Laminar Wash™ HT2000 Station 96 User Manual

Curiox Biosystems CO., Ltd.
Samsung IT Harrington Tower 2F
#211, 41 Digital-ro 9-gil,
Geumcheon-gu, Seoul,
South Korea (08511)
T: +82-70-8680-6100
F: +82-70-8280-6665
E: sales@curiox.com
W: www.curiox.com

Laminar Wash HT2000 User Manual

Document Nr: IN-CL-UD-04
Revision 11, August 2024

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

Curiox, the Curiox logo, and Laminar Wash are trademarks or registered trademarks of Curiox Biosystems. Other marks appearing in these materials are marks of their respective owners.

Table of Contents

Chapter 1:

General Information

Chapter Overview

- General Information
- Introduction to the Laminar Wash HT2000
- Chemical Compatibility
- Safety
- CE Mark
- Customer Service and Technical Support

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™ HT2000 Station. The content of this manual includes:

- How to set up and operate the HT2000
- The principle of operation and modes of function for the HT2000
- Safety features of the HT2000 and precautions to ensure safe operation.
- Troubleshooting procedures and maintenance

Introduction to the Laminar Wash HT2000

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 HT2000 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 over 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 99x 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 HT2000. Table 1-1 on page 4 lists the material composition of the main components of the HT2000 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 HT2000, 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 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 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 (MSDSs) 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 HT2000 may be listed as hazardous. Warnings are displayed on the labels of all chemicals when hazards exist.

MSDSs 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 HT2000. 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 HT2000 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 is able to accommodate electrical power in the range of 100 - 240 V. The resultant output to the HT2000 power jack is 24 V DC.

Emergency Stop

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

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 HT2000.
- Do not load more than one Laminar Wash 96-well Plate in the HT2000 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 HT2000.
- 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 HT2000.

Prior to System Operation

Ensure that all users of the HT2000 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 MSDSs.

CAUTION

Avoid using the HT2000 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

Telephone
+1 650 226 8420 (US)
+65 6908 4201 (Singapore)

Fax
+ 1 650 590 5406 (US)
+65 6908 4203 (Singapore)

E-mail
sales@curiox.com

Web
www.curiox.com

Addresses
Curiox Biosystems, Inc.
400 West Cummings Park
Suite 6650
Woburn, MA 01801
USA

Curiox Biosystems Pte. Ltd.
2 Gambas Crescent
#07-14 Nordcom II (Tower 1)
Singapore 757044

Curiox Biosystems China Co., Ltd
Room C05, Building G, No. 889
Bibo Road, Pudong District,
Shanghai, 201203
China

Curiox Biosystems Co. Ltd
Samsung Harrington IT Tower
#202, #204-213
9 Gil 41 Digital-ro
Geumcheon-gu, Seoul 08511
South Korea

Instrument Storage and Shipping

1. Ensure that all internal and external surfaces of the HT2000 washer station and BX are dry following the decontamination procedure above. See Chapter 4: *Operations – 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 airtight 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 HT2000 and the phases which outline a typical washing process.

Functional Description

The simple digital interface located on the top front panel of the HT2000 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 HT2000 are shown in Figure 2-1, Figure 2-2 and Figure 2-3.

Figure 2-1: *Front view of the Laminar Wash HT2000*

Figure 2-2: *Lateral (right side) view of the Laminar Wash HT2000*

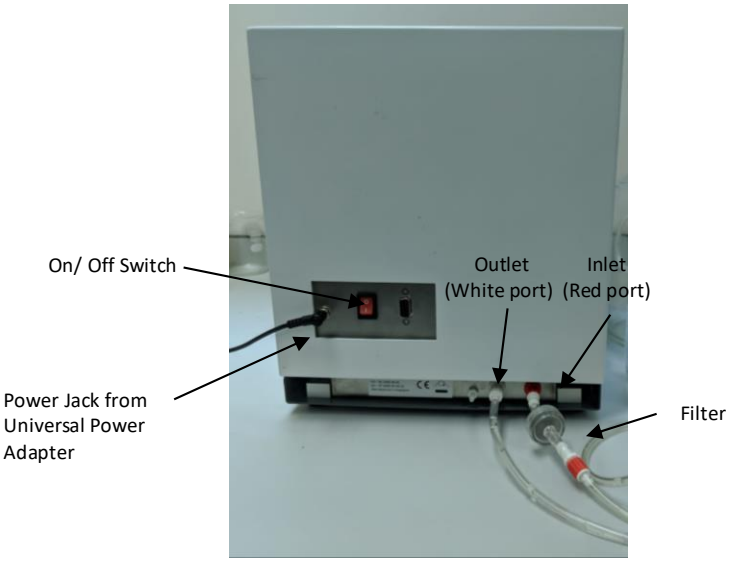


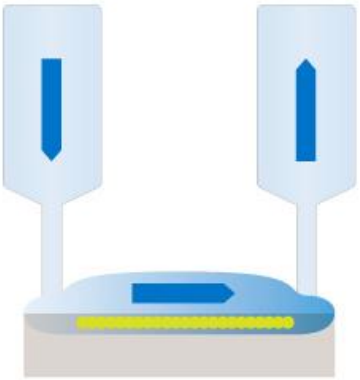
Figure 2-3: *Back view of the Laminar Wash HT2000 with main components labelled.*

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 HT2000*

Chapter 3:

Setup

Chapter Overview

- Installation
- Preparation for Operation

Installation

Upon arrival of the HT2000 package, check that all the accessories listed below are present. Some of the components of the HT2000 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 HT2000.

List of items:

- Laminar Wash HT2000 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 washer*
- Deionized (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 HT2000 on a flat surface and in an environment with temperature and humidity conditions as stipulated in “Technical Specifications” on page 3.
2. 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).*

3. 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.

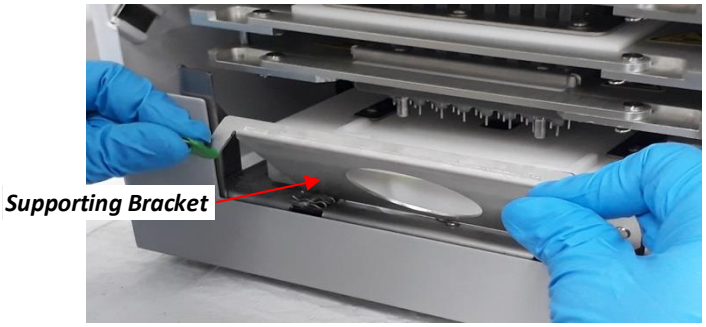


Figure 3-2: *Remove the machine supporting bracket.*

4. 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.

