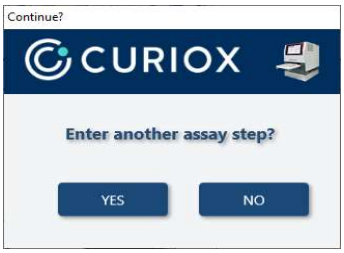


Figure 4-38. Option to continue another assay step or proceed to end protocol building.

7. Loop 2 and subsequent loops enable users to add reagents from the Reagent Plate. Refer to step 3 on the options for **General Input Parameters** and **Incubation and Wash parameters**.

Antibody / Panel Transfer Parameters enable users to define transfer of reagents from the Reagent Plate to the Laminar Wash plate.

Enter number of samples for panel *x* – indicates number of unique samples receiving panel *x* laid out in the Reagent Plate. Note that reagent addition begins in sequence from Well A1, and is applied to all technical replicates of the unique samples. Users may indicate a name for panel *x*. The volume of panel *x* to transfer is indicated under **Enter Transfer Volume (µL)** on the left panel of the same loop.

If one plate is chosen on step 1, 22 input field appears under **Antibody / Panel Transfer Parameters**. (***Figure 4-40***.)

User may input a loop name, e.g. *Surface antibody addition*.

Select **Continue** to proceed.

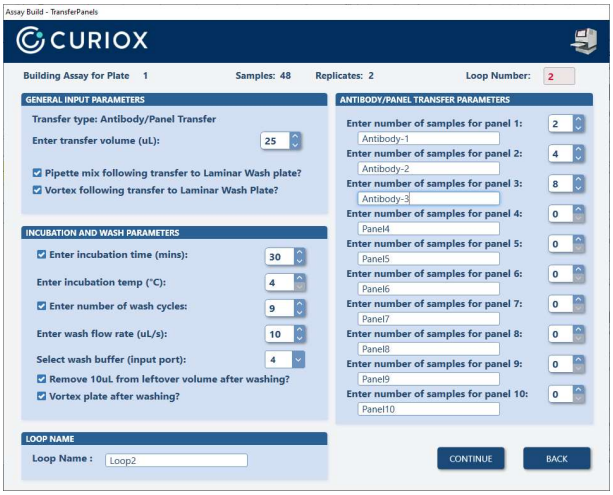


Figure 4-39. *Configure Loop 2 for reagent addition, showing screenshot for up to 10 panels / reagents, to run to up two plates in series.*

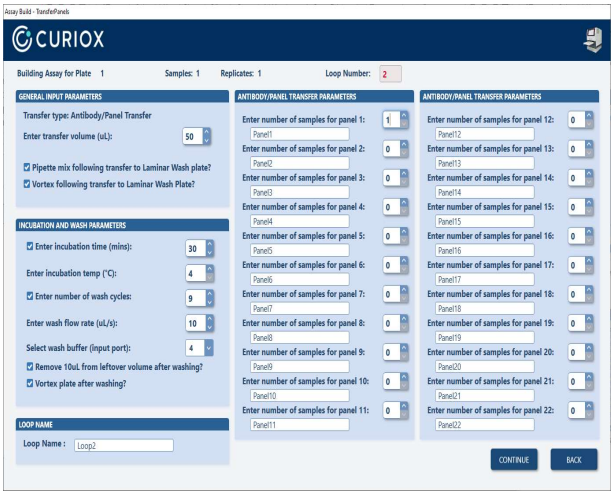


Figure 4-40. *Configure Loops 2 for reagent addition, showing screenshot for up to 22 panels / reagents to run on one plate.*

8. This screen will display the plate layout for user to confirm. The plate layout indicates the position of samples on the plate.

Reagent Panels will display a Reagent Plate Layout as configured by the users inputted parameters.

Panel Treatments will show the reagent panels and their corresponding wells on the Laminar Wash Plate. Select Continue to proceed or Back to return to the previous screen.

NOTE:

The total number of samples to add to each panel should not be greater than the total number of samples used on the Laminar Wash plate.
Antibody panels are added to the Laminar Wash plate sequentially from top to bottom, left to right starting from well A1 based on the number of samples entered to receive a given panel. Replicates are added horizontally.

9. User will then be prompted to select whether they would like to enter another assay step (continue to next loop) or continue to the sample output transfer step. To continue to next loop, user should select YES and follow through steps 6 and 7. To continue to sample transfer, select NO to continue to step 9.

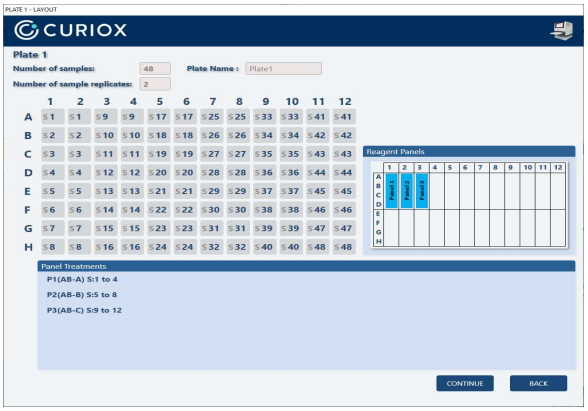


Figure 4-41. Sample Plate layout with assignment of panel reagents to respective sample wells.

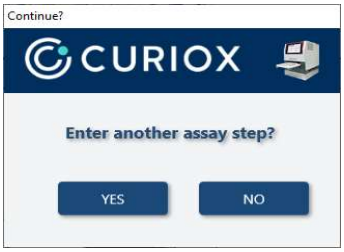


Figure 4-42. Option to continue to another assay step or proceed to end protocol building.

10. Transfer step

Users have two options for sample transfer.

Option 1

□ **Do not transfer processed sample to output plate** – samples remain on LW plate and are not transferred to output plate.

□ **Use DRG (Direct Reading Grid)** – samples remain on LW plate and are not transferred to output plate. A DRG will be added to the Laminar Wash Plate

Additional buffer can be added to the Laminar Wash plate regardless of the presence of DRG, as specified by the User under **Final volume in LW plate** (max 70µL without DRG, 300µL with DRG).

NOTE: The final volume in LW plate value should be the final output volume required for Direct Acquisition, not the additional top-up volume.

Option 2

□ **Transfer processed sample to output plate** – samples will be transferred to output

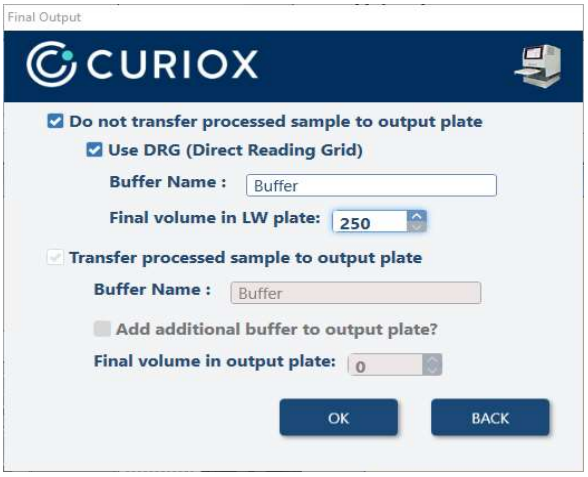


Figure 4-43. Options for output of samples at the end of assay run.

