Laminar Wash™ HT2100 Station 96 User Manual

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Laminar Wash HT2100 User Manual

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The information contained in this manual is believed to be accurate, but Curiox Biosystems Pte. Ltd. accepts no liability for any errors it contains and reserves the right to alter specifications without notice.

IMPORTANT NOTICE

Please carefully adhere to all instructions provided in this User Manual, as failure to do so may result in the invalidation of your service contract.

Trademarks

Table of Contents

Chapter 1:

General Information

Chapter Overview

- General Information
- Introduction to the Laminar Wash HT2100
- Chemical Compatibility
- Safety
- CE Mark
- Customer Service and Technical Support
- Instrument Storage and Shipping

General Information

In general, this user manual has been written for the purpose of providing technical, installation, operating and troubleshooting information to the operators of the Laminar Wash™ HT2100 Station. The content of this manual includes:

- How to set up and operate the HT2100
- The principle of operation and modes of function for the HT2100
- Safety features of the HT2100 and precautions to ensure safe operation.
- Troubleshooting procedures and maintenance

Introduction to the Laminar Wash HT2100

The Laminar Wash solution enables sample preparation for multi-color flow cytometry, single-cell sequencing, and other cellular analytical methods. It bypasses the need of centrifugation for washing cells in suspension. The Laminar Wash technology is designed to maintain samples in a 96-droplet based format instead of using a conventional microwell with walls. It is a centrifuge-less processing method using the unique wall-less features of the Laminar Wash technology combined with a fully automated washer. The Laminar Wash HT2100 generates a laminar flow for each drop on the Laminar Wash 96-well Plate via dual-nozzle action, with one nozzle dispensing liquid into the drop and the other nozzle aspirating liquid. The Laminar Wash workflow is superior to the traditional centrifugation method.

Technical Specifications

Description	Specification	
Physical		
Dimensions	310 mm H x 262 mm W x 302 mm D	
Weight	14 kg	
Electrical		
Voltage Requirement	100 - 240 V	
Equipment Ratings	Input Voltage	24.0 V
	Input Current	3.0 A
Environmental		
Indoor or Outdoor Use	Indoor	
Operating Temperature Range	4 - 28 °C	
Operating Humidity	< 80%, non-condensing	
Operation		
Plate Type	Laminar Wash Plate in 96-well format, Single-use.	
Capacity	1 plate per operation	
Performance		
Flow rate at nozzle	5-20µl/s	
Volume capacity	80µl per nozzle	
Wash sequence	96 wells simultaneous washing	
Dilution factor per cycle	Approx. 3.5 times	
Bulk flow rate for priming	Approx. 300ml/min	
Well Residual Volume	Average	26 ± 3µl
	Maximum	< 30µl
	Minimum	> 20µl
User Interface		
Display	7-inch Raspberry Pi Display	
Input control	Touch screen interface	
Number of washes per cycle	1x to 50x selectable	
External Interface		
1x fluid inlet connector (red)	A connector for incoming fresh wash buffer	
1x fluid outlet connector (white)	A connector for outgoing waste wash buffer	
1x RS232	A plug-in for updating operating parameters	

Chemical Compatibility

The components exposed to fluids and reagents are composed of materials which were selected to be resistant against common chemical substances. However, some common disinfectants should not be used when decontaminating the HT2100. Table 1-1 on page 4 lists the material composition of the main components of the HT2100 and shows some reagents which do not pose issues when in contact with these components. However, some reagents are not compatible with the component materials, and prolonged contact should be avoided to prevent corrosion and damage.

Table 1-1: Chemical compatibility between the component materials and accessories in HT2100, including common reagents and disinfectants. (Adapted from: CP LabSafety www.calpaclab.com, USPlastic www.usplastic.com/catalog/files/charts/Tygon%20CC.pdf accessed Jan 2023)

Component	Material	Approved Chemicals	Incompatible Chemicals
Internal base structure	Stainless Steel (304)	Ethanol, Benzene, Chloroform, Acetaldehyde, Propylene Glycol, Isopropanol, Formaldehyde, Phenol, Grease, Potassium Permanganate, Hydrogen Peroxide	Hypochlorite bleach, Sulfuric Acid
Some minor components of main body	Delrin (Polyoxymethylene)	Ethanol, Benzene, Soap Solutions, THF, Formaldehyde, Propylene Glycol, Isopropanol, Potassium Permanganate	Acetic acid, Grease, Ketones, Ozone, Phenol, Ammonia, Hypochlorite bleach, Iodine, Hydrogen Peroxide, Phosphoric Acid, Sodium Hydroxide (>50%)
Bottle cap	Polypropylene	Ethanol, isopropyl alcohol, detergents, ethylene glycol, propylene glycol, formaldehyde (<40%), dimethyl formamide, sodium hypochlorite, hydrogen peroxide, ozone, sulfuric acid (<75%)	Chloroform
Tubing (bottle cap, parts of washer internal structure)	Silicone	Ethanol, isopropyl alcohol, detergents, ethylene glycol, propylene glycol, formaldehyde, formamide, sodium hypochlorite, hydrogen peroxide, sulfuric acid (<3M), ozone	Chloroform

Component	Material	Approved Chemicals	Incompatible Chemicals
Tubing (parts of washer internal structure)	Tygon E-3603	Alcohols[a], detergents, ethylene glycol, propylene glycol, sodium hypochlorite, hydrogen peroxide (≤30%), ozone, sulfuric acid (≤30%)	Ketones, hydrocarbons, formaldehyde (37%), dimethyl formamide, chloroform
Internal and external Luer lock connectors	Nylon[b]	Chloroform, ethanol, detergents, ethylene glycol, propylene glycol, formaldehyde (<40%), dimethyl formamide	Isopropyl alcohol, sodium hypochlorite (<20%), Hydrogen peroxide (>10%), ozone, sulfuric acid (>10%)
Internal structural parts	Aluminum	Ethanol, Benzene, Propylene Glycol, Isopropanol, Formaldehyde, Ozone, Grease, Phenol, Hydrogen Peroxide	Hypochlorite Bleach, Soap Solutions, Sulfuric Acid, Potassium Permanganate, Phosphoric Acid
External structure			

[a] Chemical compatibility of Tygon with alcohols is rated fair.

[b] Nylon is not autoclave-safe. Do not autoclave the assembled bottle caps.

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 the users regarding potentially hazardous situations that could potentially result in 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 Hazards

!WARNING! CHEMICAL HAZARD

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

- Read and understand the Material Safety Data Sheets (MSDSs) 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 MSDS.
- Do not leave chemical containers open.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- 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 Material Safety Data Sheets (MSDS) 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.

Material Safety Data Sheets

Some chemicals used with the HT2100 may be listed as hazardous. Warnings are displayed on the labels of all chemicals when hazards exist.

MSDS provide users with safety information needed to store, handle, transport and dispose of the chemicals safely. Curiox recommends updating laboratory MSDS records periodically.

Material Safety Data Sheets for Curiox reagents are available upon request by calling 650 226-8420 in the US or +65 6507 0361 outside the US. Otherwise call the chemical manufacturer directly or visit their web site.

Instrument Safety Labels

The following safety label is located on the HT2100. The label displays a safety alert symbol indicating a potential safety hazard.

Symbol	Description
	Pinch point hazard. Keep hands clear.
	Separate collection for electrical and electronic equipment.
	Potential Biohazards. Some assays or specimens may pose a biohazard. Adequate safety precautions should be taken as outlined in the specimen's package insert.

Instrument Safety Features

Plate Detection

The HT2100 detects the presence of a Laminar Wash 96-well Plate. The machine will not begin operation if a plate is not detected on the feeder platform.

Disconnecting Device

When operating the machine, always position the power adapter at a location that is easily accessible to the user. Disconnect machine by detaching the power adapter plug.

Power Supply

The universal power adapter accepts electrical power in the voltage range of 100 - 240 V. The resultant output on the HT2100 power jack is 24 V DC.

Emergency Stop

Activating the emergency stop button in the middle of a washing cycle will cause the machine to immediately halt operations. The machine goes to home position when the emergency stop button is subsequently deactivated.

Machine Homing

In the event power to the machine is cut off in the middle of a washing cycle and subsequently switched on again, the machine will go to home position.

Spill Tray

The spill tray sensor detects excess liquid in the tray to prevent it from spilling onto the machine's interior. When this happens, the error code "E31" is prompted on the machine display panel. User may only access the draining button during this time.

Safety Precautions

- At the end of a wash process, remove the plate from the feeder platform immediately to prevent the wells from drying, stressing cells and/or deteriorating biological integrity.
- Keep the area around the power supply free from liquid.
- During active operation, keep your hands off the instrument other than using the display panel.
- If there is an unexpected error, reset the instrument by turning the power switch off and back on.

General Precautions

- Do not use any other plate except the specified Laminar Wash 96-well Plate with the HT2100.
- Do not load more than one Laminar Wash 96-well Plate into the HT2100 at a time.
- Use only the supplied power adapter cord for electrical supply to the unit.
- Do not allow particles larger than 70 µm to enter any of the liquid tubes. Ensure that the inlet filter is assembled at the inlet port.
- Be careful not to spill liquid onto the interior of the HT2100.
- Always perform a cleaning cycle with an appropriate cleaning solution at the end of an experiment.
- Keep the original packaging material in case the unit should ever need to be shipped.
- 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.
- Contact only qualified Curiox personnel for servicing of the HT2100.

Prior to System Operation

Ensure that all users of the HT2100 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 understood all related MSDS.

CAUTION

Avoid using the HT2100 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

Based on the testing described below and information contained herein, this instrument bears the CE mark.

Directive 2014/30/EU Electromagnetic Compatibility

This device has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1 for Emissions and Immunity.

Verification of compliance was conducted to the limits and methods of the following:

1. EN 61326-1: 2013 Emissions
    a. Harmonics Current Emission (Class A)
    b. Voltage Fluctuation/ Flicker
    c. Conducted Emission (Group 1 Class B)
    d. Radiated Emission (Electric Field) (Group 1 Class B)

2. EN 61326-1: 2013 Immunity
    a. Electrostatic Discharge Immunity
    b. RF Radiated Immunity
    c. Electrical Fast Transient/ Burst Immunity
    d. Voltage Surge Immunity
    e. Conducted Disturbance Immunity
    f. Voltage Dips & Interruptions Immunity

Directive 2014/35/EU Low –Voltage Device

This device has been verified and found to meet the requirements of Directive 2014/35/EU “electrical electronic equipment designed for use within certain voltage limits”.

Directive 2011/65/EU Restriction On the use of Hazardous Substances (ROHS 2)

This device has been verified and found to meet the requirements of Directive 2011/65/EU “restriction on the use of certain hazardous substances in electrical and electronic equipment”.

Directive 2012/19/EU Waste Electrical and Electronic Equipment (WEEE)

Dispose of the device according to Directive 2012/19/EU, on “waste electrical and electronic equipment (WEEE)” or local ordinances.

Customer Service and Technical Support

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Instrument Storage and Shipping

1. Ensure that all internal and external surfaces of the HT2100 washer station and BEX are dry following the decontamination procedure above. See Chapter 4, Section *Long Term Dry Storage Procedure* for decontamination procedure prior to extended storage of the instrument.
2. Stow the instruments in a regulated storage environment: Temperature: 4-28ºC, Humidity <80% RH, non-condensing.
3. If it is intended to ship the instruments, seal the machine in an air-tight bag, pack it in its original packaging prior to transportation.

Chapter 2:

Functional Description

Chapter Overview

- Introduction
- Functional Description

Introduction

This chapter gives a detailed overview of the parts of the HT2100 and the phases which outline a typical washing process.

Functional Description

The simple digital interface located on the top front panel of the HT2100 allows operators to adjust the number of desired wash cycles and the initial sample volume which will be shown numerically on the display. The main components of the HT2100 are shown in Figure 2-1, Figure 2-2 and Figure 2-3.

Figure 2-1: *Front view of the Laminar Wash HT2100*

Figure 2-2: *Lateral (right side) view of the Laminar Wash HT2100*

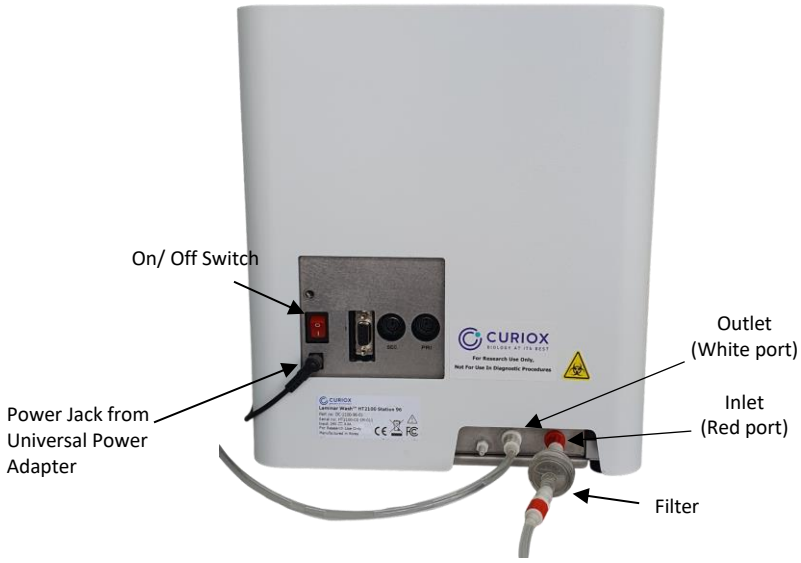
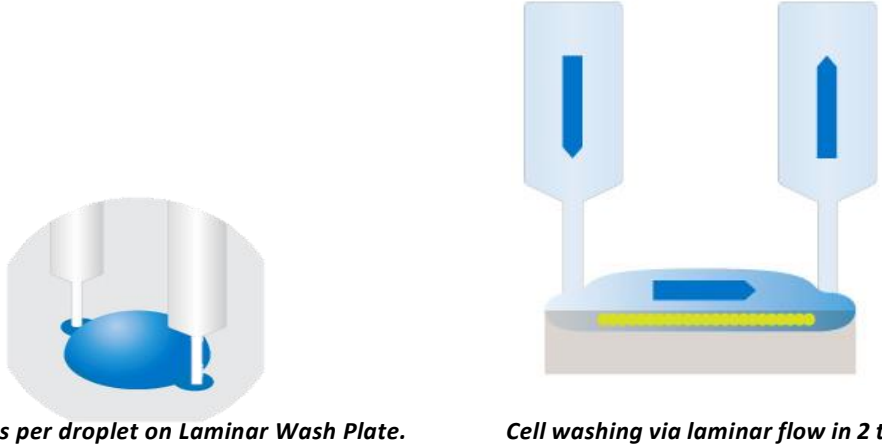


Figure 2-3: *Back view of the Laminar Wash HT2100 with main components labelled.*

Wash Cycle Process

The plate wash cycle process is as follows; a schematic representation of the process is shown in Figure 2-4.