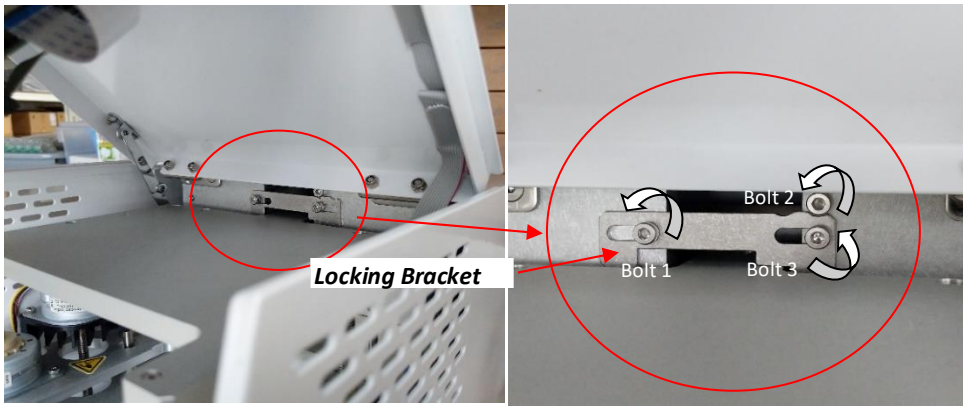


Figure 3-3: *Loosen bolts to move locking bracket to the right.*

5. 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.

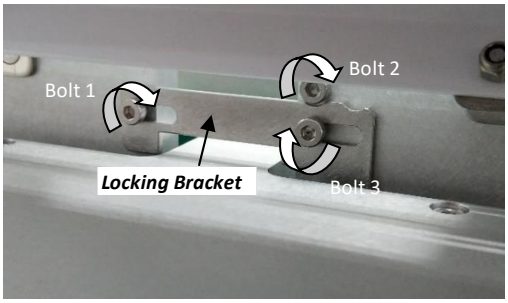


Figure 3-4: *Lock locking bracket into position by tightening bolts*

6. Detach the cotter pins and lock pins, and then flip open the metal bars.

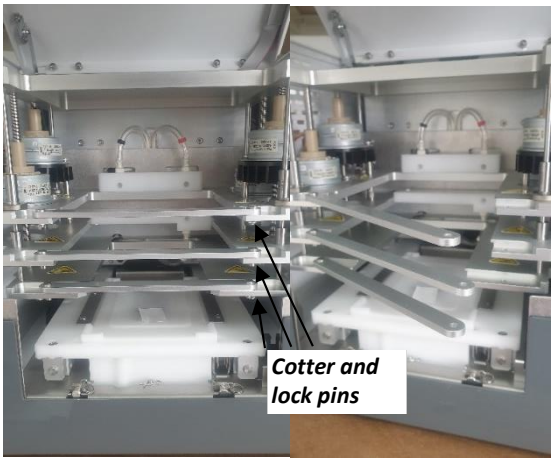


Figure 3-5: Opening metal bars

7. 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.