(contd.) plate (standard microtiter plate) on deck with the output buffer. Standard transfer protocol results in final volume of 100µL.

□ **Add additional buffer to output plate** – Indicate desired final output volume if >100µL is required.

11. Plate Layout Confirmation:

Here, User will be shown the plate layout and Panel Treatments that the user has configured. User must confirm this to continue to the next step.

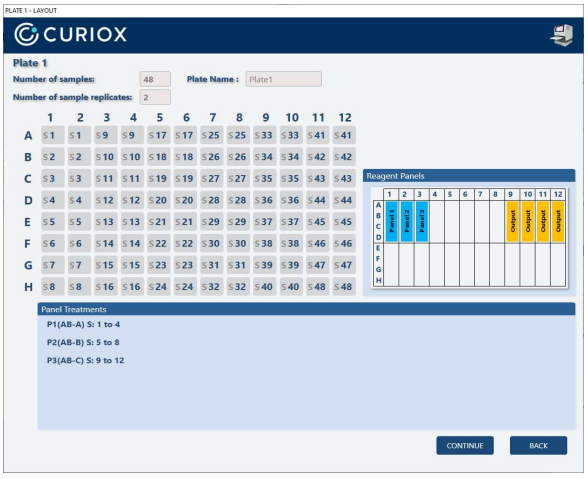


Figure 4-44. Summary Sample Plate layout with assignment of all panel / reagents to respective sample wells.

12. Save Run Parameters? User will have the option to preserve assay settings for future runs without having to re-input all parameters. Select **Continue**.

All saved run parameters are located at *C:\Auto_1000_Run_Files*

Figure 4-45. Save Run Parameters

13. Required Reagent volume confirmation
Based on the values set for the assay and the dead volume, the required reagent values to be added to the reagent plate are calculated and displayed. User can prepare the necessary buffer volume based on this information.

Figure 4-46. Calculated Reagent volume Display

14. Prior to performing assay run, the GUI will guide User in setting up the AUTO1000 deck. User will be prompted to **Empty the LVA waste**, **Emtpy Tips Waste Bin** and **Empty Tip Racks** before beginning the run. After this, User will need to check of each point to correctly load the AUTO1000 deck.

An option to **Skip all** is also provided, which will allow User to skip directly to the final confirmation page.

Select **Continue** for the confirmation before beginning the run.

Figure 4-47. Guide to prepare deck for run – empty waste from Tip Waste bin and AUTO1000 deck spaces.

15. User will then be prompted to □ **Load a Washer Maintenance Plate** in the position indicated by yellow flashing.

After this is complete, select **Continue**.

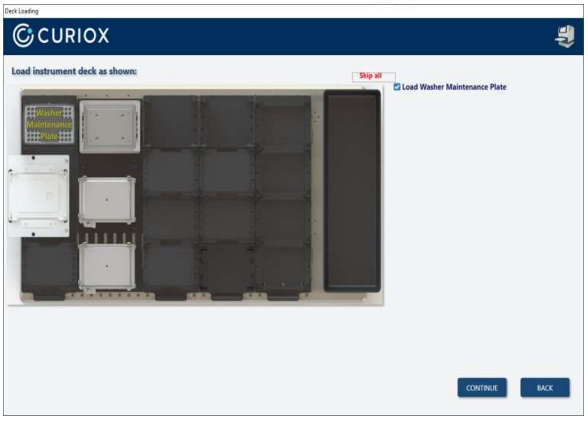


Figure 4-48. *Guide to prepare deck for run – load AUTO1000 deck with Washer Maintenance Plate.*

16. Depending on the requirements of the protocol, User will then be prompted to □ **Load 1 (or 2) Sample Input Plate** in the position indicated by yellow flashing.

After this is complete, select **Continue**.

NOTE: If user has selected Auto during the initial loop of sample transfer, this option will say "No sample input plate loaded"

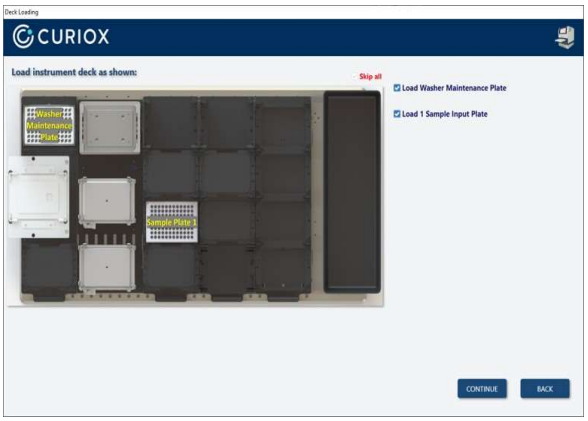


Figure 4-49. *Guide to prepare deck for run – load AUTO1000 deck with Sample Input Plate.*

17. Depending on the requirements of the protocol, User will then be prompted to □ **Load 1 (or 2) Laminar Wash Plate** in the position indicated by yellow flashing.

After this is complete, select Continue.

NOTE: If user has selected Auto during the initial loop of sample transfer, this option will not be visible.

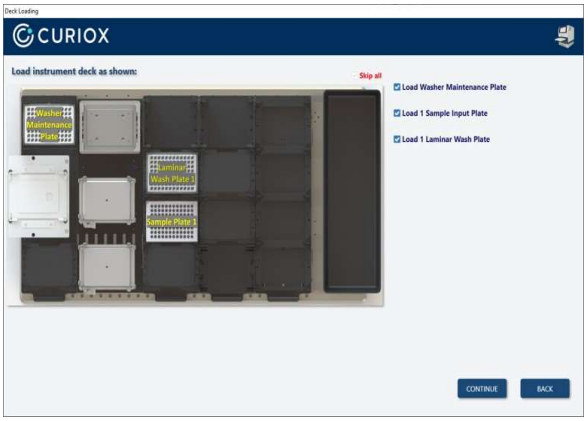


Figure 4-50. *Guide to prepare deck for run – load AUTO1000 deck with Laminar Wash plate.*

18. User will then be prompted to □ **Load Reagent Plate** in the position indicated by yellow flashing.

After this is complete, select **Continue.**

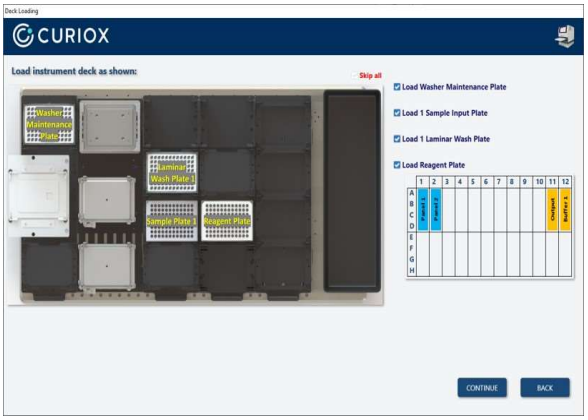


Figure 4-51. Guide to prepare deck for run – load AUTO1000 deck with Reagent Plate

19. User will then be prompted to □ **Load 300µL Stacked Tips** in the position indicated by yellow flashing. User should load stacks of 4 tip racks.