1. Feeder platform detects for presence of plate and retracts into the machine.
2. Fluidics head lowers down into plate.
3. Based on indicated initial volume, dispensing head tops up or removes each well content until 80 ± 2µl (e.g., if initial volume is 50µl, dispensing head adds 30µl to the droplet).
4. Aspirating head aspirates full volume based on internal firmware. It is intended that contact between aspirating nozzles and well droplet breaks before aspiration is completed. Once the aspiration nozzle completes the aspiration of existing drops at a given height of a nozzle, it continues to aspirate air. Resultant average residual volume should be approximately 26 ± 3µl based on the height of an aspiration nozzle.
5. Dispensing head tops up well contents based on internal firmware.
6. Steps 4 and 5 are repeated until stipulated number of washes is reached. Note: if only 1 wash is specified, the machine will only run until step 4.
7. Feeder platform extends out of the machine, returning plates with approximately 26 ± 3µl average residual volume in each well.



2 nozzles per droplet on Laminar Wash Plate. ***Cell washing via laminar flow in 2 to 4 mins.***

Figure 2-4: *Laminar Wash 96-well Plate washing process on the HT2100.*

Chapter 3:

Setup

Chapter Overview

- Installation
- Interconnection to Accessories
- Preparation for Operation

Installation

Upon receiving the HT2100 package, check that all the accessories listed below are present. Some of the components of the HT2100 have been secured with brackets to protect against damage during transportation. Remove all brackets shown in the steps below before installing and powering up the HT2100.

List of items:

- Laminar Wash HT2100 Station
- Fluidic Head
- Parts pack 1
  - Fluidic Head Spacer Jig.
  - Power Adapter (Output 24V, 3A).
  - Electrical Power Cord.
  - RS232 to USB Cable.
  - RS232 Extension Cable.
  - Allen Key Set.
  - Post card with QR Code for User Manual.
  - Declaration of Conformity (for CE compliance).
- Parts pack 2
  - Spare 3.5 ID O-rings (10x Red, 3x Black).
  - 1m Tubing, Red Coded Ring on Connector, connected to Filter.
  - 1m Tubing, White Coded Ring on Connector.
  - LW96 plate labeled 'Priming Plate'.
  - LW96 plate labeled 'Calibration Plate'.
  - 4 units Buffer Inlet Bottle Cap.
  - 10ml Manual Priming Syringe, assembled to 0.2m tubing.
  - Plunger and Nozzle tool.
  - Spare Nozzles & Plungers Set (Aspirating & Dispensing).

Additional materials required for installation:

- 70% ethanol containing 1% Tween-20 (polysorbate-20) ('Ethanol/Tween') NOTE: 300mL required f*or standard Start Up and Shutdown procedure.*

  CAUTION *Do not use isopropyl alcohol (IPA) in place of ethyl alcohol, as IPA has severe chemical incompatibility with the tubing connectors in the washers.*

- De-ionized (DI) water containing 1% Tween-20 (polysorbate-20), 0.01% sodium azide (as preservative) ('DI/Tween')

  NOTE: 300mL is required for standard Start Up and Shutdown procedure.

- Empty bottle (recommended ≥ 1L capacity) for liquid waste.

NOTE: Subject to local legislation and assay needs, it is recommended to add sodium azide (0.01-0.05 w/v%) in the wash buffer to prevent biofouling of the buffers and areas of the washer in prolonged contact with buffers.

Installation

1. Place the HT2100 on a flat surface and in an environment with temperature and humidity conditions as stipulated in “Technical Specifications” on page 3.
2. Remove the screws on the sides of the top cover .
3. Lift machine top cover and push hinge to lock the cover in the open position.

Figure 3-1: *Lift the machine top cover (left) and lock in the open position (right).*

4. Hold and pull down the two ends of the supporting bracket to remove. Please note that some force may be required for this step. The supporting bracket must be kept in a secured location for any transportation which may arise in the future. For convenience, we would suggest taping it to the back of the machine.

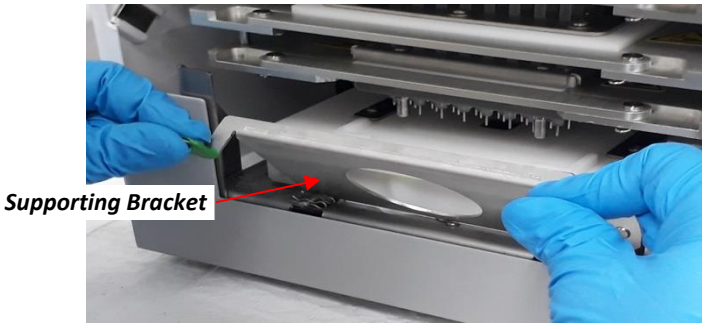


Figure 3-2: *Remove the machine supporting bracket.*

5. Using a size 3 Allen key, loosen bolts (x3) by turning to the anti-clockwise direction. Do not over loosen bolts. Move the locking bracket to the right.

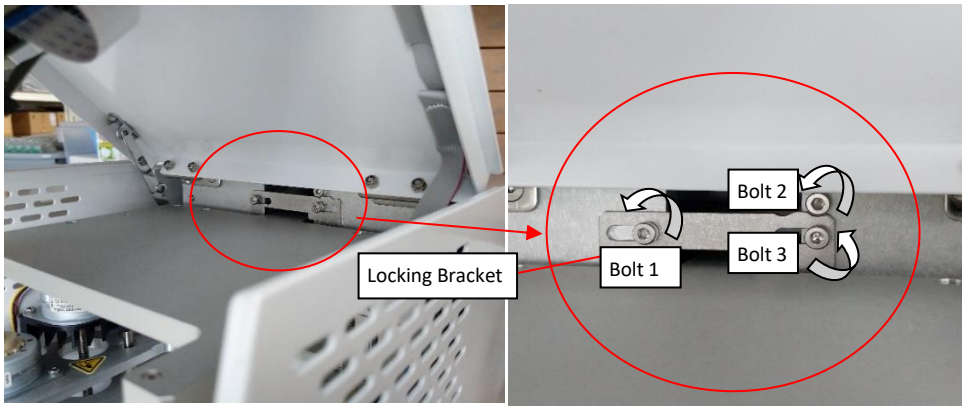


Figure 3-3: *Loosen bolts to move locking bracket to the right.*

6. Once the locking bracket is on the right, use the same Allen key to tighten bolts (x3) and lock the locking bracket into position by turning to the clockwise direction. Do not over tighten bolts.

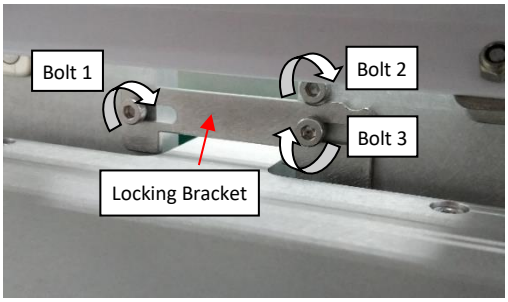


Figure 3-4: *Lock locking bracket into position by tightening bolts.*

7. Detach the cotter pins and lock pins, and then swing open the metal bars.

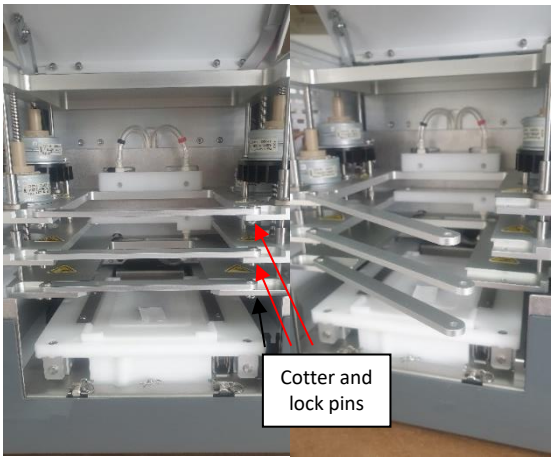


Figure 3-5: *Opening metal bars*

8. Remove the fluidic head from its packaging, and position and insert the Fluidic Head Spacer (available in the accessories pack) according to Figure 3-6a.

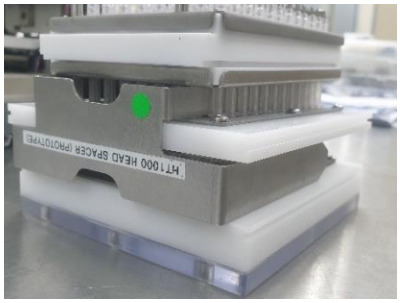


Figure 3-6a: *Fluidic Head*

Figure 3-6b is how it looks like underneath the nozzle block. Notice the fork on the right-side cuts in between the plungers.

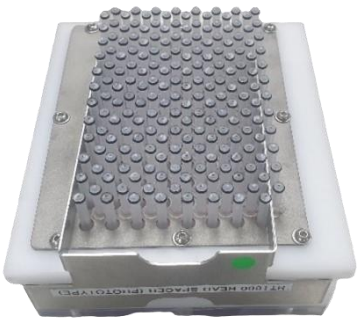


Figure 3-6b: *Fluidic Head*

9. Turn the fluidic head reservoir-side up and align the center block of the fluidic head to the machine (Figure 3-7 red arrow).

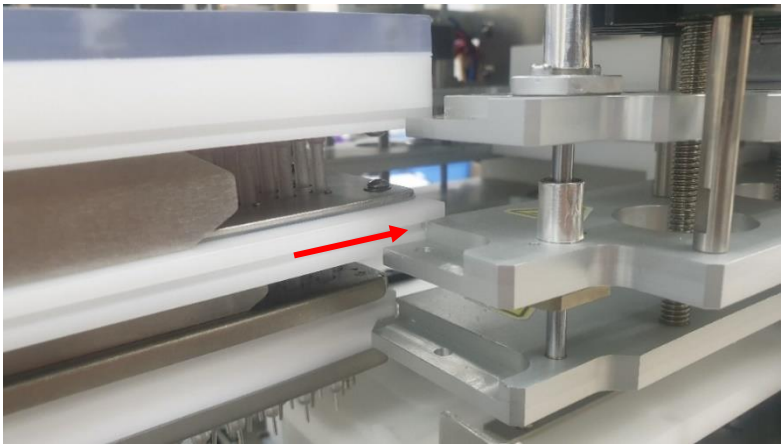


Figure 3-7: *Align Fluidic Head to Machine (step 1)*

10. Followed by the bottom block (Figure 3-8 red arrow).

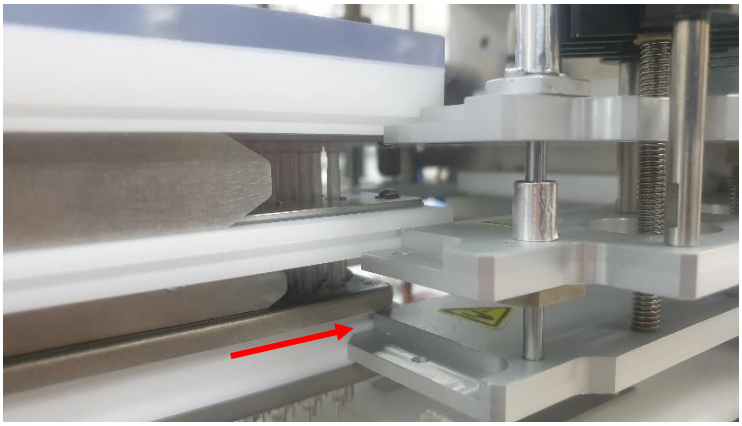


Figure 3-8: *Align Fluidic Head to Machine (step 2)*

11. And finally, the top block (Figure 3-9 red arrow).

Figure 3-9: *Align Fluidic Head to Machine (step 3)*

12. Push the fluidic head all the way in before sliding the metal spacer out.

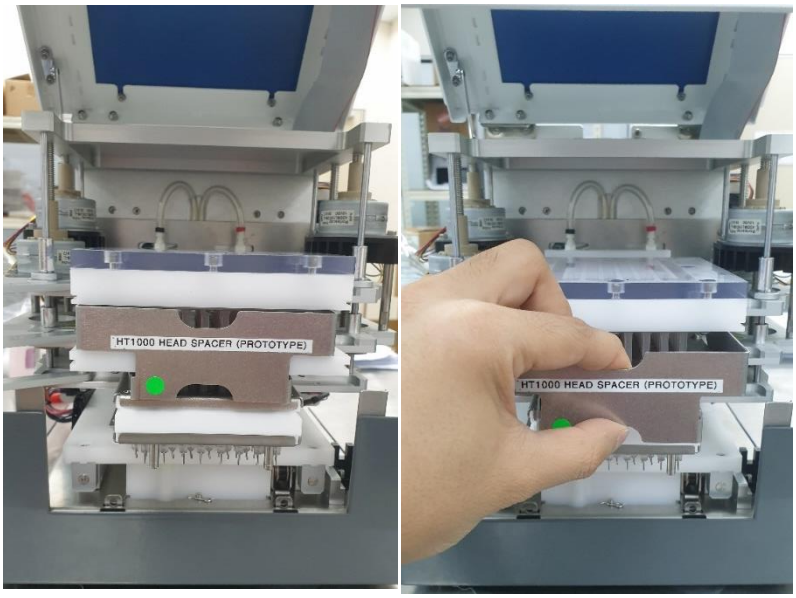


Figure 3.10: *Align Fluidic Head to Machine (step 4)*

13. Ensure the fluidic head is secured into position before closing the brackets.

Figure 3.11: *Align Fluidic Head to Machine (step 5)*

14. Close the machine cover by returning the hinge back to its original position.
15. Connect the power cable to the universal power adaptor. Insert the power jack at the back of the HT2100 as shown in Figure 2-3.
16. Insert the two delivery tubes into the respective ports on the HT2100 in the manner specified below (Figure 3-12):

i. Install a clean filter into one end of the inlet tubing and insert into the red port on the HT2100. Insert the other end into wash buffer bottle via the buffer inlet bottle cap. Refer to the next section *Buffer Inlet Bottle Cap Installation* for installation instructions.

ii. Insert one end of the outlet tubing into the white port on the HT2100 and the other end into the waste bottle.
NOTE: The buffer inlet bottle cap must not be used on the waste bottle.

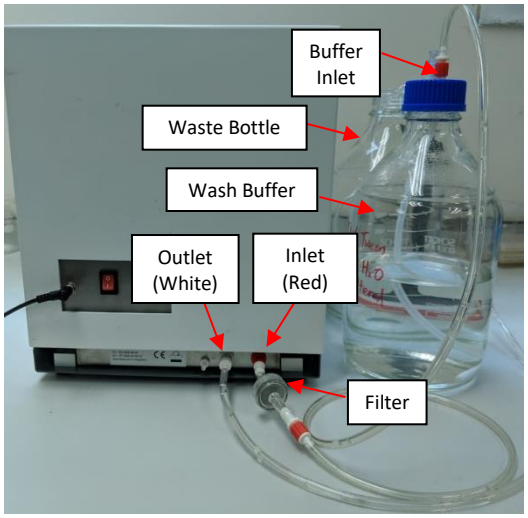


Figure 3.12: *Assembly of tubing from bottle caps to HT2100*

Buffer Inlet Bottle Cap Installation

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

1. 4 sets of buffer inlet bottle caps are packed with the machine. Each bottle cap comes with a pre-installed tubing, as shown below.

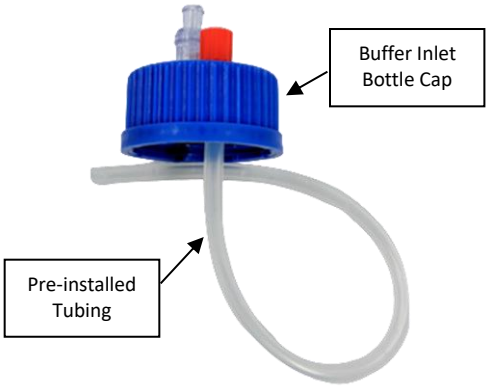


Figure 3.13: *Four sets of buffer inlet bottle caps with pre-installed tubing*

2. Connect a buffer inlet bottle cap onto a 2L glass bottle containing the wash buffer of choice, as shown in Figure 3-14.