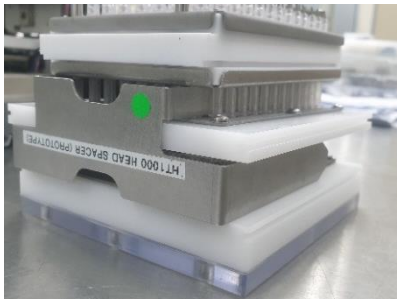


Figure 3-6a: *Fluidic Head*

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

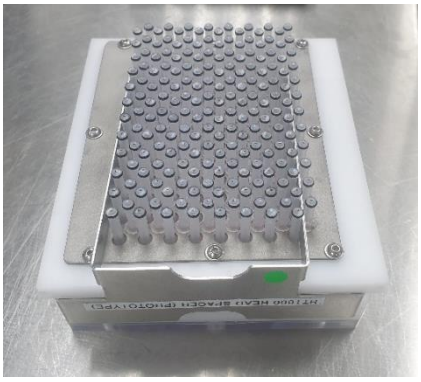


Figure 3-6b: *Fluidic Head*

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

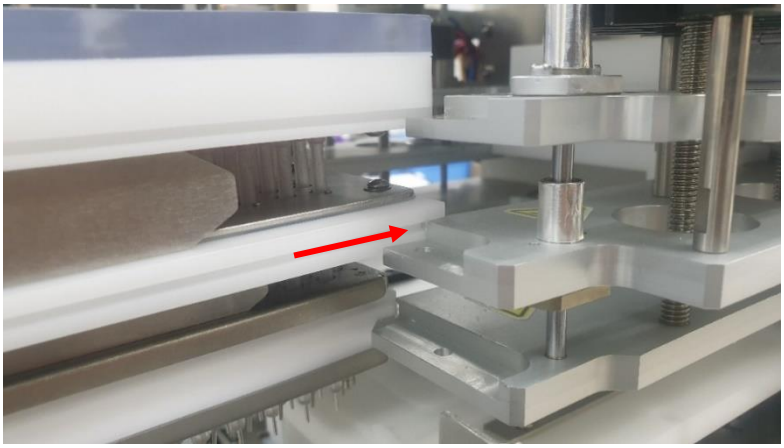


Figure 3-7: *Align Fluidic Head to Machine (step 1)*

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

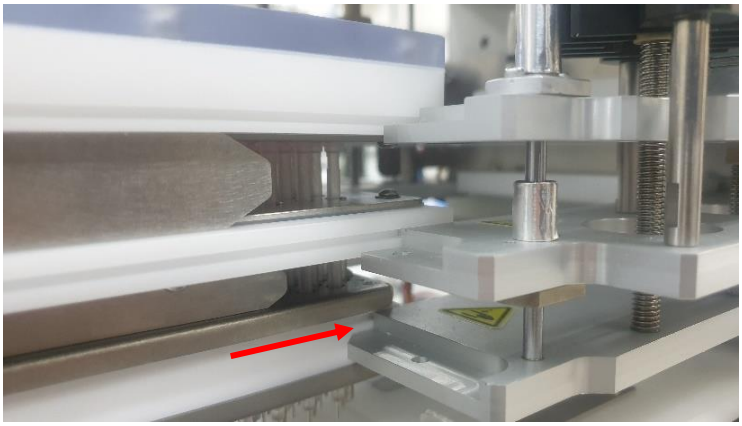


Figure 3-8: *Align Fluidic Head to Machine (step 2)*

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

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

11. Push the fluidic head all the way in before sliding the Fluidic Head Spacer out.

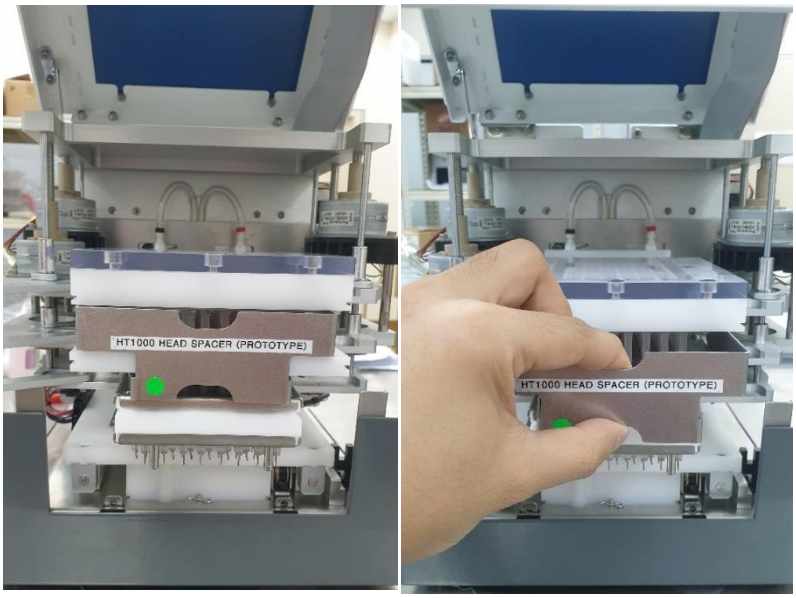


Figure 3.10: *Align Fluidic Head to Machine (step 4)*

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

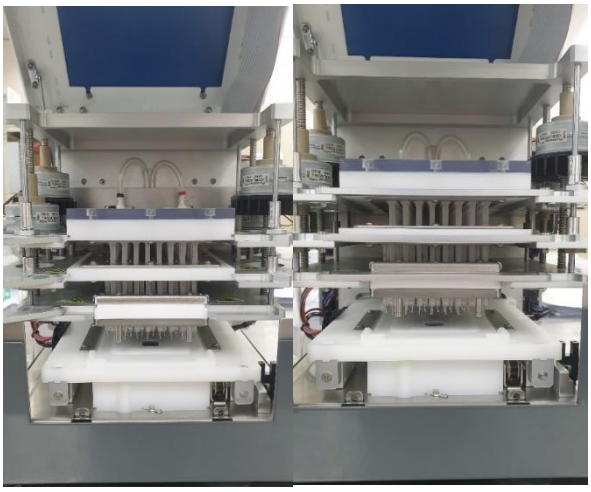


Figure 3-11: *Align Fluidic Head to Machine (step 5)*

13. Close the machine cover by returning the hinge back to its original position.
14. Connect the power cable to the universal power adaptor. Insert the power jack at the back of the HT2000 as shown in Figure 2-3.
15. Insert the two delivery tubes into the respective ports on the HT2000 in the manner specified below (Figure 3-12):

i. Connect a clean filter into the red connector end of the inlet tubing and insert into the red port on the HT2000. Insert the other end of the inlet tubinginto wash buffer bottle via the buffer inlet bottle cap. Refer to the next section “Buffer Inlet Bottle Cap Installation” for installation instructions.

ii. Connect the white connector end of the outlet tubing into the white port on the HT2000 and the other end into the waste bottle.

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

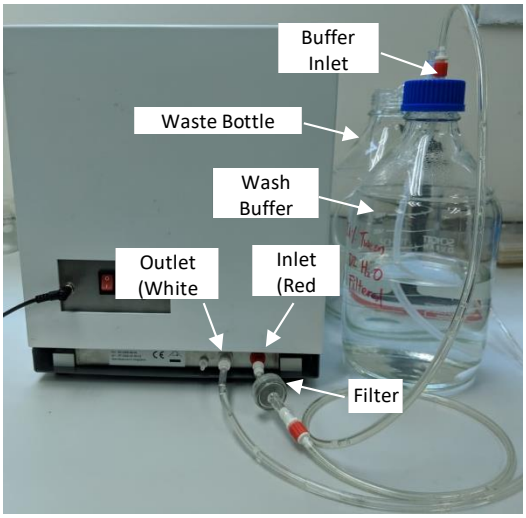


Figure 3-12: *Assembly of tubings from bottle caps to HT2000*

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.

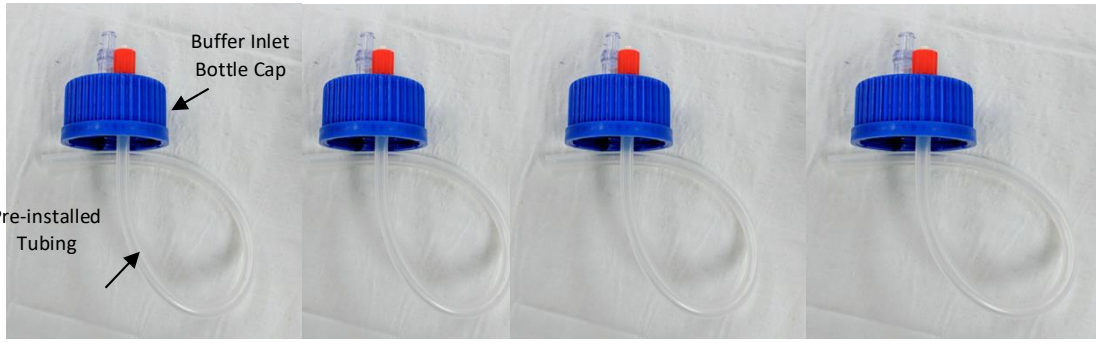


Figure 3-13: *Four sets of buffer inlet bottle caps with pre-installed tubing*

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

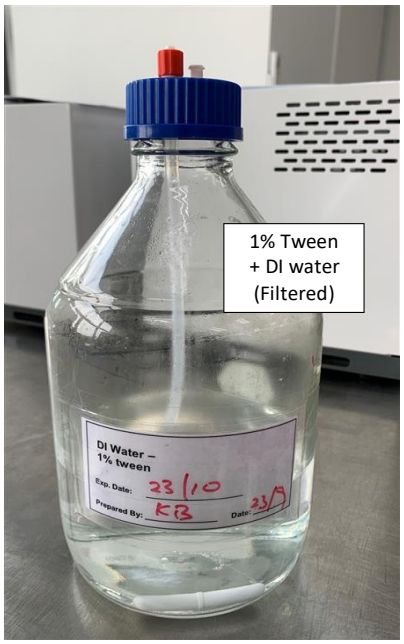


Figure 3-14: *Buffer inlet bottle cap connected to 2L bottle containing wash buffer of choice.*

3. Connect a clean filter into the red connector end of the inlet tubing and insert into the red port on the HT2000. Insert the other end of the inlet tubinginto wash buffer bottle via 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 HT2000, 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 HT2000, using two separate mini-DIN Connectors.

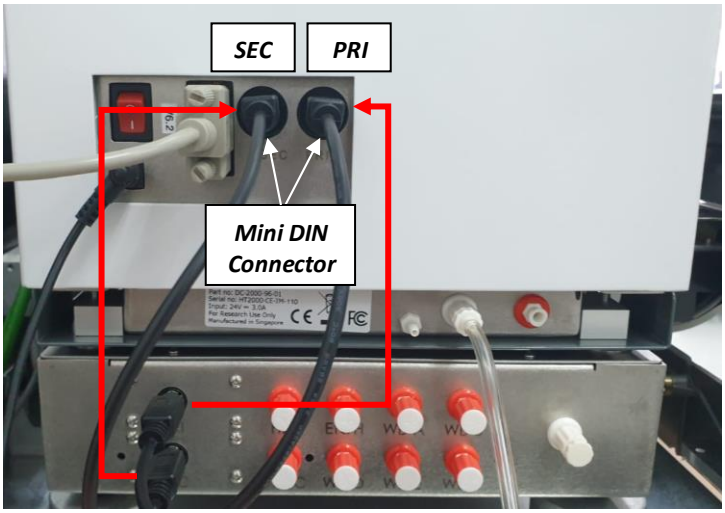


Figure 3-15: *Connection with a Buffer Exchanger using Mini DIN Connectors.*

RS232 Serial Port Connection

A computer/laptop can be connected to the HT2000 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 Computer/Laptop 7*

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 Wash Assay Buffer at the start of the day's operation. A calibration wash 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 calibration steps afterward. It is recommended to do a weekly calibration test to ensure optimal performance and recalibration will be required after moving the machine out from the cold room.