After this is complete, press **Continue**.

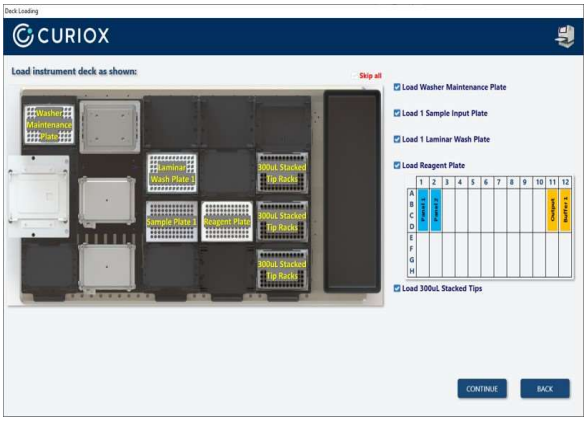


Figure 4-52. Guide to prepare deck for run – load AUTO1000 deck with 300µL Stacked Tip Racks

20. User will then be prompted to □ **Load an Opaque Lid** in the position indicated by yellow flashing.

After this is complete, press **Continue**.

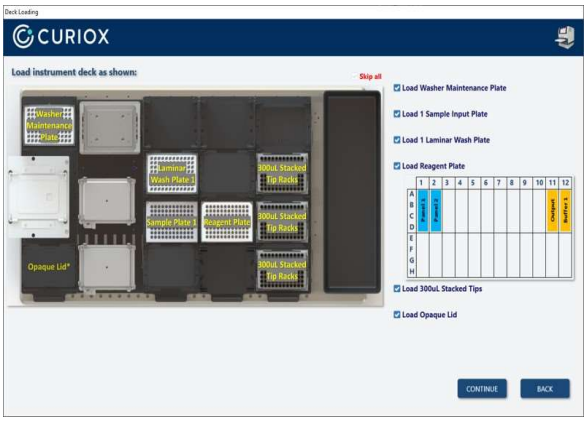


Figure 4-53. Guide to prepare deck for run – load AUTO1000 deck with Opaque Lid.

21. Depending on the requirements of the protocol, User will then be prompted to □ **Load a (or 2) Large Volume Adapter (**or **No Large Volume Adapters Required)** in the position indicated by yellow flashing.
After this is complete, press **Continue**.

NOTE: If users configured protocol does not require the use of an LVA, the option will display No Large Volume Adapters Required.

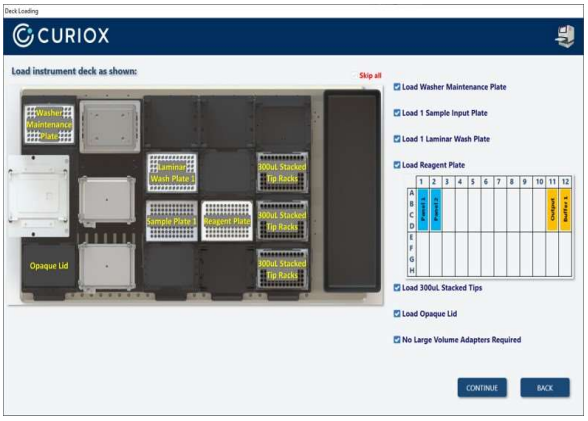


Figure 4-54. Guide to prepare deck for run – load AUTO1000 deck with Large Volume Adaptor(s), if needed.

22. If User had indicated to transfer samples from LW to output plate in the GUI, User will then be prompted to □ **Load an (or 2) Output Plate with Lid** in the position indicated by yellow flashing.

After this is complete, press **Continue**.

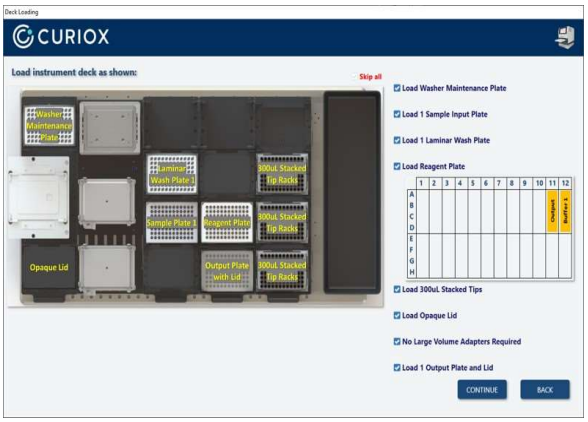


Figure 4-55. Guide to prepare deck for run – load AUTO1000 deck with Output Plate(s) with Lid, if needed.

23. If User has programmed the use of a DRG in the final loop, User will be prompted to load DRG(s) and DRG adaptor onto the DRG/Output deck.

After this is complete, press **Continue**.

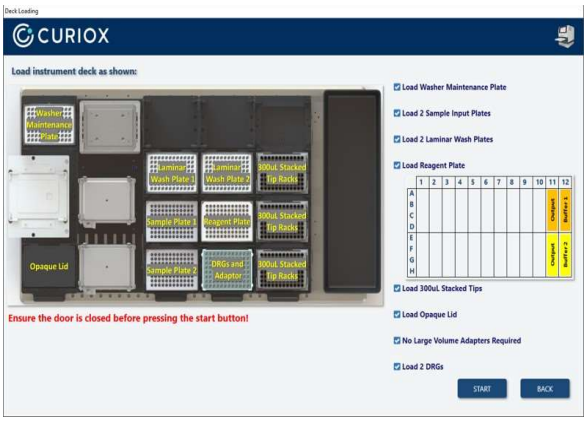


Figure 4-56. Guide to prepare deck for run – load AUTO1000 deck with Direct Reading Grid(s), if needed.

24. User will then need to ensure the door is closed before selecting the start button.

After the **Start** button is selected, the AUTO 1000 assay run will begin.

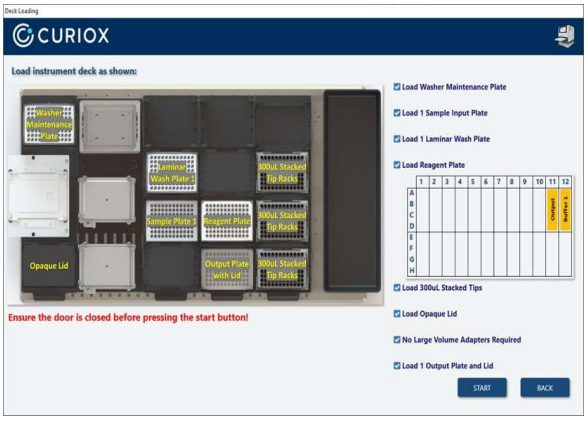


Figure 4-57. Ensure AUTO1000 door is closed prior to the start of protocol run.

Setting up the Reagent Plate

The required volume of reagent can be confirmed through the Required Reagent Volume page that appears during assay. The software automatically calculates and then displays the required volume. User should add the volume as indicated on the page. This volume is affected by dead volume, which should be adjusted to ensure uninterrupted operatioin.