Figure 3-14: *Buffer inlet bottle cap connected to 2L bottle containing wash buffer of choice.*

3. Install a clean filter onto one end of the inlet tubing and insert into the red port on the HT2100. Install the other end (white connector) into wash buffer bottle via the red connector on the buffer inlet bottle cap. Refer to Figure 3-12.

Interconnection to Accessories

PRI & SEC Port Connection

1. For connection with a Buffer Exchanger 5 (BX5), connect the ‘PRI’ ports on the BX 5 and HT2100, using a mini-DIN Connector.
2. For connection with a Buffer Exchanger 10, connect both the ‘PRI’ & ‘SEC’ ports on the BX 10 to their corresponding ports on the HT2100, using two separate mini-DIN Connectors.

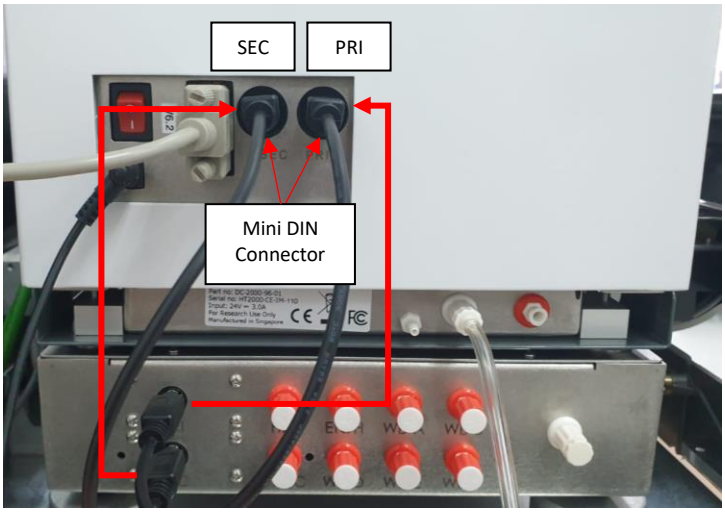


Figure 3-15: *Connection with a Buffer Exchanger using Mini DIN Connectors.*

RS232 Serial Port Connection

A computer/laptop can be connected to the HT2100 via the RS232 Serial Port as follows:

1. Connect RS232 Extension Cable to the Serial Port.
2. Connect other end of Extension Cable to the RS232 to USB Cable. USB can then be plugged in to a computer/laptop.

Figure 3-16: *RS232 Serial Port Connection to HT2100 (left) and Computer/Laptop 7 (right).*

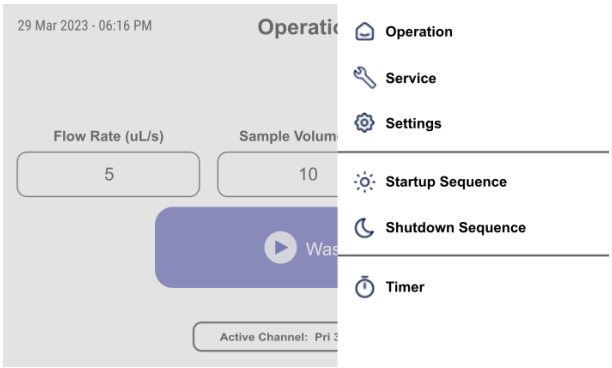
Preparation for Operation

It is mandatory to perform priming of the machine with 1% Tween 20 in 70% Ethanol, followed by 1% Tween 20 in DI water and finally with Assay Wash Buffer at the start of the day's operation. A Fluidic Verification is performed after priming for added assurance that the machine is performing to specifications prior to an actual wash. Follow the steps below to prepare the machine for operation.

If the machine installation will be carried out in a cold room, or moved into a cold room, acclimatize the machine in the cold room for at least 3 hours. Proceed to the following Fluidic Verification steps afterward. It is recommended to do a weekly Fluidic Verification to ensure optimal performance and reverification will be required after moving the machine out from the cold room.

Startup Sequence

1. Ensure the power jack is connected. Power up the HT2100 and wait for the homing sequence to complete.
2. Click on the Menu icon on the top right corner to reveal the popout side bar. Select [Startup Sequence].

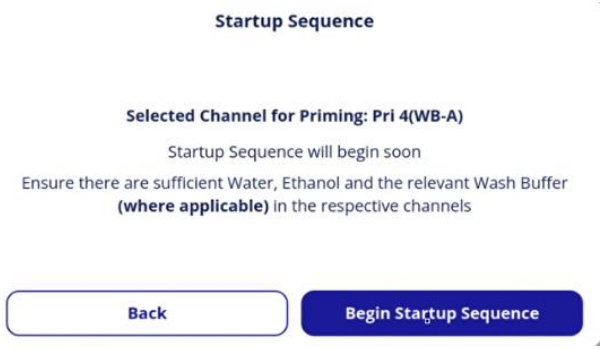


3. If Buffer Exchanger is connected to HT2100, a menu will appear to select the desired wash buffer to complete Startup Prime with.

Note: If no Buffer Exchanger is connected, select [None].

IMPORTANT: If HT2100 is connected to a Buffer Exchanger, do not select [None]. Doing so will cut off fluidic path to the fluidic head when the washer is in operation, resulting in irreversible damage.

4. A confirmation screen will appear to remind Users to ensure there are sufficient 'Water' (DI Water + 1% Tween-20), 'Ethanol' (70% ethanol + 1% Tween-20) and the relevant Wash Buffer(s) connected to the respective channels.



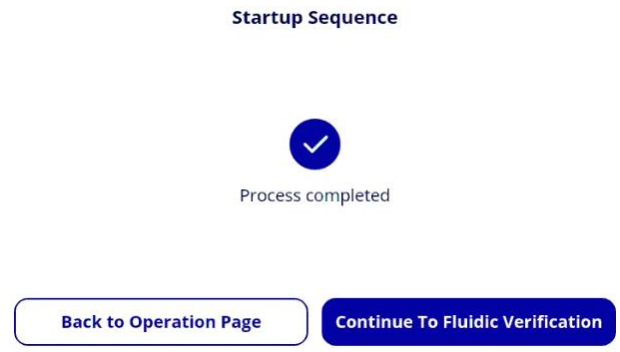
Ensure the two delivery tubes are properly connected into the respective ports (red and white) on the HT2100. A clean filter is installed into one end of the inlet tubing and inserted into the red port on the machine. The other ends of the tubes are connected to either the wash buffer bottle via the buffer inlet bottle cap (from red port) or the waste container (from white port). Refer to Figure 3-12.

5. Ensure that each bottle is filled with the appropriate fluid, connected to the buffer inlet bottle cap and properly installed into the machine. Refer to "Buffer Inlet Bottle Cap Installation" on page 24.

6. Select [Begin Startup Sequence] to initiate washer priming. The washer automatically primes the fluidic head with 'Ethanol', followed by 'Water', and finally the selected wash buffer.

   The feeder tray is extended during priming as the fluidic head primes into the priming tray below the feeder tray. The splash screen indicates the progress of fluidic head priming.

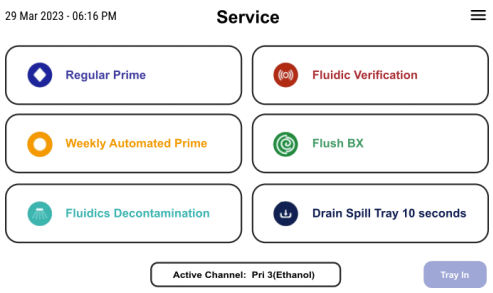
7. Upon completion of Startup Sequence, Users are prompted to perform Fluidic Verification in a notification screen. Select [Continue To Fluidic Verification].



Fluidics Verification

Users are recommended to perform visual inspection of nozzle function using [Fluidic Verification] daily prior to use.

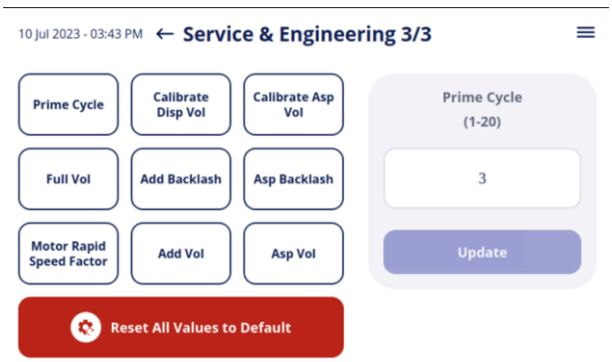
1. Select [Continue To Fluidic Verification] on the notification screen after the completion of Startup Sequence. Users may also access this function via the [Service] menu in the popup side bar.



2. Weigh the mass of the LW96 plate labeled ‘Calibration Plate’ on the mass balance. Record the initial mass.

3. Place the 'Calibration Plate' on the feeder platform and select [Dispensing Verification (Dispense 80µL)] to dispense 80µL of wash buffer into all wells.

4. Carefully remove the 'Calibration Plate on the feeder platform. Visually observe that fluid was dispensed into all wells. Obtain the mass of the filled plate on a mass balance.
5. To estimate the volume of fluid dispensed in each well, subtract the mass obtained in step 4 by the initial mass obtained in step 2. Divide the resultant value by 96. Assuming the mass density of the wash buffer is 1µg/µL, the final figure represents the average volume in each well.
6. Place the calibration plate on the feeder platform. Select [Continue to Aspirating Verification] to aspirate 50µL from all wells.
7. Carefully remove the 'Calibration Plate on the feeder platform. Visually observe that fluid was aspirated from all wells. Obtain the mass of the filled plate on a mass balance.
8. To estimate the volume of fluid remaining in each well, subtract the mass obtained in step 7 by the initial mass obtained in step 2. Divide the resultant value by 96. Assuming the mass density of the wash buffer is 1µg/µL, the final figure represents the average volume in each well.
9. Select the Menu icon on the top right corner to access [Service & Engineering] on the bottom of the popup side bar. Scroll to page 3/3 using the [→] button to adjust dispensing and aspirating volume.



10. Tap on [Calibrate Disp Vol] to input the calculated dispensed volume in step 5. Click [Update] to confirm.
11. Tap on [Calibrate Asp Vol] to input the calculated remaining volume after aspiration in step 8. Click [Update] to confirm.
12. Return to [Operation] menu using the popup side bar Menu icon on the top right corner.
13. Input the following wash parameters by tapping the relevant input bar and inputting the parameters using the keypad:

Flow rate (µL/s)	5
Sample volume (µL)	80
Number of Washes	5

14. Tap [Wash] to begin the wash cycle on the Calibration Plate.
15. Remove the Calibration Plate when the wash is completed. Visually inspect the wells to ensure all wells are aspirated uniformly.
16. Obtain the average remaining volume in each well by repeating steps 7 & 8. Ensure that the average volume is 26 ± 3 µL.
17. Refer to the section on 'Maintenance and Troubleshooting' if there are dispensing or aspirating irregularities.
18. The washer is now ready for Operation.

NOTE:

1. ***If the subsequent washing requires sterile environment, the priming plate should be sterilized by rinsing with 70% EtOH and drying under UV before use.***
2. ***If the HT has been under prolonged storage, refer to Appendix C for instructions on how to re-qualify the machine.***

Chapter 4:

Operation

Chapter Overview

- Before Operating the HT2100
- Operational Safety
- Operation Mode
- Service Mode
- Settings Mode
- Startup Procedure
- Shutdown Procedure
- Timer
- Service & Engineering
- Washer Operations
- Washer Maintenance
- Error Codes

Before Operating the HT2100

The HT2100 should be placed on a flat surface and in an environment with temperature and humidity as stipulated in “Technical Specifications” on page 3.

Operational Safety

The HT2100 possesses the following built-in safety features which prevent the machine from operating under unsafe conditions.

Plate Detection

The HT2100 detects the presence of a Laminar Wash 96-well Plate. The machine will not begin wash operation if a plate is not detected on the feeder platform.

Power Supply

The universal power adapter is able to accommodate electrical power in the range of 100 - 240 V. The resultant output to the HT2100 power jack is 24 V DC.

Emergency Stop

Activating the emergency stop button in the middle of a washing cycle will cause the machine to immediately halt its operation. The machine goes to home position when the emergency stop button is subsequently deactivated.

Machine Homing

In the event power to the machine is cut off in the middle of a washing cycle and subsequently the machine is switched on again, the machine will go into home position.

Washer Locking

Locking screws were added to both sides of the front panel. Removal of locking screws can only be done by qualified Curiox personnel for servicing of the HT2100 purposes.

Front Protective Panel

Front protective panel grill was added to prevent user to access the internal compartment of the washer.

Spill Tray

The spill tray sensor detects excess liquid in the tray to prevent it from spilling onto the machine's interior. When this happens, error code "E31" is prompted on the machine display panel. User may only access the draining button during this time.

Safety Precautions

In addition to the built-in safety features of the HT2100, below are some precautions operators are advised to take while using the HT2100 to ensure their safety and to preserve the accuracy of the experiments.

- At the end of a wash process, remove the plate from the Feeder Tray immediately to prevent the wells from drying, stressing cells and/or deteriorating biological integrity.
- Keep the area around the power supply free from liquid.
- During active operation, keep your hands off the instrument other than using the display panel.
- If there is an unexpected error, reset the instrument by turning the power switch off and back on.

Operation Mode

The Operation Mode allows an operator to set up wash parameters and initiate washes.

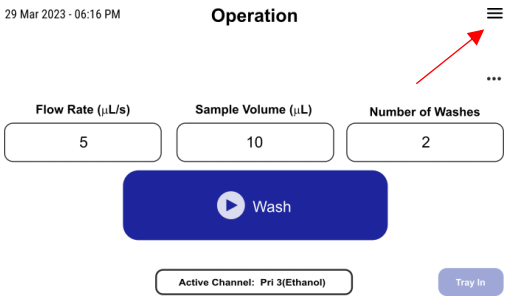
User Interface

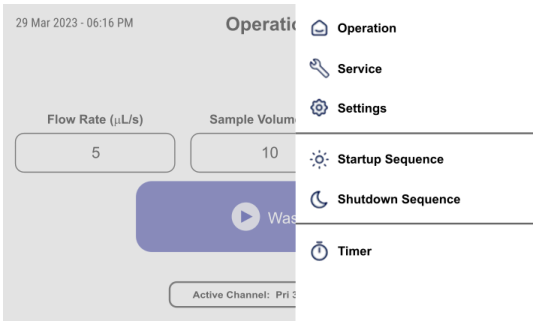
- ***"Menu" icon***

  To access other modes on the washer:
  - Operation (described in this section)
  - Service
  - Settings
  - Startup Sequence
  - Shutdown Sequence
  - Timer

- ***"Operation" menu***

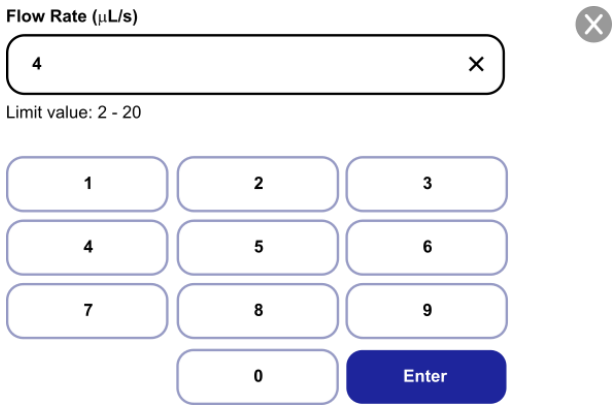
  The "Operation" menu shows 3 parameters for User Input: "Flow Rate (µL/s)", "Sample Volume (µL)", and "Number of Washes".