1. Ensure the power jack is connected. Power up the HT2000 and wait for the homing sequence to complete.
2. Select **[SERVICE MODE]**. Go to page 2 of 3 tab by tapping on **[→]**

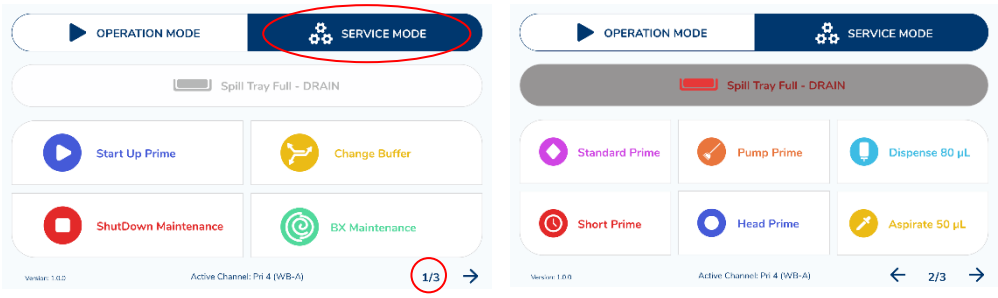


Figure 3-17: *Enter* **[Service Mode]**

3. Ensure the two delivery tubes are properly connected into the respective ports (red and white) on the HT2000. 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.
4. 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 23.

Start Up Procedure

IF YOUR HT2000 WASHER HAS GUI VERSION 1.0.0.7T, FOLLOW AS STATED BELOW.

1. Ensure 1% Tween 20 in 70% Ethanol is connected to the machine.
2. Place a Priming Plate onto the feeder platform.

Figure 3-18: *Insert priming plate into machine (in* **[Service Mode]***)*

3. Under **[SERVICE MODE]**, select **[Standard Prime]** to prime the washer with 1% Tween 20 in 70% Ethanol.

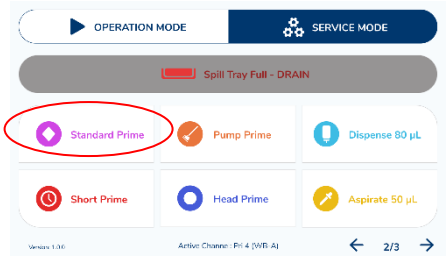


Figure 3-19: *Standard Prime in* **[Service Mode]** *(GUI V1.0.0.7T)*

4. HT2000 completes the prime with 1% Tween 20 in 70% Ethanol when the feeder platform extends.
5. Repeat steps 5 to 8, replacing fluid with 1% Tween 20 in DI water.

IF YOUR HT2000 WASHER HAS GUI VERSION 2.0.3, FOLLOW AS STATED BELOW.

1. Under **[SERVICE MODE]**, select **[Start Up Prime]** to initiate the package function that will guide User to prime the washer with 1% Tween 20 in 70% Ethanol, 1% Tween 20 in DI water, followed by wash buffer if selected.

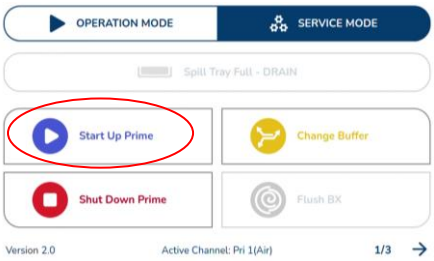


Figure 3-20: *Start Up Prime in* **[Service Mode]** *(GUI Version 2.0.3)*

2. Follow the on-screen instructions to ensure sufficient priming solution volume and connect the priming solutions accordingly.

Calibration Procedure

1. While priming the HT2000 with 1% Tween 20 in DI water, measure and record the mass of an empty Calibration Plate using a mass balance.
2. After priming the washer with 1% Tween 20 in DI water, replace the Priming Plate with Calibration Plate.
3. In **[SERVICE MODE]**, select **[Dispense 80µL]** to dispense 80µL 1% Tween 20 in DI water into all wells.

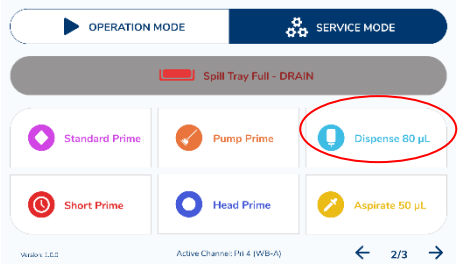


Figure 3-21: *dispense 80µL (in* **[Service Mode]***)*

4. Carefully remove the Calibration Plate from the feeder platform.
5. Visually observe that all wells are dispensed evenly, then measure and record the mass of the filled Calibration Plate on a calibrated balance.
6. Mass of empty plate is subtracted from mass of filled plate to obtain the mass of the dispensed fluid.
7. Resultant mass is divided by number of wells to obtain volume dispensed per well, with the assumption that 1mg = 1µL (mass density of water 1mg/µL) and that all wells are filled evenly.
8. Tap on **[OPERATION MODE]**.
9. Input the number of washes value as "1", the initial volume value as "80" and flow rate as "5" into the system by using the "+" or "-" symbols or the keypad.

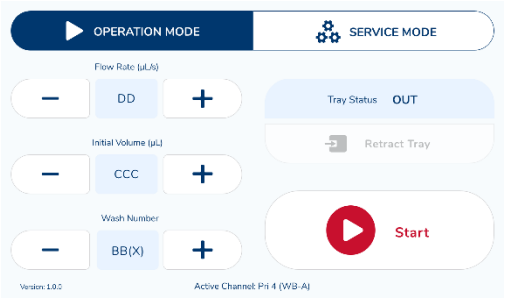


Figure 3-22**:** Input parameters *(in Service Mode)*

10. Press the **[Start]** button to start the wash cycle.
11. Remove Calibration Plate from the feeder platform.
12. Visually observe that residual volumes on all wells are even and obtain the mass of the filled Calibration Plate on a calibrated balance.
13. Mass of empty plate is subtracted from mass of filled plate to obtain the mass of the dispensed fluid.
14. Resultant mass is divided by number of wells to obtain volume dispensed per well, with the assumption that 1mg = 1µL (mass density of water 1mg/µL) and that all wells are filled evenly.
15. Ensure that average volume is 26 ± 3 µL.
16. If the dispensed or aspirated volume per well differ from the stated range, access "Service Engineering", by tapping 5 times at the bottom right corner of the GUI.

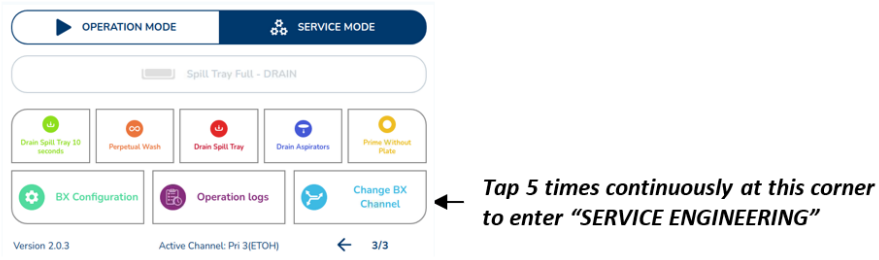


Figure 3-23: *Accessing calibration (in Service Engineering mode)*

17. Select on **[Calibrate Disp Vol]** or **[Calibrate Asp Vol]** to input the calculated value into the system and then update it by tapping on **[Update]**.