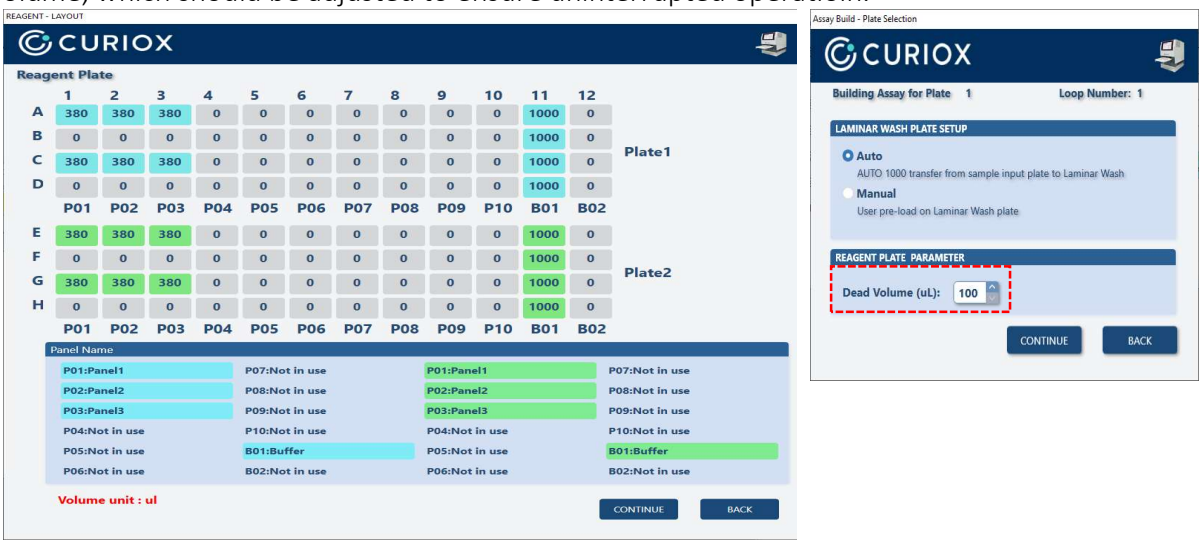


Figure 4-58. Required Reagent Volume confirmation page and dead volume set.

Shutdown Maintenance

1. After each run is complete, User may select proceed to shut down if the AUTO 1000 runs are finished for the day.

□ **Perform Shutdown Maintenance** – select to shut down HT2000/HT2100 washing station. *Refer to Step 3.*

□ **Execute Buffer Exchanger daily shutdown maintenance** – select to flush Buffer Exchange with using BEX shutdown protocol. *Refer to Step 4.*

Select **OK** to continue.

Figure 4-59. *Prompt to perform Shutdown Maintenance of HT2000/HT2100 after protocol run.*

2. The options for HT2000/HT2100 or Buffer Exchanger shutdown maintenance are also available on the landing page of the AUTO1000 GUI.

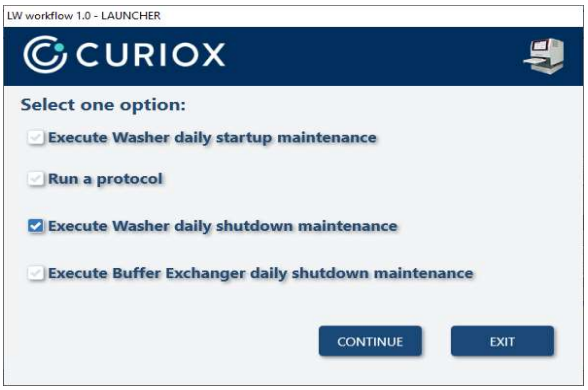


Figure 4-60. *Option for Shutdown Maintenance is also available on the initial start-up page on the AUTO1000 GUI.*

3. After selecting **Execute Washer daily shutdown maintenance**, User will be prompted to place a Washer Maintenance Plate onto the Washer Maintenance Plate Deck.

After placing the plate on the correct deck, select **Continue**.

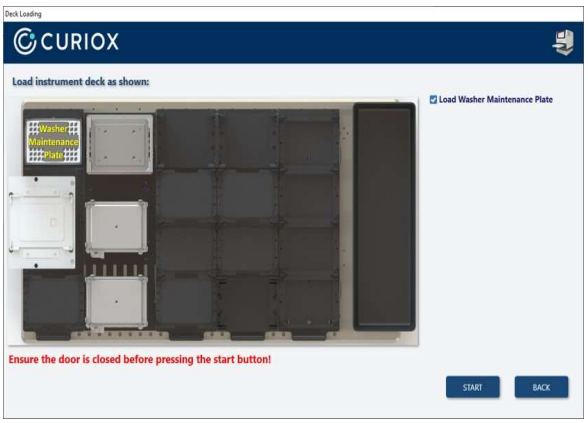


Figure 4-61. *Guide to prepare deck for Shutdown Maintenance – load AUTO1000 deck with Washer*

Maintenance Plate

4. User can then choose to **Execute Buffer Exchanger daily shutdown maintenance**, with the option to select whether a secondary buffer exchanger is in use.

After selecting this, select **Continue**.

Figure 4-62. *Prompt to perform shutdown maintenance of Buffer Exchanger after protocol run.*

5. User will be prompted to "Place all washer input lines for the primary buffer exchanger into the cleaning solution".

Externally, connect the buffer inlet lines of the primary buffer exchanger to 1-to-4 connector. The connector is then connected, in sequence, to the bottle cap of

1. DI Water + 1% Tween-20
2. 70% Ethanol + 1% Tween-20
3. Air

After this is complete, User should select **Continue**.

Figure 4-63. *Prompt for Users to set up the primary Buffer Exchanger for Shutdown Maintenance.*

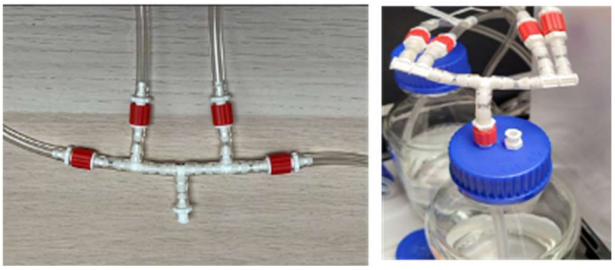


Figure 4-64. *1-to-4 connector links the four buffer inlet lines of the Buffer Exchanger to a single bottle of priming solution.*

6. After the primary buffer exchanger maintenance is completed, User should select **Continue**.

Figure 4-65. Prompt upon completion of primary Buffer Exchanger Shutdown Maintenance.

7. Here, the user will be prompted to "Place all washer input lines for the secondary buffer exchanger into the cleaning solution".

Externally, the 1-to-4 connector should be switched to connect the four inlet buffer lines of the secondary buffer exchanger, if present.

After this is complete, user should select **Continue.**

Figure 4-66. Prompt for Users to set up the secondary Buffer Exchanger for Shutdown Maintenance.

8. After the secondary buffer exchanger maintenance is completed, the user should select **Continue**.

Figure 4-67. Prompt upon completion of secondary Buffer Exchanger Shutdown Maintenance

Example Protocols

Given below are two example protocols with details on the sample plate and reagent plate layout.