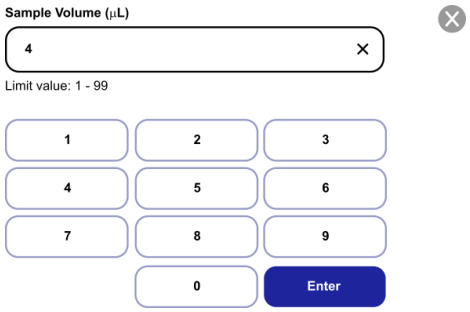
- ***"Flow Rate (µL/s)"***

  Flow rate of dispensing and aspirating fluid in µl/s. The value can be input by tapping the number to pull out a keypad. Tap Enter to confirm. The lower and upper limit is 2 and 20 respectively.



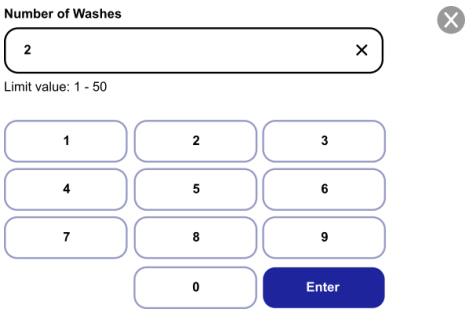
- ***"Sample Volume (µL)"***

  Initial volume of droplet on each well in the plate in µL. Tap the field to pull up a keypad for direct value input. The lower and upper limits are 1 and 99 respectively.



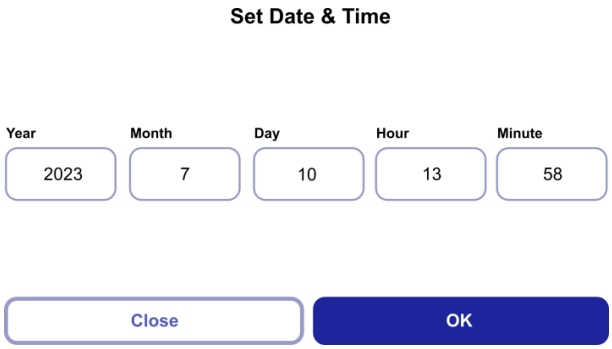
- ***"Number of Washes"***

  Number of dispense and aspiration cycles. Tap the field to pull up a keypad for direct value input. The lower and upper limits are 1 and 50 respectively.



- ***"Date and time"***

  This indicates the current date and time. This may be indicated in [Settings] menu in the popup side bar.

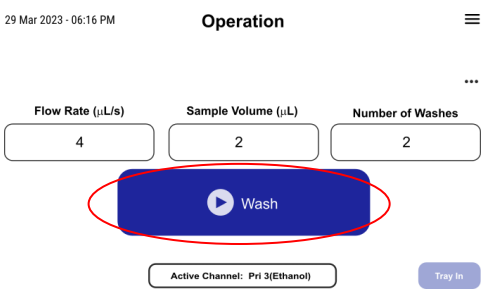


- ***"Active Channel"***
  This indicates the channel on the buffer exchanger that is currently activated, if connected to a buffer exchanger.

Function Buttons

- ***[Wash] button***

  Press the [Wash] button to initiate full wash cycle. During operation, the progress screen indicates the process of the wash sequence, with the option to halt using [Stop Operation] button. When the wash is completed, a count-up timer begins to indicate time elapsed after wash. When pressed mid-operation, it will stop the wash cycle immediately and extend the feeder platform, returning the plate.



- ***[Stop Operation] button***

  Function is available only during operation of the washer. When selected, the washer halts the wash cycle immediately. User should select [Recover] to extend the feeder tray to recover the plate.

- ***[Tray In] or [Tray Out] button***

  To retract or extend feeder tray.
  NOTE: Feeder tray cannot be retracted if a plate is detected on it.

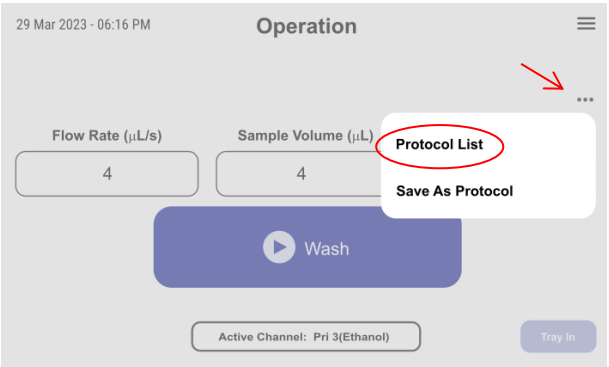
Plate not detected.

Plate detected.

- ***"Protocol List" icon***

  Opens options to retrieve and save protocols (parameters of Flow Rate, Sample Volume, Number of Washes) that will automatically appear on the "Operation" page when selected.

  - [Protocols List] button – opens list of past saved protocols for selection or editing.
  - [Save As Protocol] button – saves current parameters as new protocol.



Wash Cycle

1. Feeder platform detects for presence of plate and retracts into the machine.

2. Fluidics head lowers down into plate.

3. Based on indicated initial volume, dispensing head tops up well contents until 80µL (e.g., if initial volume is 50µL, dispensing head adds 30µl to the droplet).

4. Aspirating head aspirates full volume based on internal firmware. It is intended that contact between aspirating nozzles and well droplet breaks before aspiration is completed. Once the aspiration nozzle completes the aspiration of existing drops at a given height of a nozzle, it continues to aspirate air. Resultant average residual volume should be approximately 26µL based on the height of an aspiration nozzle.

5. Dispensing head tops up well contents based on internal firmware.

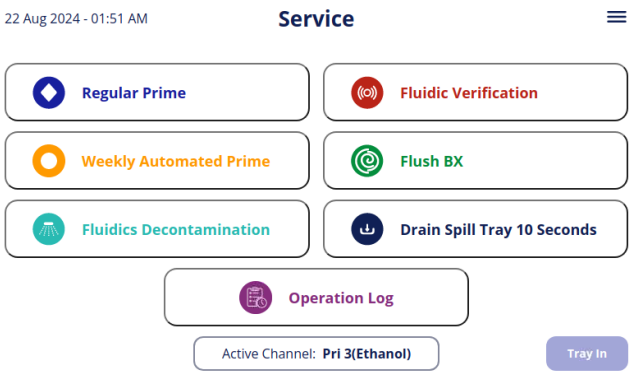
6. Steps 4 and 5 are repeated until stipulated number of washes is reached.
   NOTE: if only 1 wash is specified, the machine will only run until step 4.

7. Feeder platform extends out of the machine, returning plates with approximately 26 µL average residual volume in each well.

8. The wash cycle of 1x dispensing and aspiration consumes ~6 mL wash solution. For example, 5x wash cycle consumes ~30mL wash solution.

Service Mode

The Service Mode allows an operator to carry out priming, cleaning, draining, and changing channels operations.



User Interface

- ***"Date and time"***

  This indicates the current date and time. This may be indicated in [Settings] menu in the pop up side bar.

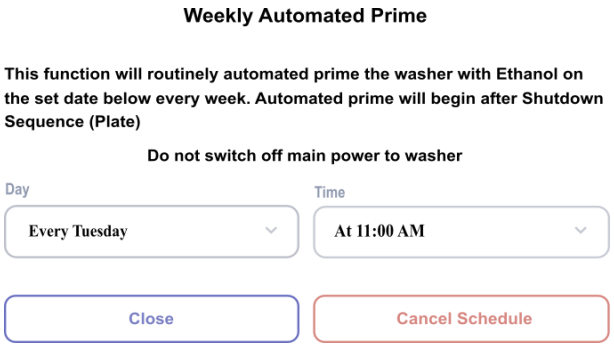
Function buttons

- ***[Regular Prime]***

  Exchanges buffer in the fluidic head with fresh inlet buffer, using the built-in priming tray under the feeder tray. The buffer will be drawn from the active channel, if BX is connected. During prime, the feeder tray will be extended. The prime ensures greater than 100x dilution of the prior fluid in the fluidic nozzles.

- ***[Weekly Automated Prime]***

  Scheduled weekly maintenance of 'Ethanol' (70% ethanol + 1% Tween-20) fluidic head prime, if the washer is idle in standby mode. User may indicate the day and time of the week to perform the automated prime, using the built-in priming tray under the feeder tray.



- ***[Fluidics Decontamination]***

  Guided program to perform decontamination of fluidic head with a Cleaning Solution such as 1% bleach. Users follow guided prompts to connect the appropriate Cleaning Solution and priming buffers either by selecting the appropriate channel on BX, or connecting directly on the washer inlet. Users may select to perform Ethanol Shutdown, subsequently setting the washer on standby mode for Weekly Automated Prime (if scheduled).

  The program performs the following packaged functions:

  - Regular prime fluidic head with DI water + 1% Tween-20
  - Regular prime fluidic head with Cleaning Solution
  - 10 minutes of Continuous Wash with Cleaning Solution
  - Regular prime with DI water + 1% Tween-20
  - 20 minutes of Continuous Wash with DI water + 1% Tween-20
  - Regular prime and shutdown with 70% ethanol + 1% Tween-20 (if selected)

  See Section *Fluidics Decontamination* on page 53 for a detailed guide.

NOTE: Scheduling of Weekly Automated Prime to be initiated prior to performing Fluidic decontamination function if Weekly Automated Prime is desired.

- ***[Fluidic Verification]***
  Guided program to perform verification of dispensing and aspirating nozzles by visual inspection and measurement of well volume. A LW96 Calibration Plate is required for Fluidic Verification. The guided program performs the following packaged functions according to User selection in the program:
  - [Dispensing Verification] – Fluidic head dispenses 80µL fluid in each well of Calibration Plate
  - [Aspirating Verification] – Fluidic head aspirates from each well, leaving approximately 30µL of fluid.

- ***[Flush BX]***

  Enables User to flush the inlet fluid through all activated channels of BX. The function will sequentially run the connected inlet fluid through all the selected channels on the BX for 10 seconds, then subsequently flush through the HT2100 fluidic head reservoir and washer outlet. A 4-to-1 inlet to outlet connector is required to perform this function.

- ***[Drain Spill Tray 10 Seconds]***

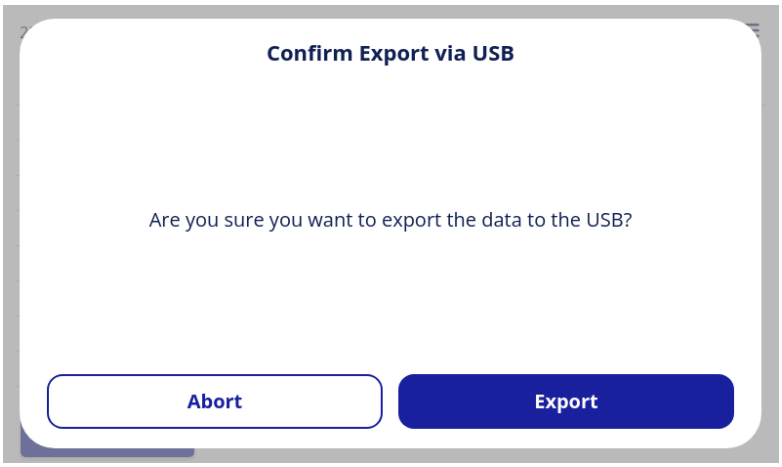
  Activates pump to drain spill tray under the Feeder Tray.

- ***[Operation Log]***

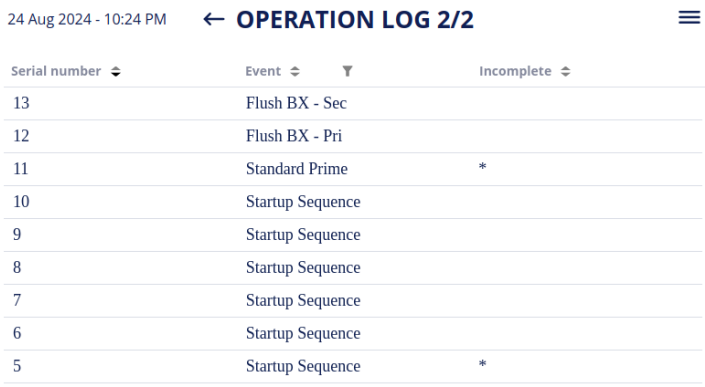
  This tab navigates to the Operations Logs page. All machine functions used by the operator will be automatically recorded by the system.

Columns	Descriptions
Process	Name of the fluidic function
Completed	Displays the total number of times the function has been successfully executed without interruption
Incompleted	Displays the total number of times the function failed to execute due to an operation stop or machine fault

And the button “Export Via USB” is to export out the machine logs history data.



Recommended to use NTFS format for USB pen drive.



Columns	Descriptions
Serial Number	Sequential number generated by system.
Event	Name of the fluidic function
Incomplete	Marked as * if the function fails to execute

- ***[Tray In] or [Tray Out] button***

  To retract or extend Feeder Tray.
  NOTE: Feeder Tray cannot be retracted if a plate is detected on it.

- ***[Active Channel] button***

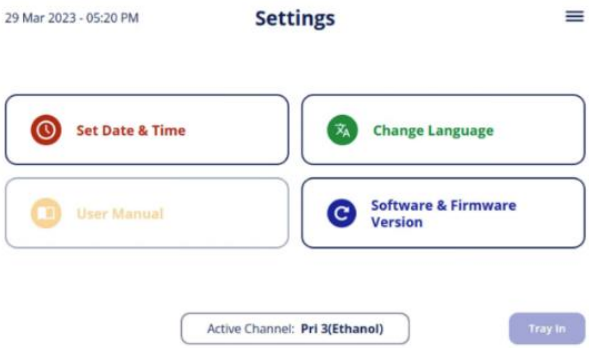
  This indicates the channel on the buffer exchanger that is currently activated, if connected to a buffer exchanger. This button may be activated to change the fluid channel.

- ***"Menu" icon***

  To access other modes on the washer:
  - Operation
  - Service (described in this section)
  - Settings
  - Startup Sequence
  - Shutdown Sequence
  - Timer

Settings Mode

The Settings Mode allows Users to set preferences related to the Graphic User Interface



User Interface

- ***"Date and time"***

  This indicates the current date and time. This may be indicated in [Settings] menu in the popup side bar.

Function buttons

- ***[Set Date & Time] button***

  Allows Users to set current date and time. This will be performed during installation with Curiox personnel.

- ***[User Manual] button***

  Opens a PDF of the HT2100 Washing Station User Manual for quick reference.

- ***[Change Language] button***

  To change language settings of the entire GUI.

- ***[Software & Firmware Version] button***

  To view the version number of the currently installed software and firmware.

- ***[Tray In] or [Tray Out] button***

  To retract or extend Feeder Tray.
  NOTE: Feeder Tray cannot be retracted if a plate is detected on it.