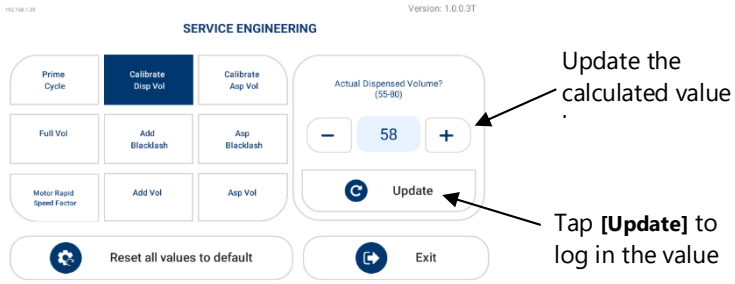


Figure 3-24: Calibration *(in Service Engineering mode)*

18. Repeat steps 1 to 15 until the desired dispensed and aspirated volumes are achieved.
19. Tap on **[Exit]** to exit Service Engineering tab.
20. Refer to 'Maintenance & Troubleshooting' Section if there are any dispensing/aspirating problems.
21. The machine is now ready for operation.

NOTE:

1. ***If the subsequent washing requires sterile environment, the Priming Plate and Calibration Plate may 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 HT2000
- Operational Safety
- Operation Mode
- Service Mode
- Shutdown Procedure
- Preventive Maintenance Procedure
- Fluidics Decontamination Protocol
- Long Term Dry Storage Procedure
- Error Codes

Before Operating the HT2000

The HT2000 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 HT2000 possesses the following built-in safety features which prevent the machine from operating under unsafe conditions.

Plate Detection

The HT2000 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.

Power Supply

The universal power adapter is able to accommodate electrical power in the range of 100 - 240 V. The resultant output to the HT2000 power jack is 24 V DC.

Emergency Stop and Recovery

Activating the **[STOP OPERATION]** button in the middle of a washing cycle will cause the machine to immediately halt its operation. The machine returns to home position and extends the feeder platform to return the plate when the **[RECOVER]** button is subsequently selected.

Machine Homing

In the event power to the machine is disrupted in the middle of a washing cycle and subsequently the machine is switched on again, the machine will go into 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, error code “E31” is prompted on the machine display panel. User may only access the spill tray draining button during this time.

Safety Precautions

In addition to the built-in safety features of the HT2000, below are some precautions operators are advised to take while using the HT2000 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 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.

Operational Mode

The Operation Mode allows an operator to set up parameters as well as washes.

User Interface

- ***User Interface LCD***

  The user interface shows 3 values: “Flow Rate”, “No. of Washes” as well as “Initial Volume”.

- ***“Flow Rate”***

  Flow rate of dispensing and aspirating fluid in µl/s. The value can be adjusted by tapping the **[+]** or **[-]** sign beside the value. The lower and upper limit is 2 and 20 respectively. In GUI v2.0, a keypad for direct numerical input may also be called by selecting the number window.

- ***“Initial Volume”***

  Initial volume of droplet on each well in the plate in µl. The value can be adjusted by tapping the **[+]** or **[-]** sign beside the value. The lower and upper limit is 1 and 99 respectively. In GUI v2.0, a keypad for direct numerical input may also be called by selecting the number window.

- ***“Wash Number”***

  User-set number of wash cycles the plate should undergo. A description of a wash cycle may be found in the next section on page 34. The value can be adjusted by tapping the **[+]** or **[-]** sign beside the value. The lower and upper limit is 1 and 50 respectively. In GUI v2.0, a keypad for direct numerical input may also be called by selecting the number window.

- ***“Tray Status”***

  Indicate if the tray is **[Retract]** or **[Extend]**

- ***"Version"***

  This indicates the current version of the Graphical User Interface.

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

Function Buttons

- ***[Retract] or [Extend]***
  To move the feed tray in or out of the washer.
  Note: feed tray cannot be retracted if a plate is detected on it

- ***[Start]***
  To begin a wash using the wash parameters as displayed in this tab.

- ***[SERVICE MODE]***
  Tap to switch to Service tab.

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 80 ± 2µl (e.g., if initial volume is 50µl, dispensing head adds 30µl to the droplet).

4. Aspirating head aspirates the 80µL droplet, leaving approximately 26µL residual volume. 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 nozzles top up well contents with primed buffer 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.

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

- ***User Interface LED***

  The user interface in Service Mode will show 3 pages of functions

- ***"Version"***

  This indicates the current version of the Graphical User Interface.

- ***"Active Channel"***

  This indicate the channel that is currently activated, if connected to a buffer exchanger.

- **[OPERATION MODE]**

  Tab to switch to Operation Mode

- **[Spill Tray Full – Drain]**

  This tab is usually greyed out and inactive. However, if the spill tray full sensor is activated, all other functions in the Service tab will be greyed out, with only this tab active. User must press and hold this tab to activate pump to remove fluid in the spill tray.

- **[➔]**

  Flip to the next page

- **[➔]**

  Return to previous page

Function tab (1st of 3 tabs)

- **[Start-up Prime]**

  Enter the guided packaged function to start-up the washer by priming with ethanol / Tween-20, followed by DI / Tween-20, and if indicated, followed by selected wash buffer.

- **[Change Buffer]**

  Enter guided packaged function to switch wash buffer.

- **[Shutdown Maintenance]**

  Enter guided packaged function to prime with DI water / Tween-20 followed by ethanol / Tween-20 to prepare washer for shutdown.

  For GUI v2.0, this packaged function guides users to complete Shutdown Maintenance with ethanol storage.

For GUI v1.0.0.7T, refer to page 40 *Daily Shutdown Procedure* on completing Shutdown Maintenance with ethanol storage.

Note: For the 3 packaged functions above, **[Standard Prime]** *is utilized for the prime functions. Each prime cycle will used approximately 190ml of fluid. See function* **[Standard Prime]** *for more information.*

- **[BX maintenance]**
  To flush the inlet buffer through all channels of the selected BX. The function will sequentially run fluid through all the channels in the selected BX, each activated for 10 seconds, then subsequently flush through the HT2000 fluidic head reservoir and washer outlet.

Function tab (2nd of 3 tabs)

- **[Standard Prime]**
  Perform a standard prime by the washer. A plate is required to be present for this function to be activated. The operation will consume approximately 190ml of fluid and will achieve greater than 1000X dilution of the prior fluid.

- **[Short Prime]**
  Perform a short prime by the washer. A plate is required to be present for this function to be activated. The operation will consume approximately 130ml of fluid and will achieve approximately 40X dilution of the prior fluid.

- **[Pump Prime]**
  Run the pump only to fill the head reservoir for duration of 2 seconds. A plate is not required to be present for this function to be activated. Multiple sequential runs can pump fluid into the reservoir of the dispensing head, but it will not run the fluid through the nozzles. Hence this function cannot be used as a substitute for **[Standard Prime]**. However, if a set-up requires an inlet tubing that is too long for **[Standard Prime]** to adequately fill the reservoir on the dispensing head, Pump Prime may be activated prior to **[Standard Prime]**to pre-fill the reservoir.

- **[Head Prime]**
  Activate to prime the dispensing head for 5X dispense strokes, followed by aspirating head for 7X aspirate strokes. A Priming Plate is required to be present for this function to be activated. The function runs the fluid from the dispensing head reservoir through the nozzle. Unlike **[Standard Prime]**, this function is not preceded by pump activation that pulls fluid into the dispensing head reservoir.