Example 1 – Surface staining protocol with viability and unstained controls

1. User planned to configure a Laminar Wash plate pre-loaded with 16 samples, consisting of 1 unstained and one viability only control, distributed as 3 replicates. (Select Manual on the first screen of General Input Parameters.)

On the Reagent Plate,

- Panel 1 will consist of Viability dye only,
- Panel 2 will contain PBS as a control reagent,
- Panel 3 will consist of Surface Antibody Stain and
- Panel 4 will consist of PBS as a control reagent.

	1	2	3	4	5	6	7	8
A	Sample 1 Viability Surface Ab	Sample 1 Viability Surface Ab	Sample 1 Viability Surface Ab	Sample 9 Viability Surface Ab	Sample 9 Viability Surface Ab	Sample 9 Viability Surface Ab		
B	Sample 2 Viability Surface Ab	Sample 2 Viability Surface Ab	Sample 2 Viability Surface Ab	Sample 10 Viability Surface Ab	Sample 10 Viability Surface Ab	Sample 10 Viability Surface Ab		
C	Sample 3 Viability Surface Ab	Sample 3 Viability Surface Ab	Sample 3 Viability Surface Ab	Sample 11 Viability Surface Ab	Sample 11 Viability Surface Ab	Sample 11 Viability Surface Ab		
D	Sample 4 Viability Surface Ab	Sample 4 Viability Surface Ab	Sample 4 Viability Surface Ab	Sample 12 Viability Surface Ab	Sample 12 Viability Surface Ab	Sample 12 Viability Surface Ab		
E	Sample 5 Viability Surface Ab	Sample 5 Viability Surface Ab	Sample 5 Viability Surface Ab	Sample 13 Viability Surface Ab	Sample 13 Viability Surface Ab	Sample 13 Viability Surface Ab		
F	Sample 6 Viability Surface Ab	Sample 6 Viability Surface Ab	Sample 6 Viability Surface Ab	Sample 14 Viability Surface Ab	Sample 14 Viability Surface Ab	Sample 14 Viability Surface Ab		
G	Sample 7 Viability Surface Ab	Sample 7 Viability Surface Ab	Sample 7 Viability Surface Ab	Viability Ctrl Viability PBS	Viability Ctrl Viability PBS	Viability Ctrl Viability PBS		
H	Sample 8 Viability Surface Ab	Sample 8 Viability Surface Ab	Sample 8 Viability Surface Ab	Negative Ctrl PBS PBS	Negative Ctrl PBS PBS	Negative Ctrl PBS PBS		

Figure 4-68. User intended layout of samples and reagents allocation

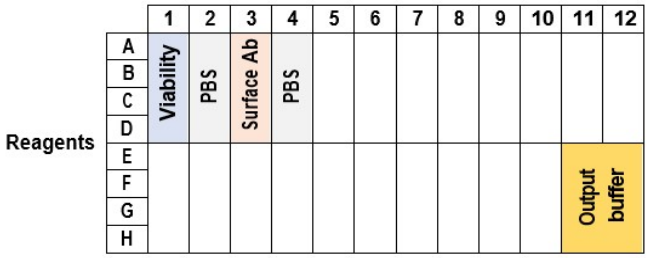


Figure 4-69. Layout of reagent distribution on Reagent Plate.

2. Loop 1:

This Loop will consist of the initial washing step with no Reagent panel added to the sample. User specified the samples preloaded on Laminar Wash plate as 25µL in each well, with 16 unique samples and 3 technical replicates each.

User configured the AUTO1000 to incubate the samples for 30min at 4ºC before placing the plate on HT2000/HT2100 for 9x wash at 10µL/s using buffer from input port 4. At the end of the wash, AUTO1000 will transfer the plate to be vortexed prior to return to the deck.

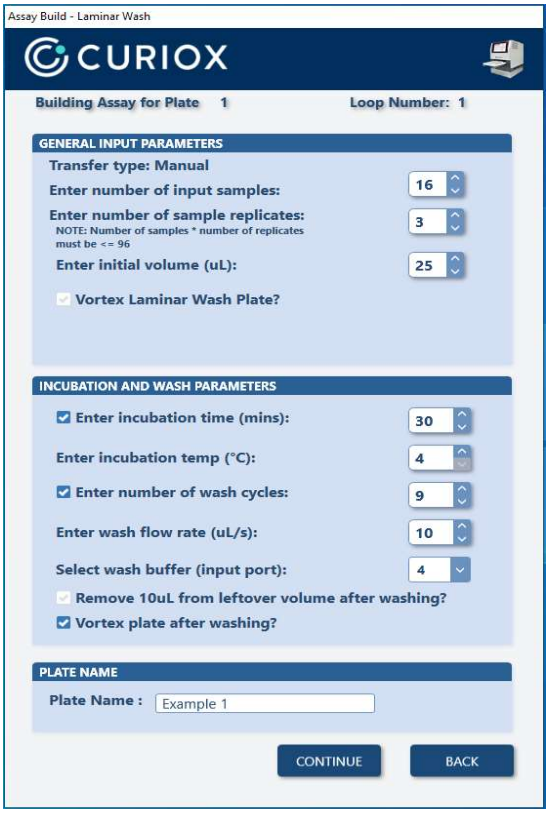


Figure 4-70. Configuring Loop 1 on the AUTO1000 GUI, informing the GUI of sample volume pre-loaded on LW plate.

3. Loop 2:

Following the HT2000/HT2100 wash in Loop 1,

- 50µL of Panel 1 (Viability) will be distributed to each of samples 1-15.
- Panel 2 (PBS) will be distributed to sample 16 as unstained control.

The incubation and wash parameters are as indicated in the loop.

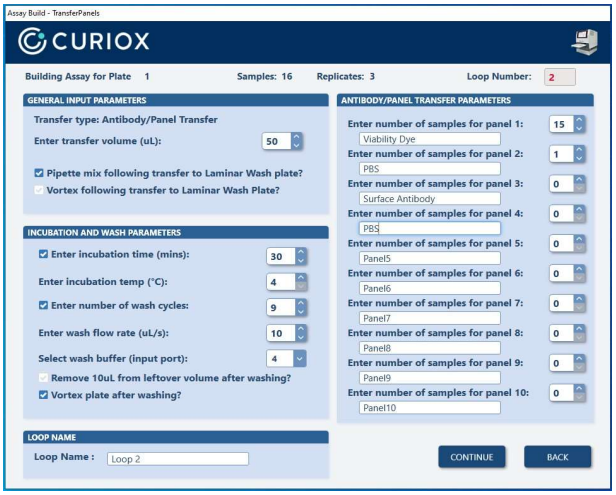


Figure 4-71. Configuring first panel / reagent (Viability dye) addition to samples and the second panel / reagent (PBS) to the control samples.

4. Loop 3:

Following the HT2000/HT2100 wash in Loop 2,

- 50µL of Panel 3 (Surface Antibody Stain) will be distributed to each of samples 1-4.
- Panel 4 (PBS) will be distributed to sample 15-16 as controls.

The incubation and wash parameters are as indicated in the loop.

Figure 4-72. Configuring the third panel / reagent (Surface Antibody) addition to samples and the fourth panel (PBS) to the control samples.