- ***[Active Channel] button***

This indicates the channel on the buffer exchanger that is currently activated, if connected to a buffer exchanger. This button may be activated to change the fluid channel.

- ***"Menu" icon***

  To access other modes on the washer:
  - Operation
  - Service
  - Settings (described in this section)
  - Startup Sequence
  - Shutdown Sequence
  - Timer

Startup Sequence

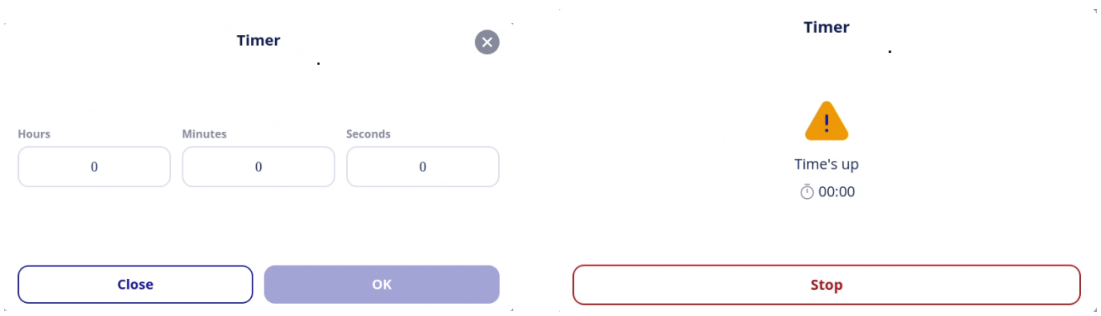
Startup Sequence is a guided program that primes the fluidic head at the start of the day, beginning with 70% ethanol + 1% Tween-20, followed by DI water + 1% Tween-20, and finally ending with the User-selected wash buffer in the washer, ready for operation. Priming takes place in the priming tray below the feeder tray. Refer to Chapter 3 Section *Preparation for Operation* on page 27 for a detailed walkthrough of the guided program.

Shutdown Sequence

Shutdown Sequence is a guided program that primes the fluidic head at the end of the day, preparing the washer for storage in ethanol. The fluidic head is first primed with DI-water + 1% Tween ends with 70% ethanol + 1% Tween-20, storing the fluidic head with ethanol in the nozzles. Priming takes place in the priming tray below the feeder tray. Prior to commencement of Shutdown Sequence, Users will be able to review or edit the scheduled Weekly Automated Prime. Refer to Section *Daily Shutdown Procedure* on page 48 for a detailed walkthrough of the guided program.

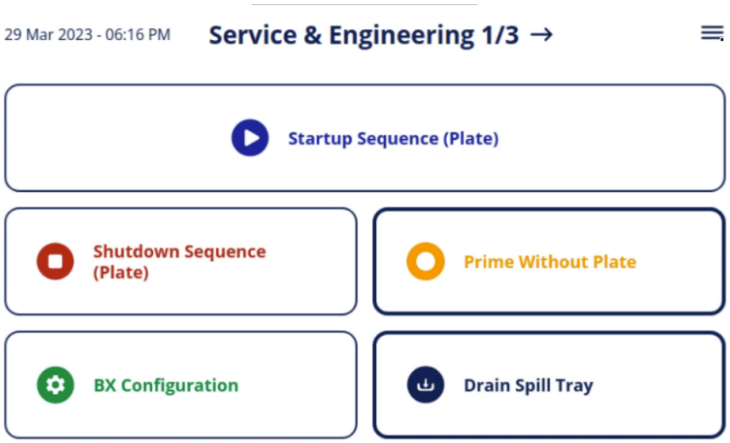
Timer

Allows Users to set up an independent countdown timer for purposes of off-board incubation or timing. A pop-up notification and audible alert is activated when the timer is completed. Timer begins count-up if the pop-up notification is not acknowledged.



Service & Engineering

Contains fluidic functions not typically performed by Users. These functions allow redundancy in the event of washer service.



Function buttons (1st of 3 pages)

- ***[Startup Sequence (Plate)] button***

  Startup Sequence (Plate) is a guided program that primes the fluidic head at the start of the day, beginning with 70% ethanol + 1% Tween-20, followed by DI water + 1% Tween-20, and finally ending with the User-selected wash buffer in the washer, ready for operation. Priming takes place on a *LW96 Priming Plate* placed on the Feeder Tray.

- ***[Shutdown Sequence (Plate)] button***

  Shutdown Sequence (Plate) is a guided program that primes the fluidic head at the end of the day, preparing the washer for storage in ethanol. The fluidic head is first primed with DI-water + 1% Tween and ends with 70% ethanol + 1% Tween-20, storing the fluidic head with ethanol in the nozzles. Priming takes place on a *LW96 Priming Plate* placed on the feeder tray. Prior to commencement of Shutdown Sequence, Users will be able to review or edit the scheduled Weekly Automated Prime. [Shutdown Sequence (Plate)] is also integrated as an option upon the completion of [Fluidic Decontamination].

- ***[BX Configuration] button***

  Enables User to indicate the configuration of Buffer Exchanger connection to the washer. The available options are:

- [None] – no buffer exchanger connected. If HT2100 is connected to a Buffer Exchanger, do not select [None]. Doing so will cut off fluidic path to the fluidic head when the washer is in operation, resulting in irreversible damage.
- [Primary] – To activate five channels on primary BX.
- [Primary + Secondary] – To activate ten channels on primary and secondary BX (for BX10 model only).

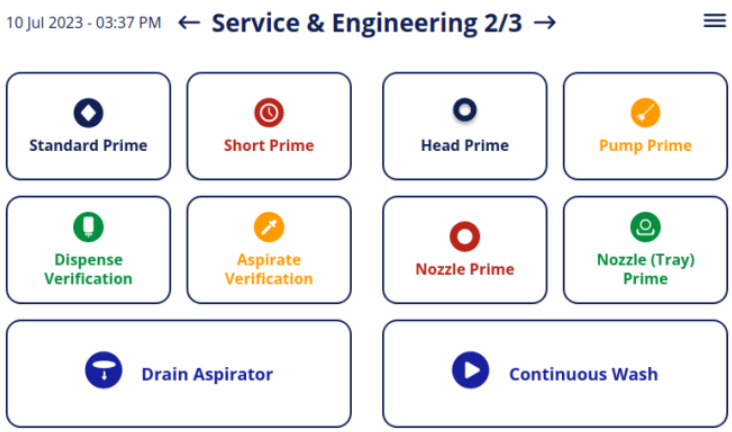
- ***[Prime Without Plate]***

  Enables priming of fluidic head without a plate or priming tray. Press and hold to activate pump to draw fluid into the fluidic head. Upon release of the button, the dispensing head will perform three strokes of dispense before ending the function. Dispensed fluid will be collected in the Auto-prime Tray. Repeated use of this function will fill the Spill Tray, resulting in an alert to drain the spill tray.

- ***[Drain Spill Tray]***

  Drains fluid collected in the Auto-prime Tray and spill tray. Press and hold to activate draining pump. The process will end once the touch is lifted.

Function buttons (2nd of 3 pages)



- ***[Standard Prime]***

  Primes the fluidic head with the fluid that is connected to the washer inlet, achieving at least 100x dilution of the previous fluid in the fluidic head. The function requires a Priming Plate on the Feeder Tray.

- ***[Short Prime]***

  Primes the fluidic head with the fluid that is connected to the washer inlet, using an abbreviate prime sequence that achieves at least 40x dilution of the previous fluid in the fluidic head. The function requires a Priming Plate on the Feeder Tray.

- ***[Head Prime]***

  Primes the dispensing and aspirating *nozzles* with the fluid present in the fluidic head reservoir. The dispensing head performs 5 strokes of dispense, followed by 7 strokes of aspirate performed by the aspirating head. The priming is performed using the Auto-prime Tray. Unlike other prime functions, this function is not preceded by pump activation that pulls fluid into the dispensing head reservoir.

- ***[Pump Prime]***

  Activates pump for 2 seconds to draw fluidic into fluidic head reservoir. Multiple runs of [Pump Prime] may be activated to manually draw fluid prior to activating [Standard Prime], in the event which the setup requires an inlet tubing that is too long for Standard Prime to fill the reservoir on the dispensing head. The function does not prime fluid through the nozzles (see [Head Prime]), and hence cannot be substituted for [Standard Prime].

- ***[Dispense Verification]***

  Dispense 80µL of prime fluid into each well on the plate. This function is used to check the working status of the dispensing head and requires a Calibration Plate on the Feeder Tray. [Dispense Verification] function is also integrated in [Startup Sequence].

- ***[Aspirate Verification]***

  Aspirates 50µL of fluid from each well on the plate. This function is used to check the working status of the aspirating head, and is typically activated after [Dispense Verification], with 80µL of fluid in each well of the plate. The function requires a Calibration Plate on the Feeder Tray. [Aspirate Verification] function is also integrated in [Startup Sequence].

- ***[Nozzle Prime]***

  Fills the aspirating nozzles with primed fluid, typically 70% ethanol + 1% Tween-20, as the final step after washer shutdown. A Priming Plate is required for this procedure. The washer performs 5 strokes of dispense and 3 strokes of aspirate, without a final aspirate pump, leaving the aspirating nozzles filled with ethanol for short-term storage.

- ***[Nozzle (Tray) Prime]***

  Fills the aspirating nozzles with primed fluid, typically 70% ethanol + 1% Tween-20, as the final step after washer shutdown. The Auto-prime Tray is required for this procedure. The washer performs 2 strokes of dispense, followed by alternate single stroke of aspirate, dispense and aspirate. The Spill Tray Pump is then activated to drain the Auto-prime Tray. [Nozzle (Tray) Prime] function is also integrated in [Shutdown Sequence].

- ***[Drain Aspirator]***

  Drains the aspirating nozzles of filled fluid with 3 aspirating strokes, followed by 2 seconds of aspirating pump activation.

- ***[Continuous Wash]***

  Perform continuous loops of 1x wash cycle (dispense to 80µL, aspirate to 25µL, pump activation, feeder tray extend and retract), until the [Stop Operation] button is activated. A Calibration Plate is required for this procedure.

Washer Operations

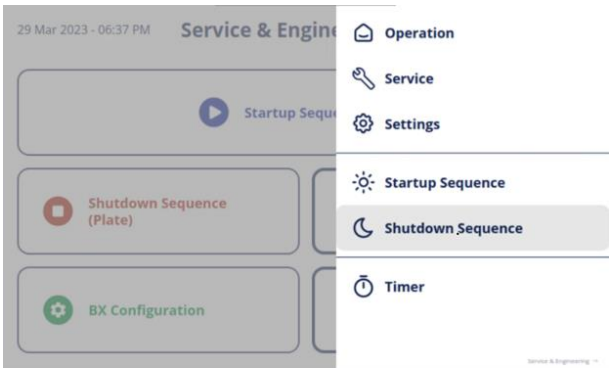
Volume Calibration

It is recommended to perform volume calibration of the machine during new machine installation, quarterly and after long term storage. Refer to Chapter 3 Section *Preparation for Operation* page 27, step 11 to 17 to calibrate the machine.

Daily Shutdown Procedure

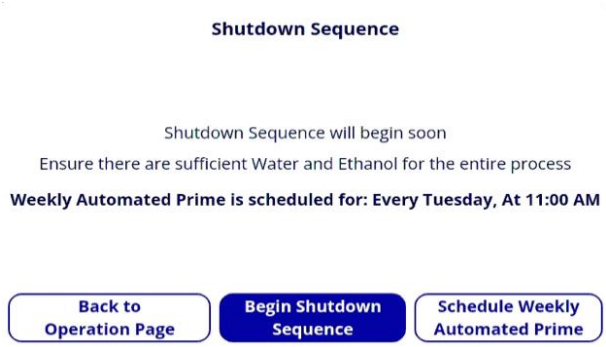
It is recommended to perform priming of the machine at the end of the day's operation. Follow the steps below to prepare the machine for operation.

1. Select [Shutdown Procedure] by tapping on the "Menu" icon on the top right of the screen.

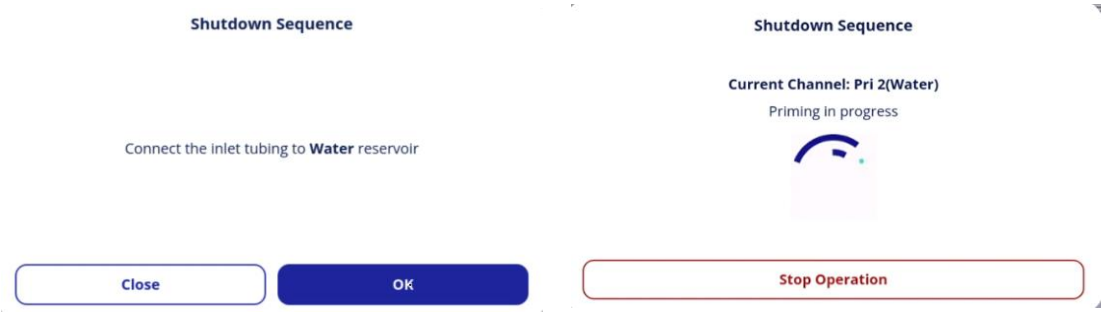


2. Prior to initiating the Shutdown Sequence, the program will remind users to ensure that the solutions required for Shutdown – 'Water' (DI water + 1% Tween-20) and 'Ethanol' (70% Ethanol + 1% Tween-20) are prepared in adequate volumes to complete the sequence. Users are also reminded on the User-set schedule for Weekly Automated Prime. If it is desired to edit the

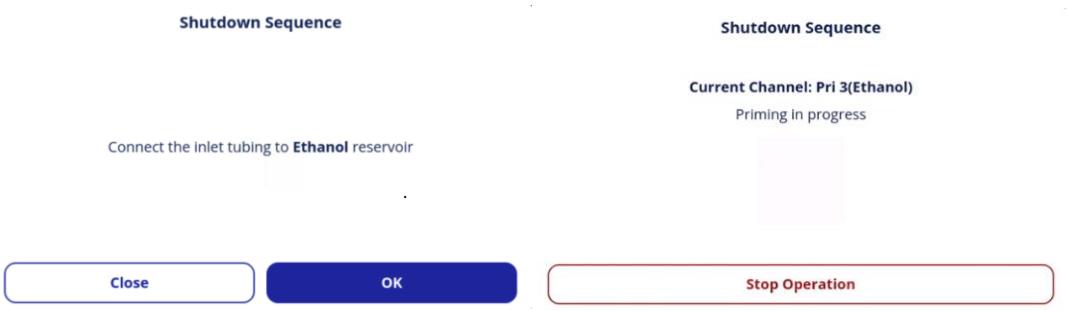
schedule of Weekly Automated Prime, User may select the button [Schedule Weekly Automated Prime]. User may initiate Shutdown Sequence by selecting [Begin Shutdown Sequence].



3. Upon initiation of [Shutdown Sequence], the program prompts users to connect the washer inlet tubing to the bottle containing 'Water' (DI Water + 1% Tween-20), if no BX connection was indicated, before selecting [OK] to continue with priming. The sequence proceeds automatically if BX connection was indicated.