  Note: In case that the pump in the washer fails, this function can act as an backup prime function, albeit there will be higher wash buffer wastage in the process due to the high number of **[Head Prime]** *required to pull the buffer though the washer reservoir.*

- **[Nozzle Prime]** *(New function in GUI v2.0)*
  Activate to prime the dispensing head for 5X dispense strokes, followed by aspirating head for 3X aspirate strokes. A Priming Plate is required to be present for this function to be activated. The function is intended for use after the final **[Standard Prime]** with Ethanol / Tween during Shutdown, and serves to ensure that the aspiration nozzles are filled with fluid instead of air.

- **[Dispense 80µl]**

Dispense 80µl of fluid onto each well on the plate. A Calibration Plate is required to be present for this function to be activated. This function is used during calibration to check the working status of the dispensing head.

- **[Aspirate 50µl]**
  Aspirate 50µl of fluid from each well on the plate. A Calibration Plate is required to be present for this function to be activated. This function is used during calibration to check the working status of the aspirating head.

Function tab (3rd of 3 tabs)

- **[Drain Spill Tray 10 seconds]**
  Tap once to activate this function. The spill tray draining pump will be activated for 10 seconds to drain fluid from the spill tray.

- **[Perpetual Wash]**
  Tap once to activate. A Priming Plate is required to be present for this function to be activated. This function will run a wash cycle (dispense, aspirate, tray extend, tray retract) continuously until User disrupts the process.

- **[Drain Spill Tray]**
  Select and hold to activate. Will run pump to drain the spill tray as long as the function is activated. The process will end once the touch is lifted.

- **[Drain Aspirator]**
  Tap once to activate. Aspiration block will cycle up/down for 3 times, followed by 2 seconds of pump aspiration.

- **[Prime Without Plate]**
  Press continuously to activate. While selected, the pump will continue to prime fluid into the head reservoir. Upon release, the dispensing head will cycle up/down 3X before ending the function. Fluid that was primed through the nozzles will be collected in the spill tray.

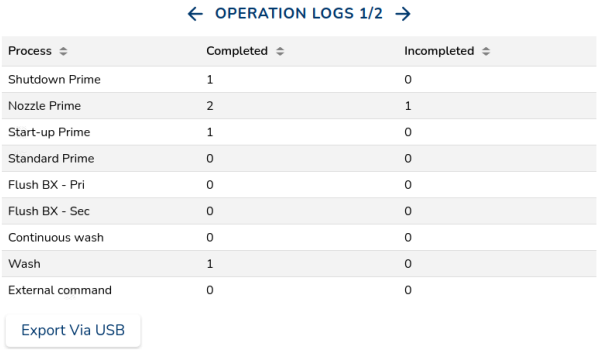
  NOTE: If the spill tray was previously filled with wash buffer, this function can be used to fill the spill tray with water or ethanol for the purpose of cleaning it. The water/ethanol can subsequently be drained off the spill tray using **[Drain Spill Tray 10 seconds]** *or* **[Drain Spill Tray].**

- **[BX Configuration]**
  This tab is to enter the page to inform the washer of the Buffer Exchanger configuration. Certain process & option in the service tab may change according to the configuration selection.

  IMPORTANT: *If the HT2000 is connected to a buffer exchanger, do not set the BX Configuration selection as* **[None]**. *A* **[None]** *selection will dictate that none of the BX channel is selected, thereby cutting any fluid flow path to the washer when the washer is in operation. This situation can cause extreme stress on the fluidic head on the washer, resulting in irreversible damage.*

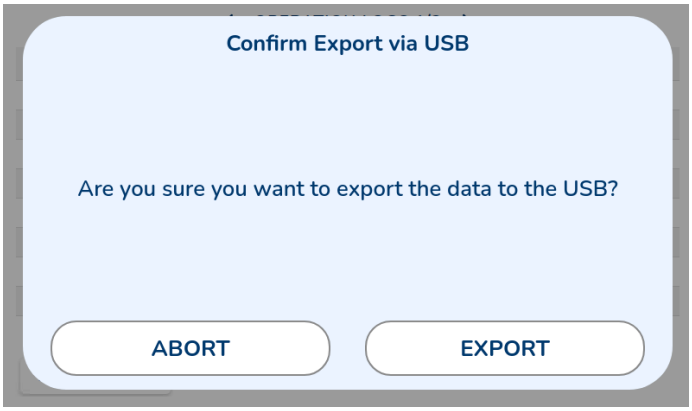
  IMPORTANT: *For the same reason as above, the appropriate cable must be inserted into the correct electrical ports that link the HT2000 washer to the BX, to ensure the appropriate channel is activated. Otherwise, damage to the washer head may occur during operation.*

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

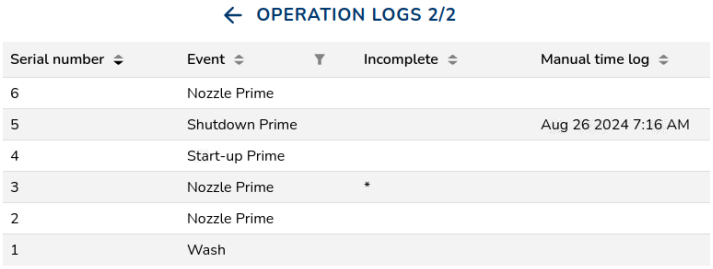


Column	Descriptions
Process	Name of the fluidic function
Completed	Displays the total number of times the function has been successfully executed without interruption
Incompleted	Displays the total number of times the function failed to execute due to an operation stop or machine fault

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



IMPORTANT: *Recommended to use NTFS format for USB pen drive.*



Column	Descriptions
Serial Number	Sequential number generated by system.
Event	Name of the fluidic function
Incomplete	Marked as * if the function fails to execute
Manual time Log	Date and time recorded by operator during the Shutdown Sequence. (This time log is only applicable to the Shutdown Sequence function)

- **[Change BX Channel]**
  Make an immediate switch to the selected channel on the BX.

  NOTE: **[Change BX Channel]** *is not the same as* **[Change Wash Buffer]. [Change Wash Buffer]** *will activate a channel switch, followed by a* **[Standard Prime]** *to ready the washer with new wash buffer.* **[Change BX Channel]** *will only switch the channel, but does not prime the washer.*

Calibration

It is recommended to perform volume calibration of the machine during new machine installation quarterly and after long term storage. Refer to page 28 to calibrate the machine.

Daily Startup Procedure

It is mandatory to perform priming of the machine and volume verification at the start of the day's operation.

1. Ensure ethanol / Tween (1% Tween 20 in 70% Ethanol) is connected to the machine.
2. Place a Priming Plate onto the feeder platform.