5. Following confirmation of the parameters in each loop, a confirmation page displays the layout of samples and allocated panel treatment to each sample. *Figure 4-72* shows the confirmation page at the end of Loop 3.

Figure 4-73. Confirmation of the Sample Plate layout and panel / reagent distribution for all loops.

6. Here, User has configured the addition of a Direct Reading Grid on the Laminar Wash plate with a final volume of 150µL in the plate for direct reading. The system will transfer a DRG onto the Laminar Wash plate after the final washing step is completed and top up to 150µL with the output buffer in the Reagent Plate.

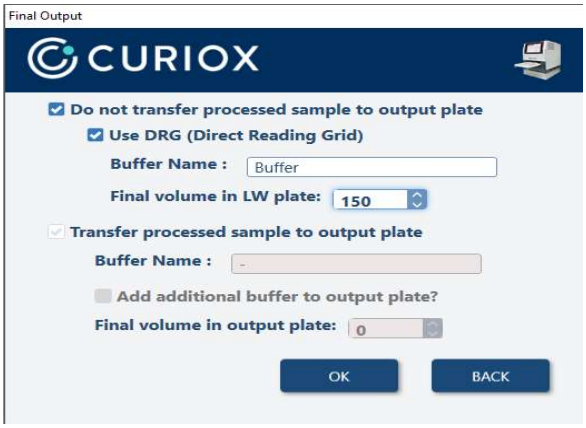


Figure 4-74. Configuring the sample transfer step using a DRG with final output volume of 150µL.

Example 2 – Surface and intracellular staining protocol

1. User planned to configure a sample input plate with 4 samples with 2 replicates and enters 4 panels to be transferred to the samples. For this example, the samples will first be transferred into an empty Laminar Wash plate in the AUTO1000 before beginning the initial wash cycle. (Select **Auto** for first General Input Parameter)

- Panel 1 will consist of the Viability dye,
- Panel 2 will consist of a Surface Antibody,
- Panel 3 will consist of Fix/Perm solution and
- Panel 4 will consist of the Intracellular Antibody.

	1	2	3	4	5	6
A	Sample 1 Viability Surface Ab Fix/Perm Intracellular Ab	Sample 1 Viability Surface Ab Fix/Perm Intracellular Ab				
B	Sample 2 Viability Surface Ab Fix/Perm Intracellular Ab	Sample 2 Viability Surface Ab Fix/Perm Intracellular Ab				
C	Sample 3 Viability Surface Ab Fix/Perm Intracellular Ab	Sample 3 Viability Surface Ab Fix/Perm Intracellular Ab				
D	Sample 4 Viability Surface Ab Fix/Perm Intracellular Ab	Sample 4 Viability Surface Ab Fix/Perm Intracellular Ab				
E						

Figure 4-75. User intended layout of samples and reagents allocation.

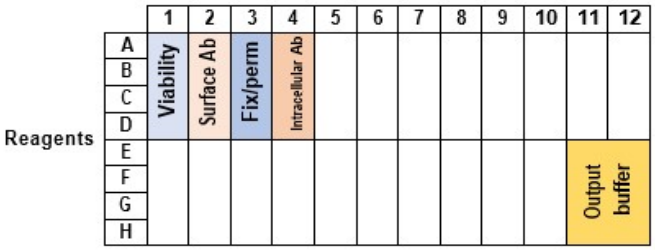


Figure 4.76. Layout of reagent distribution on Reagent Plate.

2. Loop 1:

This loop will consist of the initial transfer from a Sample Input Plate to a Laminar Wash plate followed by incubation and washing.

Here, the user specified 25µL of samples to be transferred from the Sample Input Plate to the Laminar Wash Plate. There are four unique samples to be transferred in two technical replicates.

The AUTO1000 is configured to incubate the samples for 30min at 4ºC before placing the plate on HT2000/HT2100 for 9x wash at 10µL/s using buffer from input port 4. At the end of the wash, AUTO1000 will transfer the plate to be vortexed prior to return to the deck.

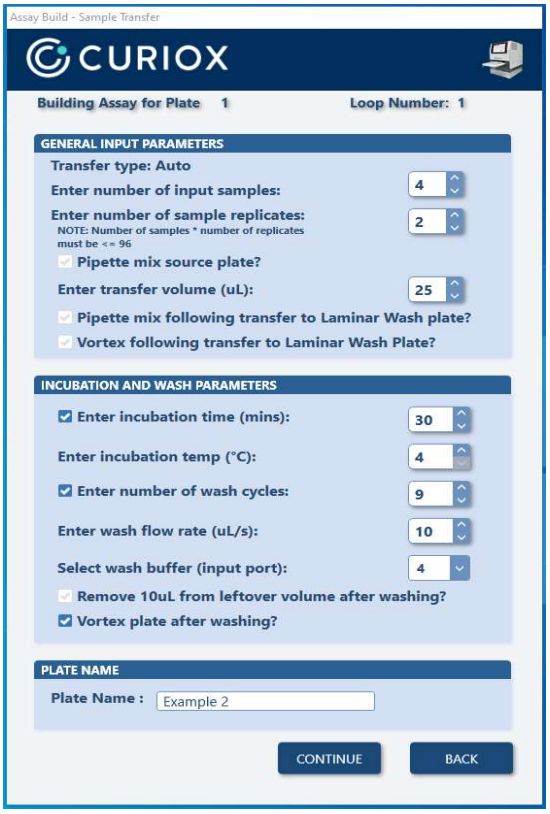


Figure 4-77. Configuring Loop 1 on the AUTO1000 GUI, informing the GUI of sample volue pre-loaded on LW plate.

3. Loop 2:

Following the HT2000/HT2100 wash in Loop 1

- 50µL of Panel 1 (Viability) will be distributed to each of samples 1-4.

The incubation and wash parameters are as indicated in the loop.

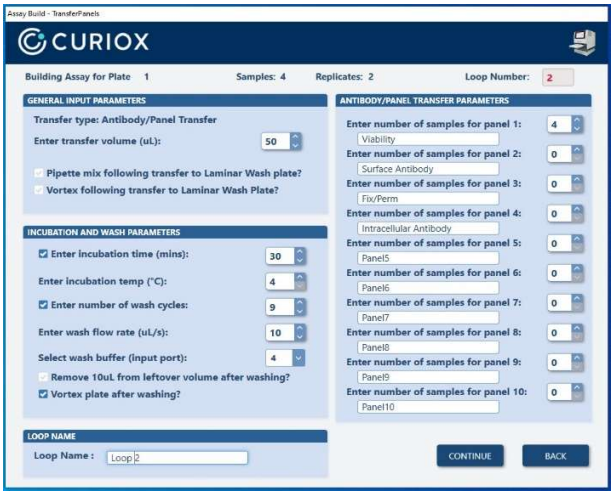


Figure 4-78. Configuring first panel / reagent (Viability dye) addition to samples

4. Loop 3:

Following the HT2000/HT2100 wash in Loop 2

- 50µL of Panel 2 (Surface antibody) will be distributed to each of samples 1-4.

The incubation and wash parameters are as indicated in the loop.