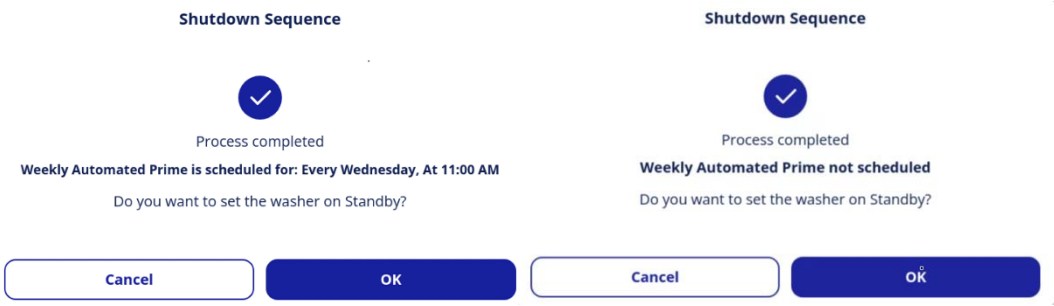


4. Upon completion of priming with 'Water', the program prompts Users to connect the washer inlet tubing to the bottle containing 'Ethanol' (70% Ethanol + 1% Tween-20), if no BX connection was indicated, before selecting [OK] to continue with priming. The sequence proceeds automatically if BX connection was indicated. The 'Ethanol' priming sequence ends with aspiration nozzles filled with 'Ethanol' for short-term storage of the washer.



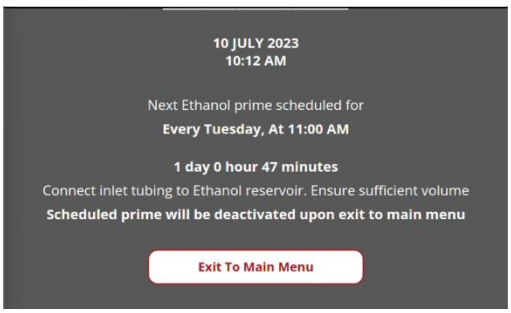
5. Upon completion of priming with 'Ethanol', the [Shutdown Sequence] is completed. The screen enters Standby mode if the Weekly Automated Prime was scheduled. If Weekly Automated Prime was not scheduled, the program prompts User to confirm setting the washer on Standby mode without Weekly Automated Prime (select [OK]) or to [Cancel] to return to Operation screen to set up the Weekly Automated Prime.

   NOTE: It is strongly recommended to schedule Weekly Automated Prime and ensure sufficient 'Ethanol' priming solution is available for optimal performance of the fluidics.



6. The Standby screen indicates the next scheduled Weekly Automated Prime, as well as a countdown to the scheduled prime. Users are reminded to ensure that there is sufficient 'Ethanol' connected to the washer. Users may exit Standby mode, and thus deactivating Weekly Automated Prime, by selecting [Exit to Main Menu].

   Once exited to the Main Menu, Users may navigate back to the Standby screen by press-and-holding the soft power button on the top right of the washer.



Washer Maintenance

Cleaning and Maintenance of Auto-prime Tray

The Auto-prime Tray acts as a built-in Priming Plate, allowing priming of the fluidic head without the use of an external plate. This also enables Weekly Automated Prime to proceed.

It is required to remove, clean and maintain the Auto-prime Tray regularly to prevent microbial growth which can result in fluidic errors in the fluidic head.

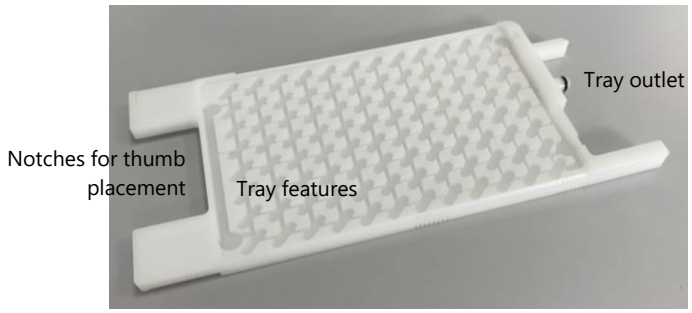


Figure 4.1: *Auto-prime Tray*

1. To remove the Auto-prime Tray, open the tray cover. Holding the right end of the lever, lift lever up and swing forward, away from the washer. Ensure lever holds down the tray cover.

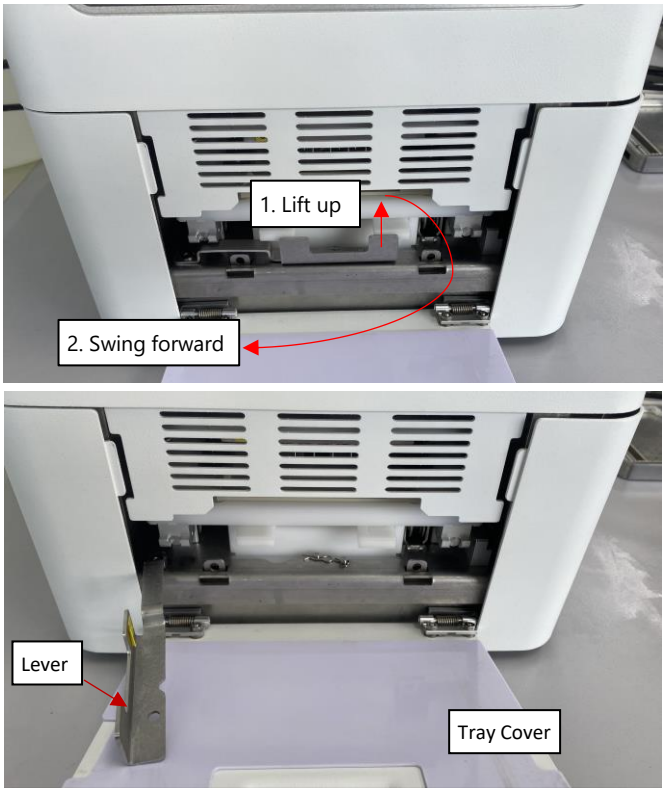


Figure 4.2: *Identify the lever and swing the lever from behind the tray cover (top). Lever holds the tray cover open (bottom).*

2. With both thumbs on the Auto-prime Tray notches, push in and lift up to release the Auto-prime Tray. Slide Auto-prime Tray out of the washer carefully.

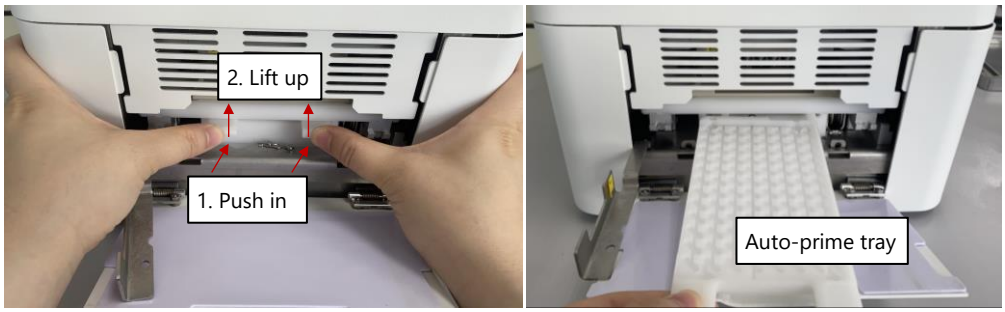


Figure 4.3: *Disengage the Auto-prime tray from the spill tray (left). Slide the Auto-prime Tray out of the washer (right).*

3. Regular cleaning and maintenance (perform fortnightly)
   a. Place the Auto-prime Tray under running tap water, ensuring all surfaces are wetted.
   b. Use a soft brush to clean around the Auto-prime Tray features thoroughly to prevent build-up of precipitated salts and reagents.
   c. Remove from running water. Flick dry to remove excess moisture.
   d. Allow the Auto-prime Tray to air dry prior to re-installation in the washer.

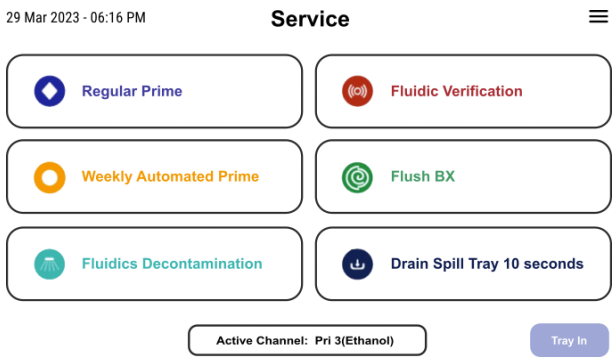
4. Corrective cleaning and maintenance (perform when stains are present)
   a. Place the Auto-prime Tray under running tap water, ensuring all surfaces are wetted.
   b. Apply a gentle detergent to the stained area and use a soft brush to clean around the Auto-prime Tray features thoroughly to remove build-up of precipitated salts and reagents.
   c. Rinse under running tap water and continue to brush the affected areas.
   d. Remove from running water. Flick dry to remove excess moisture.
   e. Allow the Auto-prime Tray to air dry prior to re-installation in the washer.

5. Decontamination of Auto-prime Tray (perform monthly)
   a. Fill a shallow tray with 1% bleach solution and fully submerge the Auto-prime Tray for 30 minutes.
   b. Remove the Auto-prime Tray from the bleach soak and use a soft brush to clean around the Auto-prime Tray features thoroughly.
   c. Rinse under running tap water and continue to brush the affected areas.
   d. Remove from running water. Flick dry to remove excess moisture.
   e. Allow the Auto-prime Tray to air dry prior to re-installation in the washer.

6. To re-install the Auto-prime Tray, slide it under the Feeder Tray above the Spill Tray, with the features facing up and tray outlet facing the washer. With both thumbs on the Auto-prime Tray notches, push the Auto-prime Tray against the Spill Tray wall, then push down to fix. A click will indicate proper placement of the Auto-prime Tray.

Fluidics Decontamination

Procedure to flush the washer fluidics with fluidics Cleaning Solution such as 1% bleach solution, with the option to continue with shutdown of the washer. [Fluidics Decontamination] requires a Priming Plate to be placed on the Feeder Tray.

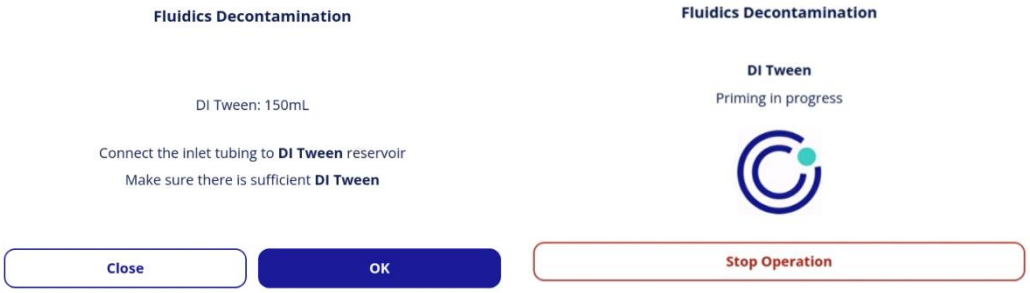


1. Select [Fluidics Decontamination] under [Service], which is accessible via the Menu icon.

2. If BX connection was indicated, Users are required to select the BX channel that is connected to the cleaning solution.

3. Users both with and without BX are then prompted to place a Priming Plate on the Feeder Tray, and to ensure that there is sufficient ‘DI Tween’ (DI water + 1% Tween-20), ‘Ethanol’ (70% Ethanol + 1% Tween-20) and Cleaning Solution prepared for the process.

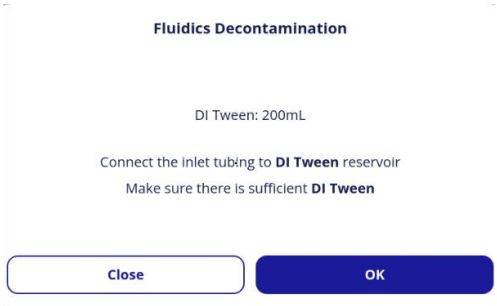
4. Fluidics Decontamination is initiated when Users select [Begin]. Users are then prompted to connect the buffer inlet on the washer to the bottle or reservoir containing the 'DI Tween' (DI water + 1% Tween-20). The washer then primes the fluidic head with the fluid, replacing the last wash buffer with 'DI Tween' to prevent reactivity between the wash buffer and the Cleaning Solution. This step proceeds automatically if BX connection was indicated.



5. After the fluidic head is primed with DI Water, Users are then prompted to connect the buffer inlet tube on the washer to the bottle or reservoir containing the Cleaning Solution. The washer proceeds to prime with fluidic head with the Cleaning Solution. This step proceeds automatically if BX connection was indicated.

6. After the fluidic head is primed with Cleaning Solution, the program commences 10 minutes of Continuous Wash with the primed fluid. This continually moves the Cleaning Solution along the entire fluidic path to ensure complete decontamination of all wetted surfaces in the fluidic head. A countdown timer appears to indicate the time left in this step of the program.

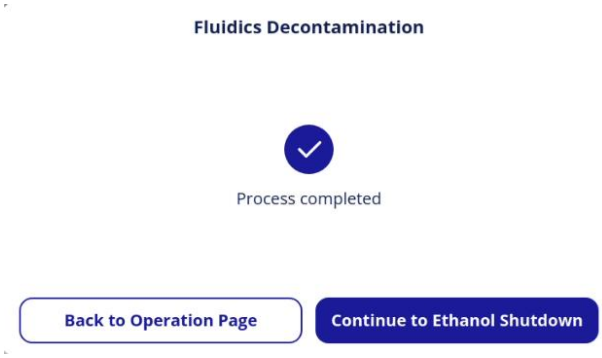
7. When 10 minutes of Continuous Wash with Cleaning Solution has been completed, Users are prompted to connect the inlet tubing on the washer to the bottle or reservoir containing 'DI Tween' (DI water + 1% Tween-20). The washer then primes the fluidic head with the fluid. This step proceeds automatically if BX connection was indicated.



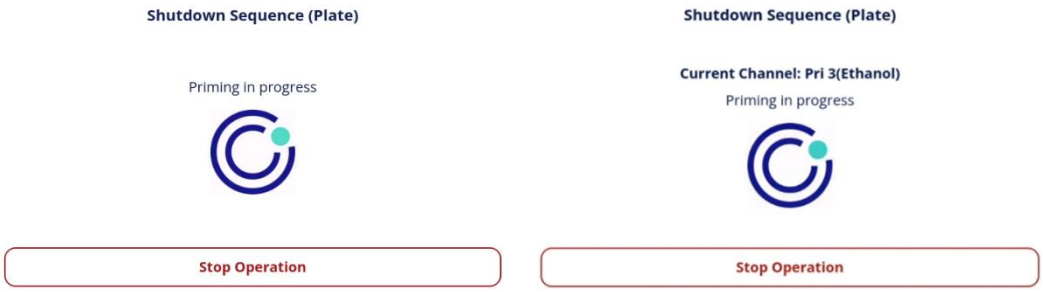
8. After the fluidic head is primed with 'DI Tween', the program commences 20 minutes of Continuous Wash with the primed fluid. This continually moves 'DI Tween' along the entire fluidic path to ensure complete removal of Cleaning Solution from all wetted surfaces in the fluidic head. A countdown timer appears to indicate the time left in this step of the program. This is the final step in the Fluidics Decontamination program.

9. Users are alerted when the program is completed. Users may return to Operation page or select to proceed with priming and shutdown with 'Ethanol' (70% ethanol + 1% Tween-20).