Figure 4-1: *Insert Priming Plate into machine (in* **[SERVICE MODE]***)*

3. Under **[OPERATION MODE]**, select **[Standard Prime]**to prime the washer with ethanol / Tween.

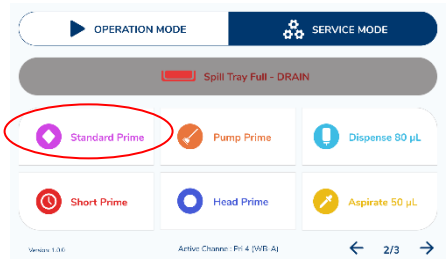


Figure 4-2: **[Standard Prime]** *in* **[SERVICE MODE]** *(GUI V1.0.0.7T)*

4. HT2000 completes the prime with ethanol / Tween when the feeder platform extends.
5. Repeat steps 1 to 4, replacing fluid with DI / Tween (1% Tween 20 in DI water).
6. Repeat steps 1 to 4, replacing fluid with wash buffer.

IF YOUR HT2000 WASHER HAS GUI VERSION 2.0.3, FOLLOW AS STATED BELOW.

1. Under **[OPERATION MODE]**, select **[Start Up Prime]** to initiate the package function that will guide User to prime the washer with 1% Tween 20 in 70% Ethanol, 1% Tween 20 in DI water, followed by wash buffer if selected.

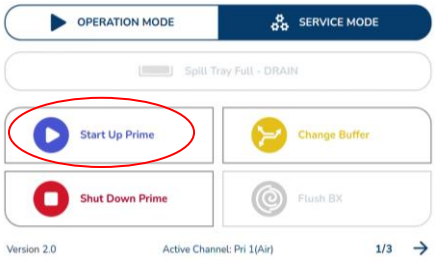


Figure 4-3: **[Start Up Prime]** *in* **[SERVICE MODE]** *(GUI Version 2.0.3)*

2. Follow the on-screen instructions to ensure sufficient priming solution volume and connect the priming solutions accordingly.

Volume Verification Procedure

1. While priming the HT2000 with 1% Tween 20 in DI water, obtain and record the mass of an empty Calibration Plate using a mass balance.
2. After priming the washer with 1% Tween 20 in DI water, replace the Priming Plate with Calibration Plate.
3. Select **[Dispense 80µL]** to dispense 80µL 1% Tween 20 in DI water into all wells.

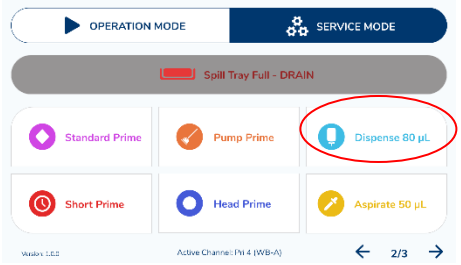


Figure 4-4: *Dispense 80µL (in* **[Service Mode]***)*

4. Carefully remove the Calibration Plate from the feeder platform.
5. Visually observe that all wells are dispensed evenly and obtain the mass of the filled Calibration Plate on a calibrated balance.
6. Select **[OPERATION MODE]**.
7. Input the number of washes value as “1”, the initial volume value as “80” and flow rate as “5” into the system by using the “+” or “-” symbols.

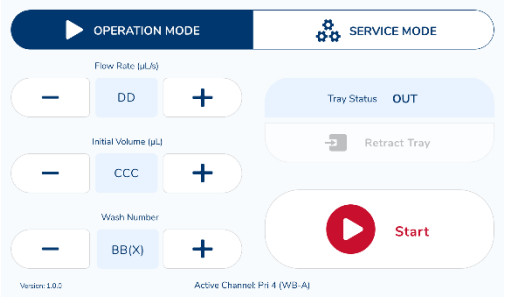


Figure 4-5: *Input parameters (in* **[Operation Mode]***)*

8. Press the **[Start]** button to begin the wash cycle.
9. Upon completion of wash, the feeder platform will extend. Remove Calibration Plate from the feeder platform.
10. Visually observe that residual volumes on all wells are even and obtain the mass of the filled Calibration Plate on a calibrated balance.
11. Refer to 'Maintenance & Troubleshooting' Section if there are any dispensing/aspirating problems.
12. The machine is now ready for operation.

Daily Shutdown Procedure

It is mandatory to perform priming of the machine at the end of the day's operation. Follow the steps below to shut down the machine.

1. Ensure the power jack is connected. Power up the HT2000 and wait for the homing sequence to complete.
2. Select **[SERVICE MODE].**
3. Ensure the two delivery tubes are properly connected to their respective ports (red and white) on the HT2000. 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.
4. Ensure that each bottle is filled with the appropriate fluid, connected to the buffer inlet bottle cap, and properly installed into the machine, as per "Bottle Cap System Installation" on page 24.

IF YOUR HT2000 WASHER HAS GUI VERSION 1.0.0.7T, FOLLOW AS STATED BELOW.
NOTE: Video illustrating ethanol shutdown protocol available at https://youtu.be/xa9UN_WBSBM.

IMPORTANT*:* If Buffer Exchanger (BX) cleaning is scheduled, perform **[Flush BX]** first using 4-to-1 connectors (flush BX with DI/Tween[1], followed by Ethanol/Tween[2], and Air). The BX is now clean.

Disconnect the BX from HT2000 and prime HT2000 manually for the remainder of the shutdown protocol.

1. Connect HT2000 inlet to DI/Tween[1] (manually or select **[Pri 2: water]** channel on BX if BX is in use).
2. Perform **[Standard Prime]** (**[SERVICE MODE]** page 2/3).
3. Connect HT2000 inlet to Ethanol/Tween[2] (manually or select **[Pri 3: ethanol]** channel on BX if BX is in use).
4. Perform **[Standard Prime]** (**[SERVICE MODE]** page 2/3).
5. With HT2000 inlet still connected to Ethanol/Tween[2], perform another **[Standard Prime]** (**[SERVICE MODE]** page 2/3). Lift the washer cover to observe the movement of the fluidic head. Following a period of pump prime where the ethanol is drawn into the reservoir, the dispensing block (top, see page 3) will commence 5 movements to dispense into the priming plate. This is followed by 7 movements of the aspirating block (middle) to aspirate from the priming plate. Do not allow the 7 movements to go to completion. Upon completion of third aspiration stroke, select **[STOP OPERATION]** on the GUI (Graphic User Interface).

NOTE: This prevents the Standard Prime from completing its programmed run which replaces ethanol with air in the aspiration nozzles.

6. **[Recover]** and remove plate, **[Retract]** the tray (**[OPERATION MODE]**).
7. Turn off HT2000 using the on/off rocker switch at the back of the instrument, *without performing Standard Prime with air.*

IMPORTANT*: Do not use the packaged* **[Shutdown Maintenance]** *function on HT2000.*

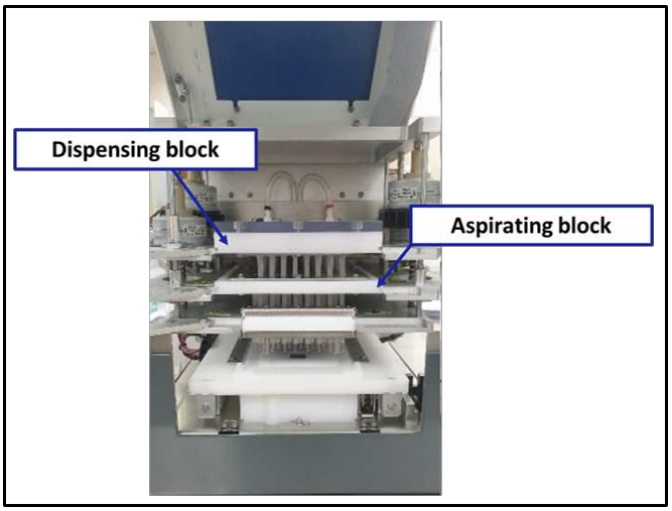
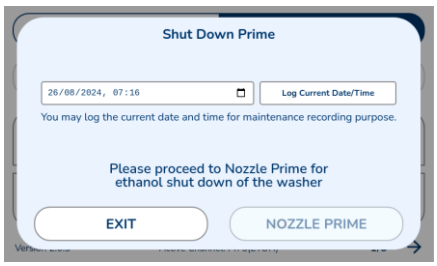


Figure 4-6: *Illustration of dispensing block and aspirating block placement in HT2000*

IF YOUR HT2000 WASHER HAS GUI Version 2.0.3, FOLLOW AS STATED BELOW.