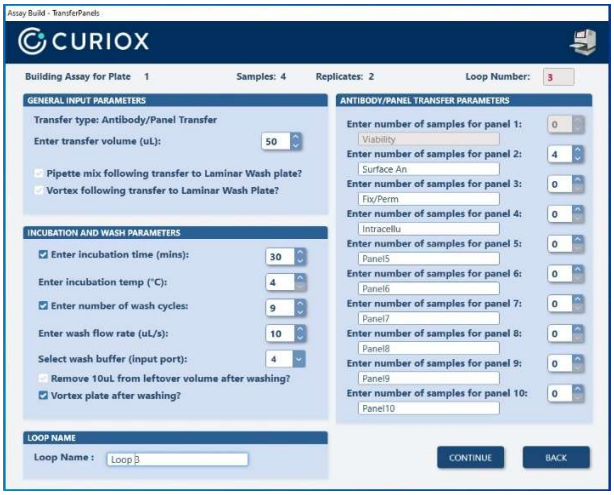


Figure 4-79. Configuring second panel / reagent (Surface Antibody) addition to samples.

5. Loop 4:

Following the HT2000/HT2100 wash in Loop 3, 55µL of Panel 3 (Perm/fix solution) will be distributed to each of Samples 1-4. The incubation and wash parameters are as indicated in the loop.

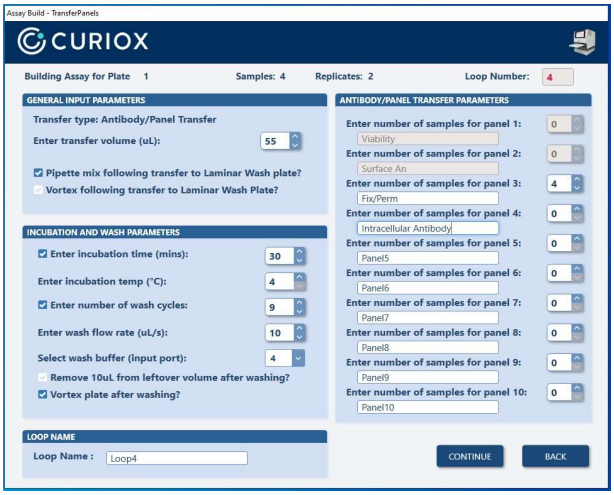


Figure 4-80. Configuring third panel / reagent (Fix/Perm) addition to samples.

6. Loop 5:
Following the HT2000/HT2100 wash in Loop 4, 50µL of Panel 1 (Intracellular antibody) will be distributed to each of Samples 1-4. The incubation and wash parameters are as indicated in the loop.

Figure 4-81. Configuring fourth panel / reagent (Intracellular Antibody) addition to samples.

7. Following confirmation of the parameters in each loop, a confirmation page displays the layout of samples and allocated panel treatment to each sample. *Figure 4-81* shows the confirmation page at the end of Loop 5.

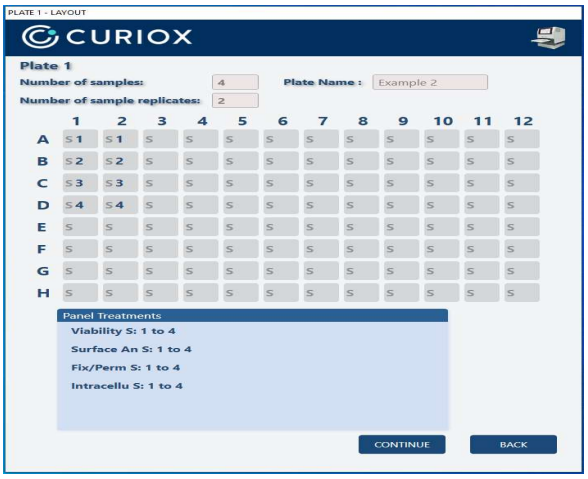


Figure 4-82. Confirmation of Sample Plate layout and panel / reagent distribution for all loops.

8. Here, the user has configured the GUI to transfer the sample from the Laminar Wash plate to the output plate on the deck. The AUTO1000 will add buffer to the output plate following the sample transfer, for final output volume of 200µL.

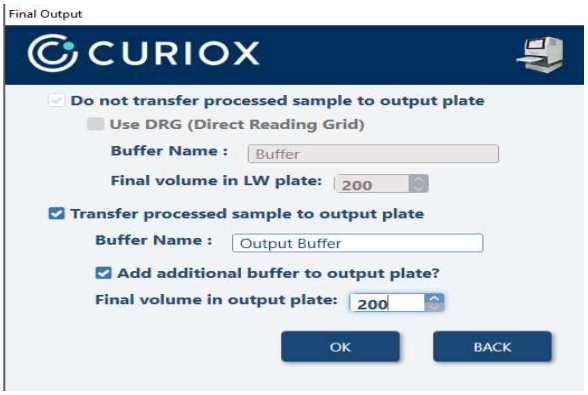


Figure 4-83. Configuring the sample transfer step using an Output Plate with a final output volume of 200µL.

5. Troubleshooting and Maintenance

Chapter Overview

Troubleshooting Checklist

Technical Support

Maintenance Schedule

Control Panel

System Log Files

Decontamination Procedure AUTO1000

Decontamination Procedure HT2000/HT2100

Troubleshooting Checklist

Refer to the following product specific user manual for the troubleshooting checklist.

- Laminar Wash™ HT2000/HT2100 System User Manual
- Hamilton Robotics NIMBUS Operator's Manual

Technical Support

If you require technical support or advice, kindly visit us at www.curiox.com and select the "Need Support?" button in the footer or on the product's page, or email us at support@curiox.com.

To find your product serial number please refer to the installation support form.

Maintenance Schedule

Below is the recommended maintenance schedule to ensure that the AUTO 1000 runs smoothly and efficiently.

Action	Daily	Weekly	Monthly	As
Perform NIMBUS daily maintenance in the calibration software.	✓	✓		
Perform HT2000/HT2100 daily maintenance via the user GUI for start-up and shut-down.	✓	✓		
Wash Filter under running DI water on a weekly basis.		✓		
Bleach run for cleaning of HT internal fluidics*: 1. Run continuous wash with 0.5 % Bleach for strictly 10 minutes. 2. Run continuous wash with 1% Tween 20 in DI water for minimum 20 minutes. **Caution: Bleach is corrosive agent, perpetual wash with 1% Tween 20 in DI water should be run immediately after the 10 minutes bleach run. Failure to do so may cause damage to the machine's component.**				✓
Perform HT2000/HT2100 volume calibration weekly. Refer to the following product specific user manual for the calibration procedure: *- Laminar Wash™ HT2000/HT2100 System User Manual*		✓		

Preventative maintenance of the AUTO1000 every 6 months.*				✓

* Contact Curiox Support for further guidance.

Control Panel

If the method is interrupted, the control panel may be used to release plates from the grippers, park the grippers and gantry, eject tips, and lock or unlock the door as required.

NOTE: using the control panel to move the gantry/channels mid-run aborts the protocol and should only be used when resetting the system. Locking and unlocking the doors allows protocol continuation so long as the gantry has not moved.