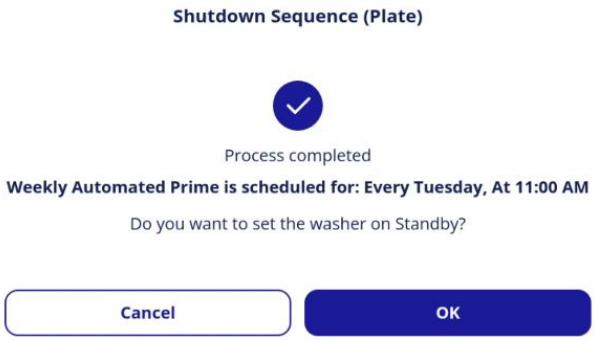
CAUTION: Empty the waste containing the Cleaning Solution prior to initiating 'Ethanol Shutdown'. Some chemicals such as bleach produce toxic chemicals when mixed with alcohols.



10. If [Continue to Ethanol Shutdown] is selected, the washer proceeds to prime the fluidic head with 70% ethanol + 1% Tween-20 for shutdown with the Priming Plate.

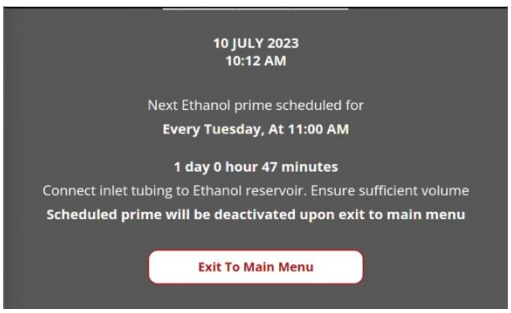


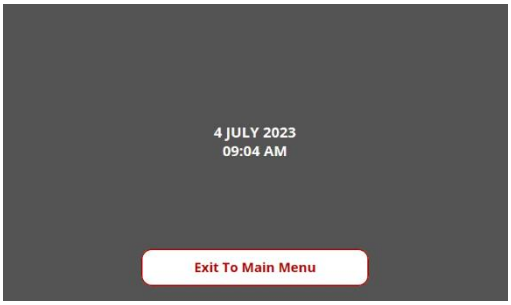
11. When Ethanol Shutdown process has been completed, Users may select to proceed to set the washer on Standby mode by selecting [OK] or return to Operation page by selecting [Cancel].



12. Upon setting the washer to standby mode, the Standby screen indicates the next scheduled Weekly Automated Prime prime, as well as a countdown to the scheduled prime. Users are reminded to ensure that there is sufficient 'Ethanol' connected to the washer. Users may exit Standby mode, and thus deactivating Weekly Automated Prime, by selecting [Exit to Main Menu].

NOTE: Once exited to the Main Menu, Users may navigate back to the Standby screen by press-and-holding the soft power button.



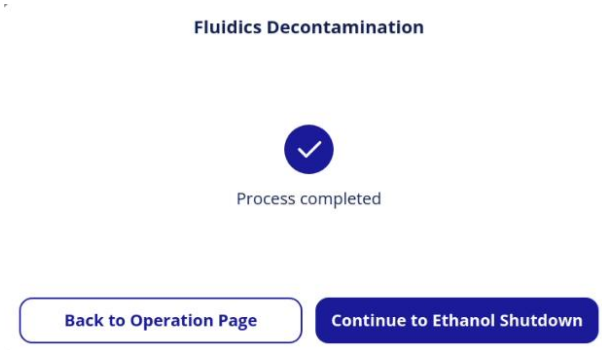


Long Term Dry Storage Procedure

Procedure for the storing of washer station for protracted period of a month or longer. Users are recommended to perform [Fluidics Decontamination] and [Shutdown Sequence] of the washer, followed by priming the fluidics with air.

1. Perform [Fluidics Decontamination] as described in Section *Fluidics Decontamination*, page 52.

2. Following the completion of [Fluidics Decontamination],
   - If there is no BX installed, select [Continue to Ethanol Shutdown] and continue the procedure from step 8.
   - If the BEX is connected and the installed BEX is intended for storage, select [Cancel] (do not continue to 'Ethanol Shutdown') and proceed steps 3 to 7 to prepare BX for storage.



3. Using the 4-to-1 connector accessory, first connect the bottle or reservoir containing 'DI Tween' (DI water + 1% Tween-20) to four inlet channels on the BX. Ensure that the BX is connected to the inlet tube on the washer.

NOTE: If the BX has both primary and secondary channels, complete steps 3 to 6 with the 4-to1-connector linked to the primary channels first. Re-connect the 4-to-1 connector to the secondary channels and repeat steps 3-6.

4. Select [Flush BX] on [Service] mode. The washer flushes buffer from the inlet bottle through the four connected BX channels for cleaning.

5. Repeat steps 3 and 4 by connecting the 4-to-1 connector accessory to the bottle or reservoir containing 'Ethanol' (70% Ethanol + 1% Tween-20) to flush all channels with 'Ethanol'.

6. Repeat steps 3 and 4 by disconnecting the 4-to-1 connector accessory from the BX inlet, flushing and drying the BX with ambient air. The BX may now be disconnected from the washer and stowed for storage.
   NOTE: Repeat steps 3-6 with the 4-to-1 connector linked to the BX secondary channels, if present.

7. Proceed to perform [Regular Prime] (found in [Service] mode using Menu icon) on the washer with 'ethanol' (70% ethanol + 1% Tween-20) connected directly to the washer.

8. Upon completion of the Ethanol Shutdown (from step 2) or [Regular Prime] (from step 7), disconnect the washer inlet tube from any fluid bottle or reservoir. From the Menu icon on the top right corner, enter [Service] mode, select [Regular Prime]. The washer is primed with ambient air from the inlet tube, displacing any fluid in the fluidic head.

9. The washer is now decontaminated and dry, ready for long-term storage. It is recommended to wipe down the external surfaces of the washer with 70% ethanol for surface decontamination.

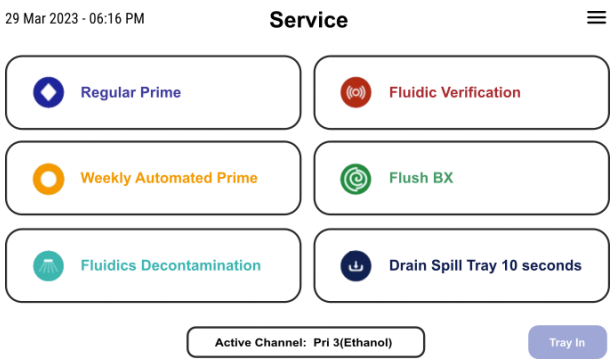
Preventive Maintenance – Weekly Automated Prime

If the product is intended to be unused for extended periods of time, it is strongly recommended to schedule a [Weekly Automated Prime] to maintain the nozzles in optimal condition.

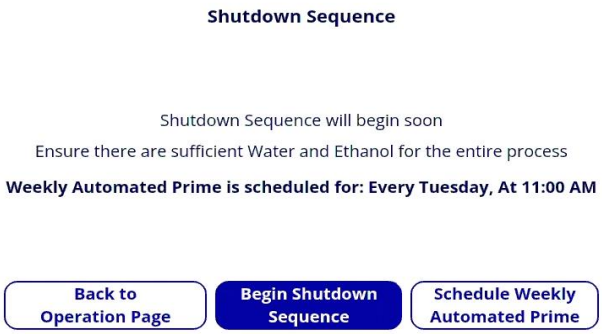
[Weekly Automated Prime] allows Users to schedule a day and time of the week to prime the fluidic head with 'Ethanol' (70% ethanol + 1% Tween-20) for optimal performance. The program does not require the use of a Priming Plate. Ensure that there is sufficient 'Ethanol' in the inlet bottle or reservoir.

The schedule for [Weekly Automated Prime] may be set and edited by

1. Accessing [Weekly Automated Prime] through [Service] mode found in the Menu icon on the top right corner.



2. Selecting [Schedule Weekly Automated Prime] at initiation of [Shutdown Sequence]



Error Codes

The HT2100 will prompt various Error Codes on the machine display panel in the event of an error. Table 4-1 lists the error codes and messages of HT2100.

Error Codes	Error Message/Description
E01	No plate
E02	Plate present, unable to retract plate feeder
E11	Head home error – Head home sensor not active when supposed to
E12	Upper right home error – Only right sensor did not activate on upper block homing move
E13	Upper left home error – Only left sensor did not activate on upper block homing move
E14	Lower right home error – Only right sensor did not activate on lower block homing move
E15	Lower left home error – Only left sensor did not activate on lower block homing move
E16	Plate feeder home error – Plate feeder home sensor not active when supposed to
E21	Load plate error – Feeder home sensor still active when feeder is retracted
E22	Head home signal/motor error – Head home sensor not activated as head moves up
E23	Plate feeder signal/motor error – Feeder home sensor not activated as feeder moves to home position
E24	Upper block home error (both left & right) – Upper block sensors did not activate on homing
E25	Lower block home error (both left & right) – Lower block sensors did not activate on homing
E31	Spill Tray Full error
E41-44	Backlash Limit errors

Chapter 5:

Maintenance and Troubleshooting

Chapter Overview

- Maintenance Schedule
- Common Problems for Troubleshooting
- Technical Support
- Decontamination Procedure

Maintenance Schedule

Below is the recommended maintenance schedule to ensure that the HT2100 runs smoothly and efficiently.

Action	Daily	Weekly	Monthly	As Required
Perform [Startup Sequence] to prime the machine with 1% Tween 20 in 70% Ethanol, followed by 1% Tween 20 in DI water and finally with Assay Wash Buffer at the start of the day's operation.	✓			
Perform [Shutdown Sequence] to prime the machine with 1% Tween 20 in DI water, followed by 1% Tween 20 in 70% Ethanol at the end of the day's operation.	✓			
Wipe all surfaces of the unit enclosure with a cloth moistened with 70% ethanol	✓			✓
Wash Filter under running DI water on a weekly basis.		✓		
Remove to clean Auto-prime Tray.			✓	✓
Perform regular [Fluidics Decontamination] to with a suitable Cleaning Solution (e.g. 1% bleach) to ensure optimal performance of fluidics Note: Weekly [Fluidics Decontamination] is highly recommended to users with sticky or clumpy samples.				✓
It is recommended to perform volume calibration during new machine installation, quarterly and after storage for long period. Refer to "Calibration" on page 35.				✓
Sending unit to Curiox Biosystems for service. Decontaminate washer and complete Appendix B.				✓
Flush bottle caps (including inlet and outlet connectors) and empty buffer bottles with 70% ethanol. Rinse thoroughly under running water.	✓			✓
Disconnect tubing from bottle caps to perform deep cleaning with 70% ethanol. Rinse all components thoroughly under running water before reassembly.			✓	

Common Problems for Troubleshooting

Problem 1: Display panel is not lit up/ Machine has no power/ Failure to initialize/ Motor error

Possible cause: Power is not switched on, or HT2100 is not plugged into electricity supply, or wrong power adaptor is used.

- Ensure the jack is properly connected to the HT2100, and that the power is switched on.
- Ensure that the correct adaptor is used and that it is working.

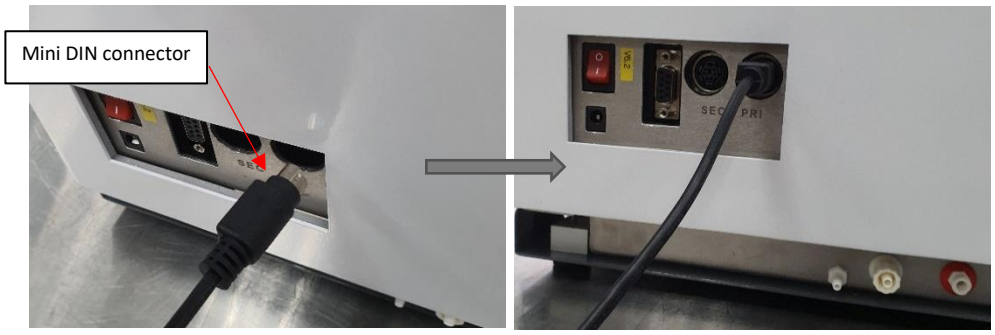
Problem 2: No plate detected

Possible cause: Plate sensor cannot detect the plate properly due to wet feeder tray, or condensation, or wet bottom of the plate.

- Ensure that the bottom of the plate is clean and dry.
- Ensure that the feeder tray is clean and dry.

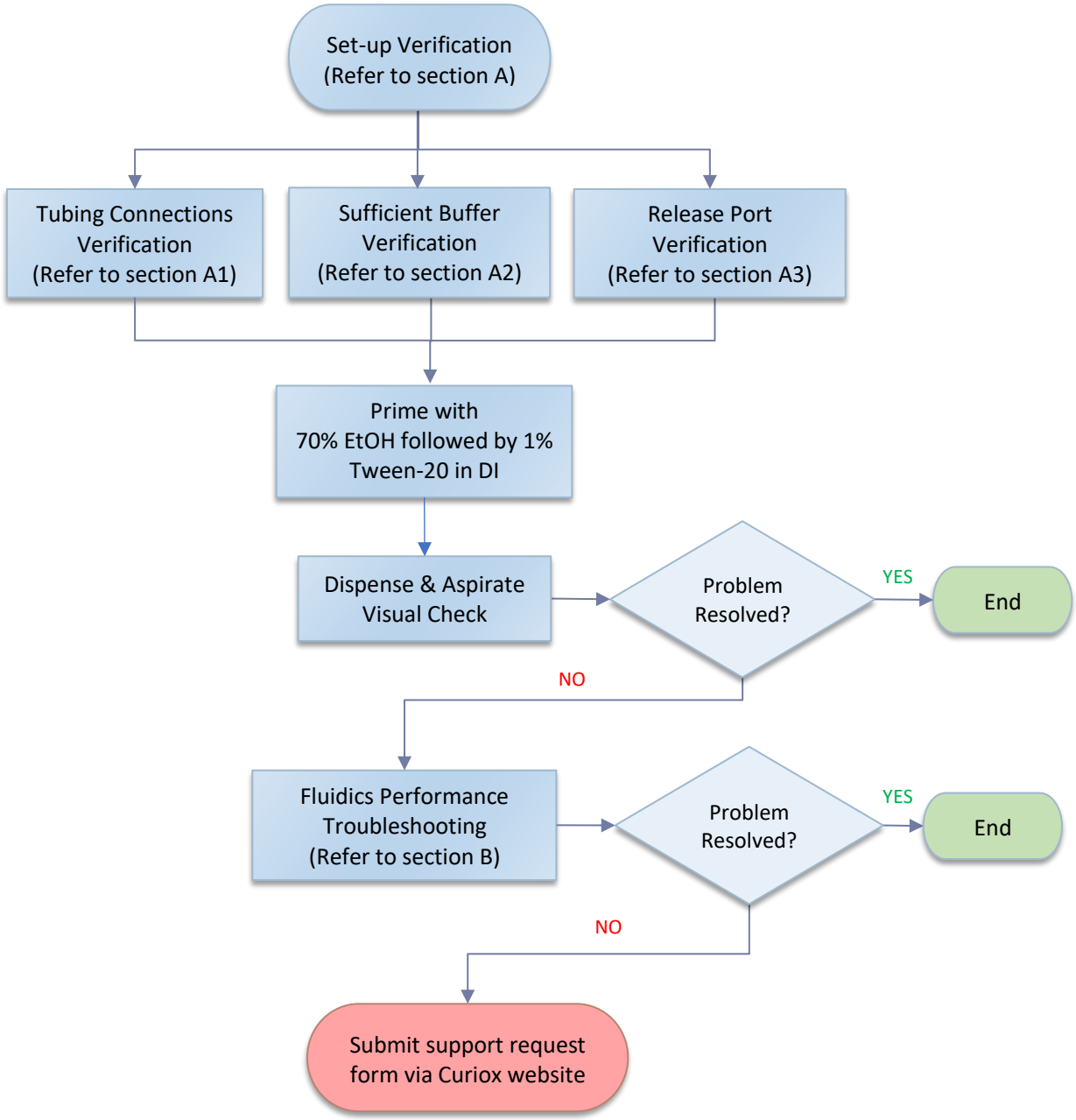
Problem 3: Failure to dispense and aspirate properly/ Washer not drawing buffer/ Inconsistent residual volume

- If buffer exchanger is used, ensure that the Mini DIN connector is fully inserted and connected properly. A subtle click sound can be heard when changing between the different channels on the buffer exchanger.



- If the problem persists, proceed with the following troubleshooting.

- Refer to the flowchart below for the overall troubleshooting process. Perform set-up verification followed by fluidics performance troubleshooting as described in the corresponding sections A and B to resolve the problem.



A. Set-up verification:

A1) Tubing connections verification

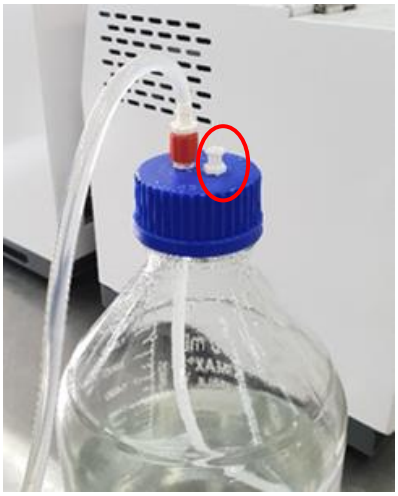
Check for any signs of leaking on the machine's inlet and outlet tubing. Also, check the tubing connections for any leakage. Where leakage is present, disassemble and carefully reassemble the affected tubing connection.

A2) Sufficient buffer verification

Verify that buffer being supplied to the machine is sufficient and that tubing is completely submerged in buffer.

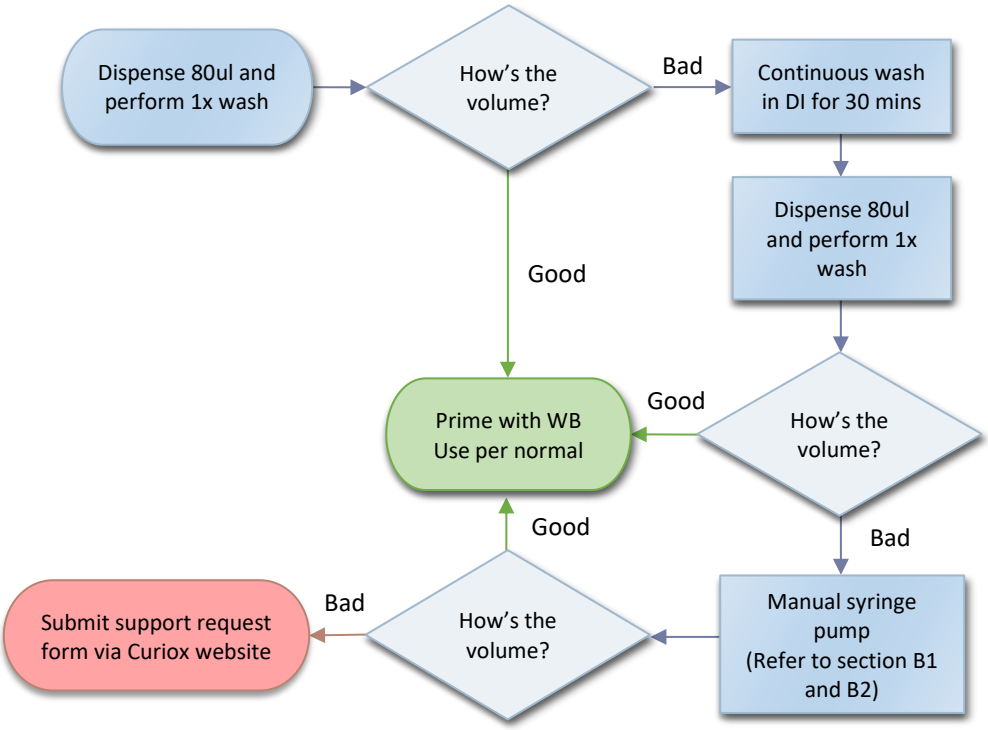
A3) Release port verification

Verify that the release port located on the bottle cap is not blocked.



A4) Perform standard prime with 1% Tween-20 in 70% EtOH followed by 1% Tween-20 in DI water. Do a dispense and aspirate visual check to see if the issue is resolved. Where inaccuracies are dispensed and aspirated volume is still observed, proceed to the next section for fluidics performance troubleshooting.

B. Fluidics performance troubleshooting flowchart

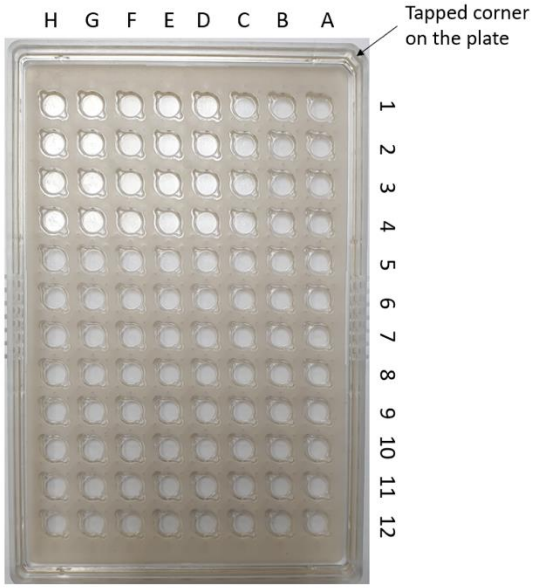


B1) Identify the affected dispensing and aspirating nozzles corresponding to the affected well(s) using the plate and nozzle mapping.

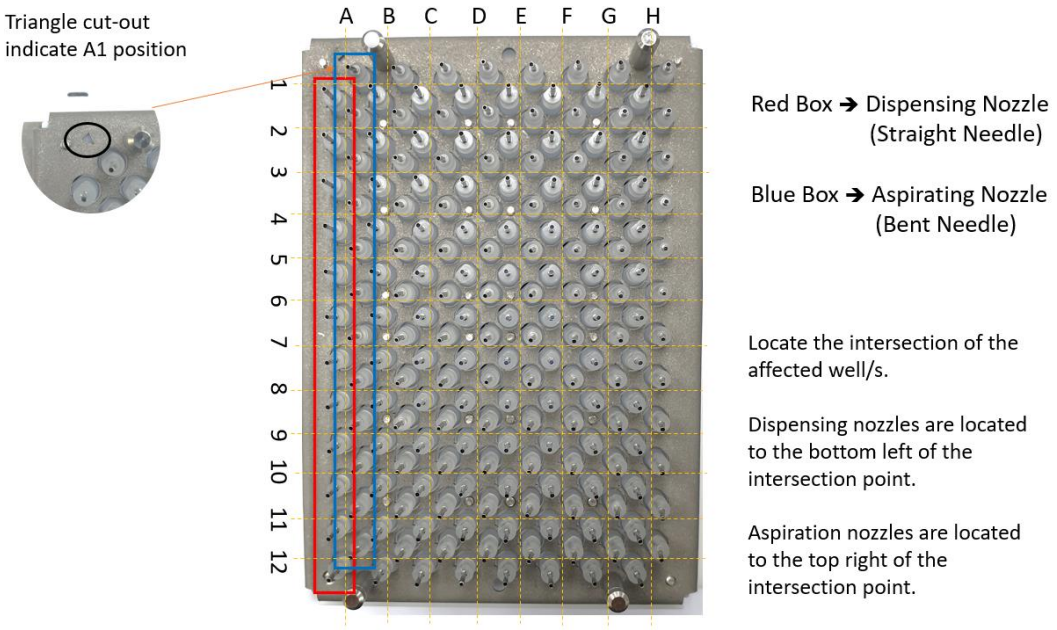
B1.1. Plate mapping:

Locate affected well according to this plate mapping

Refer to nozzle mapping to test the affected nozzle



B1.2. Nozzle mapping:

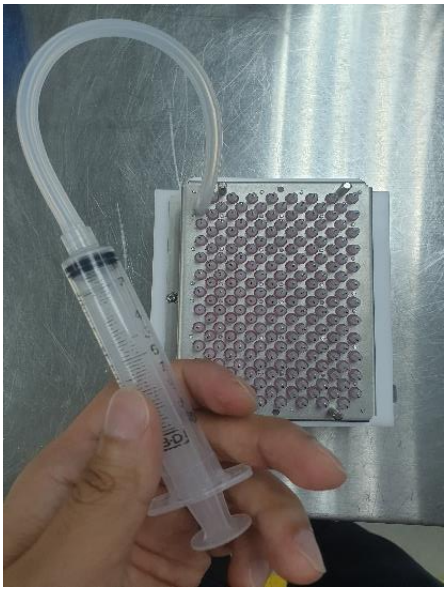


B2) Execute manual syringe pump

B2.1 Prime the fluidic head with air to remove any liquid from the fluidic pathways.

B2.2 Take out the fluidic head from the washer and turn it over.

B2.3 Fit the tubing connected with the syringe to the affected dispensing nozzle.



B2.4 Pull the plunger fully to the back. There should be minimal resistance.

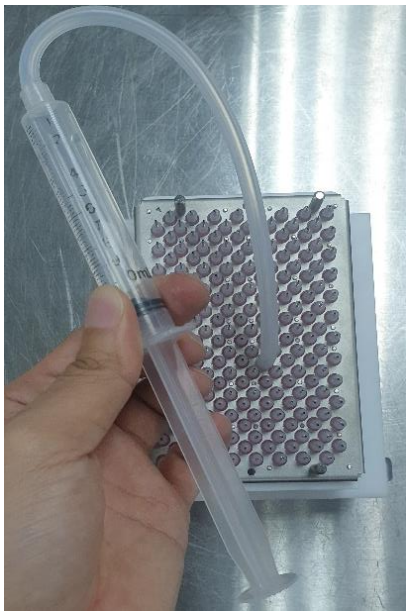
Note: If there is significant resistance, there is clogging. Please submit a support request form via Curiox website

B2.5 Try to push in the plunger. You should feel resistance upon pushing the plunger.

B2.6 Remove the tubing from the dispensing nozzle.

B2.7 Pull the plunger fully to the back to fill the syringe with air.

B2.8 Fit the tubing connected with the syringe to the affected aspirating nozzle.



B2.9 Push the plunger fully into the syringe. There should be minimal resistance.

Note: If there is significant resistance, there is clogging. Please submit a support request form via Curiox website.

B2.10 Try to pull the plunger. You should feel resistance upon pulling the plunger.

B2.11 Use the head spacer to ensure that the three blocks of the fluidic head are aligned and slot the fluidic head into the washer (For installation of fluidic head refer to Chapter 3, pg. 18-21).

B2.12 Perform a standard prime with 1% Tween-20 in 70% EtOH.

B2.13 Perform a standard prime with 1% Tween-20 in DI water.

B2.14 Do a dispense and aspirate visual check to see if the issue is resolved. If the problem persists, please submit a support request form via Curiox website.

Technical Support

Kindly visit us at www.curiox.com to submit a support request form or email us at *sales@curiox.com* if you require technical support or advice.

Decontamination Procedure

For environmental health and safety reasons, it is imperative that operators decontaminate the 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 HT2100.

1. Refer to Chapter 4 Section *Long Term Dry Storage Procedure*, page 56 to prepare the fluidics of HT2100 for storage and transportation.
2. Remove all biological contents from the HT2100. Turn off and unplug the HT2100 from the power supply and disconnect the power jack.
3. Wearing gloves, clean all surfaces of the 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.
4. Moisten (do not soak) a cloth with 70-85% EtOH solution. Wipe all surfaces of the HT2100 enclosure. Wait for 20 minutes.
5. Moisten a cloth with deionized water and wipe all the surfaces previously cleaned with formaldehyde/EtOH. Dry the wet surfaces with a clean cloth. Ensure all used cloths are disposed of in a bio- hazard bag/container.
6. **If intended for transport:** Seal the machine in an airtight bag prior to transport. If you intend to return the device to Curiox Biosystems for servicing or maintenance, please fill out the Acknowledgment of Decontamination form found on Appendix B).

Appendix A:

Acknowledgment of Decontamination

Acknowledgment of Decontamination Form

Decontamination is required prior to HT2100 return to Curiox Biosystems for reasons such as servicing and maintenance. It is required that you fill out this form to acknowledge that decontamination had been conducted on the instrument. Failure to do so may result in the return of the instrument to your address for decontamination.

PRODUCT SERIAL NO.	
CONTACT INFORMATION	
Dr./Mr./Mrs./Ms. (Please circle accordingly)	JOB TITLE
NAME	EMAIL ADDRESS
COMPANY	PHONE NUMBER
DECONTAMINATION INFORMATION	
DECONTAMINATION METHOD	
DECONTAMINATION DATE	
ACKNOWLEDGEMENT	
I hereby acknowledge that this piece of equipment has been decontaminated and sealed in accordance to the procedure recommended in this manual prior to shipment to Curiox Biosystems. To the best of my knowledge, the equipment is safe to handle by the receiving personnel. Name _____ Signature and Date _____	

Appendix B:

Purchase Information and Feedback

Purchase Information and Feedback Form

PURCHASE INFORMATION	
PRODUCT SERIAL NO.	
PURCHASED BY	**PURCHASED FROM**
COMPANY	DISTRIBUTOR
ADDRESS	DATE OF PURCHASE
PHONE	DATE OF DELIVERY
FAX	
CONTACT INFORMATION	
Dr./Mr./Mrs./Ms. (Please circle accordingly)	JOB TITLE
NAME	EMAIL ADDRESS

FEEDBACK (PLEASE CHECK/COMMENT ACCORDINGLY)					
	Excellent	Good	Average	Poor	Comments
User Guide					
Ease of Use					
Reliability					
Operating Costs					
Overall Experience					

Do you have any other comments/suggestions about the Laminar Wash™ HT2100? If so, please let us know below.

End of Document

Revision History

Rev No	Date	Change Description	Author(s)
01	28 Jul 2023	First Release	Mun Wai Lee
02	14 Sep 2023	Update Curiox China office address Update all bleach concentration from 0.5% to 1%	Mun Wai Lee
03	10 January 2024	Change of headquarters address	JaeYoung Shin
04	29 Aug 2024	Added Operations Log	Andriyono Candra

Author	Designation	Signature	Date
Andriyono Candra	Software Engineer		29 Aug 2024

Review & Approval

Name	Designation	Signature	Date
Kong Leong Cheng	A/Dir Engineering		29 Aug 2024
Chyan Ying Ke	Dir Bioapplications		29 Aug 2024
Yanli Li	Associate Product Manager		03 Sep 2024