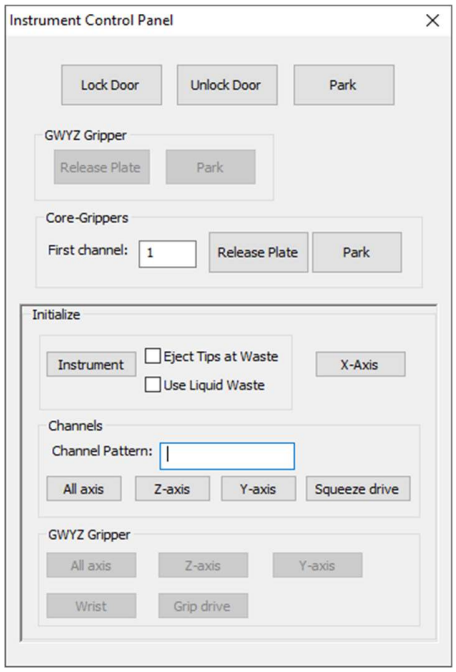


Figure 5-1. Control Panel

*If power goes out, tips or grippers may be removed manually by turning the squeeze drive screw on the channel head. Refer to NIMBUS Operators Manual for further detail.

System Log Files

If support is required, please send the relevant trace file along with your inquiry. Trace files are .trc extension files found in C:/Program Files(x86)/Hamilton/LogFiles

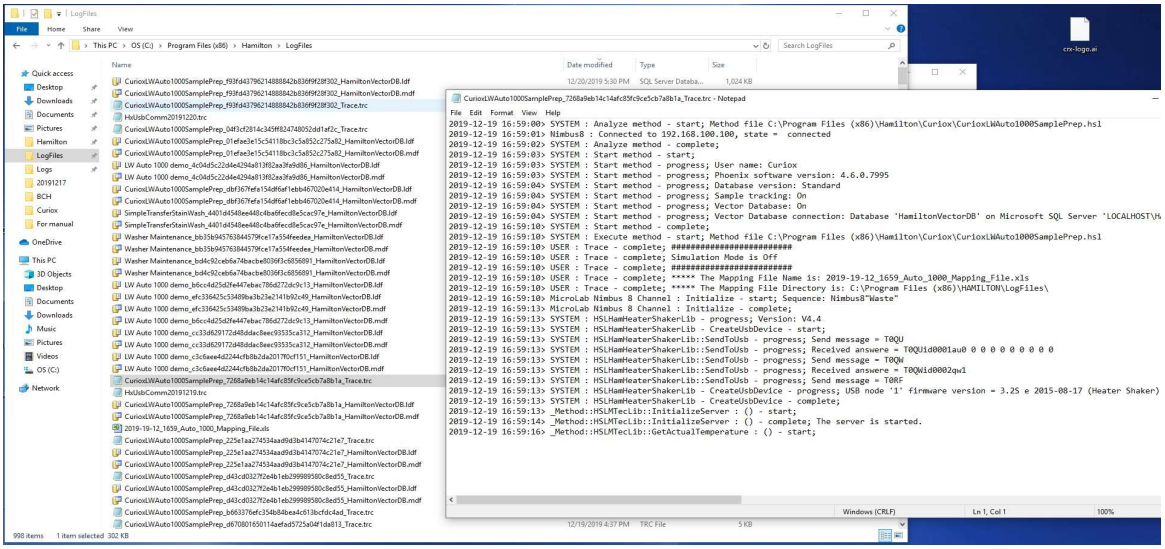


Figure 5-2. Run Log Files

Decontamination Procedure AUTO1000

For the purposes of cleaning, Microcide SQ, Deconex 61 DR, or Deconex SOLARSEPT are the recommended reagents for decontamination of the AUTO1000 enclosure. 70% Isopropyl Alcohol or 70% Ethanol is generally the recommendation for cleaning the deck, waste bar, carriers/pedestals, and channel sleeves if you're not using Deconex or Microcide. Please **do not** use alcohol/ethanol on the o-rings or any gaskets that may be present on any instrument components, as it'll dry and crack the rubber, causing any sealing action to lose performance or fail. Do not use alcohole/ethanol on the plastic enclosure, either, as doing so causes microfractures in the plastic.

Using hypochlorite solutions (bleach) on any of the metal components may cause discoloration or corrosion depending on the specific component. Anything chemical that generates a significant amount of corrosive aerosols is not recommended, as it may potentially damage the internal components (e.g. channel boards).

To decontaminate:

- Spray the front, back and side covers with Microcide SQ.
- Spray the deck with Microcide SQ.
- Remove the tip eject plate of the tip waste station and clean it.
- Spray Microcide SQ directly onto the surface of the tip waste station.
- Remove the frame that holds the plastic bag in place, and discard the plastic bag in the laboratory's contaminated waste. Put the tip eject plate back in place.

- Clean all pedestals with Microcide SQ and leave them to dry.

Decontamination Procedure HT2000/HT2100

For environmental health and safety reasons, it is imperative that operators decontaminate the HT2000/HT2100 prior to transport to a different laboratory, or back to Curiox Biosystems for servicing and maintenance. The step-by-step procedure below is a generic decontamination guide, using chemicals which are compatible with the materials in the HT2000/HT2100.

- Remove all biological contents from the HT2000/HT2100. Turn off and unplug the HT2000/HT2100 from the power supply and disconnect the power jack.
- Wearing gloves, clean all surfaces of the HT2000/HT2100 with warm soap solution. This initial step in decontamination is important as it ensures that any surface microbes will not contaminate and reduce the efficiency of the chemical disinfectant in the next step.
- Moisten (do not soak) a cloth with 70-85% EtOH solution. Wipe all surfaces of the HT2000/HT2100 enclosure. Wait for 20 minutes.
- Moisten a cloth with deionized water and wipe all the surfaces previously cleaned with EtOH. Dry the wet surfaces with a clean cloth. Ensure all used cloths are disposed of in a bio-hazard bag/container.
- If intended for transport: Seal the machine in an airtight bag prior to transport.

Appendix A. 21 CFR Part 11 Setting Guide.

Appendix A. provides explanations regarding the configuration and feature required for using 21 CFR Part 11. Users who want to utilize the 21 CFR Part 11 functionality refers to Appendix A, the 21 CFR Part 11 Setting Guide document.

Purpose

This document provides instructions on how to configure and utilize software for implementing 21 CFR Part 11 regulatory requirements in the auto1000 equipment.
The AUTO1000 Software includes Security Protection and Audit Trail features, which are the primary requirements of 21 CFR Part 11, and this document outlines how to configure and utilize these functions.

Scope

This document serves as a guide for the setup of the AUTO 1000, utilizing 21 CFR Part 11 Feature.

Terms

Configuration

Step-1> Software Installation for 21 CFR Part 11.

To enable the 21 CFR PART 11 functionality in AUTO1000, the following Software must be installed.

- Hamilton.HSLExtensions.Setup_v1.1.0.exe
- HSLMlStarCfgKeys.hs_
- HSLMlStarCfgKeys.STP

1> Install Hamilton. HSLExtensions.Setup_v1.1.0.exe file

- Double click the "***Hamilton.HSLExtensions.Setup_v1.1.0.exe***" file.

Click the "***Next***" or "***Yes***" button until "***Close***" button appears.

Close the Software.

2> Hamilton Configure Key file copy.
After copying the two files below, paste them into the C:\Program Files (x86)\Hamilton\Library folder.