IMPORTANT*:* If BX cleaning is scheduled, perform **[Flush BX]** using 4-to-1 connectors (flush BX with DI/Tween[1], followed by Ethanol/Tween[2], and Air). The BX is now clean. Disconnect the BX from HT2000 and prime HT2000 using the packaged **[Shutdown Maintenance]** function for the remainder of the shutdown protocol.

1. Select **[Shutdown Maintenance]** (**[SERVICE MODE]** page 1/3). Press **[Begin]**.
2. If BX is in use, the packaged program will complete the shutdown to leave the washer primed with Ethanol/Tween.
3. If BX is not in use, follow the prompting on the GUI to complete shutdown.
    a. Connect HT2000 inlet to DI/Tween[1] manually.
    b. Select **[OK]** to perform DI/Tween prime.
    c. At the end of the prime, connect HT2000 inlet to Ethanol/Tween[2] manually.
    d. Select **[OK]** to perform Ethanol/Tween prime.
    e. When Ethanol/Tween prime is completed, follow the prompt on the screen, set date and time follow the current date and time and press button “Log Current Date/Time” to manual log date and time for Shutdown prime function.



    Press Nozzle Prime button to perform **[Nozzle Prime]** with Ethanol/Tween.
    f. **[Recover]** and remove plate, **[Retract]** the tray (**[OPERATION MODE]**).
    g. Turn off HT2000 using the on/off switch at the back of the instrument, *without performing* **[Standard Prime]** *with air.*

Preventive Maintenance Procedure

If the product is intended to be unused for extended periods of time (≥ 7 days), preventive maintenance shall be carried out on the machine. The preventive maintenance procedure should be performed **once a week**, as follows.

IF YOUR HT2000 WASHER HAS GUI VERSION 1.0.0.7T, FOLLOW AS STATED BELOW.

HT2000 fluidic head is currently primed with Ethanol/Tween[2] following the last Shutdown Prime.

1. Connect HT2000 inlet directly to Ethanol/Tween[2] (manually or select **[Pri 3: ethanol]** channel on BX if BX is in use).

2. Perform **[Standard Prime]** (**[SERVICE MODE]** page 2/3). Lift the washer cover to observe the movement of the fluidic head. Following a period of pump prime where the ethanol is drawn into the reservoir, the dispensing block (top) will commence 5 movements to dispense into the priming plate. This is followed by 7 movements of the aspirating block (middle) to aspirate from the priming plate. Do not allow the 7 movements to go to completion. Upon completion of third aspiration stroke, select **[STOP OPERATION]** on the GUI (Graphic User Interface).

   NOTE: This prevents the Standard Prime from completing its programmed run which replaces ethanol with air in the aspiration nozzles.

3. **[Recover]** and remove plate, **[Retract]** the tray (**[OPERATION MODE]**).
4. Turn off HT2000 using the on/off switch at the back of the instrument, *without performing Standard Prime with air*

   IMPORTANT*: Do not use the packaged* **[Shutdown Maintenance]** *function on* GUI v1.0.0.7T.

   IMPORTANT*:* Recommended to perform *Preventive Maintenance Procedure* on a weekly basis to keep nozzles soaked in Ethanol/Tween. Failure to do so may result in excessive Tween-20 build-up in the nozzles.

IF YOUR HT2000 WASHER HAS GUI Version 2.0.3, FOLLOW AS STATED BELOW.

HT2000 fluidic head is currently primed with Ethanol/Tween[2].

1. Connect HT2000 inlet directly to Ethanol/Tween[2] (manually or select **[Pri 3: ethanol]** channel on BX if BX is in use).
2. Perform **[Nozzle Prime]** (**[SERVICE MODE]** page 2/3).
3. **[Recover]** and remove plate, **[Retract]** the tray (**[OPERATION MODE]**).
4. Turn off HT2000 using the on/off switch at the back of the instrument, *without performing Standard Prime with air.*

   IMPORTANT*:* Recommended to perform *Preventive Maintenance Procedure* on a weekly basis to keep nozzles soaked in Ethanol/Tween. Failure to do so may result in excessive Tween-20 build-up in the nozzles.

Fluidics Decontamination Protocol

Perform deep decontamination of washer fluidics regularly to ensure functioning of fluidic channels. If the samples used on the washer are prone to clumping and aggregation, it is recommended to perform deep contamination with bleach.

Estimated buffer consumption:

- 1% Bleach – 190mL to prime, 80mL for 10min perpetual wash

CAUTION: *Do not use bleach concentration greater than 1%. Most household bleach contains 5-9% sodium hypochlorite.*

- DI/Tween[1] – 190mL to prime, 160mL for 20min perpetual wash

A. Bleach prime and wash

1. Connect HT2000 to inlet bottle containing 1% bleach.
2. Perform **[Standard Prime]** (**[SERVICE MODE]** page 2/3).
3. With HT2000 inlet still connected to 1% bleach, select **[Perpetual Wash]** (**[SERVICE MODE]** page 3/3). Set a timer to run continuously with bleach for exactly 10min.
4. Empty the waste containing bleach.

CAUTION: *Do not mix bleach and alcohols as the reaction produces chloroform, which is highly toxic.*

B. DI/Tween[1] prime and rinse

5. Connect HT2000 to bottle containing DI/Tween[1] (manually or select **[Pri 2: water]** channel if a buffer exchanger is installed).
6. Perform **[Standard Prime]** (**[SERVICE MODE]** page 2/3).
7. With HT2000 inlet still connected to DI/Tween[1], select **[Perpetual Wash]** (**[SERVICE MODE]** page 3/3). Set a timer to run continuously with DI/Tween[1] for a minimum of 20min.
8. Continue with shutdown procedures with Ethanol/Tween[2] (see next section).

Long Term Dry Storage Procedure

Procedure for the storing of washer station for period longer than 1 month

If the washer station is expected to be left unused for protracted period (one month or more), it is recommended to perform a bleach decontamination of the unit prior to stowing it.

Additional solutions required for bleach decontamination (above standard reagent and volumes required for washer Start Up and Shutdown):

- 1% bleach – 190mL to prime, 100mL for 10min perpetual wash

CAUTION: *Do not use bleach concentration greater than 1%. Most household bleach contains 5-9% sodium hypochlorite.*

- DI water/Tween[2] – 180mL to prime, 200mL for 20min perpetual wash

A. Bleach prime and wash

1. Connect HT2000 to inlet bottle containing 1% bleach.
2. Perform **[Standard Prime]** (**[SERVICE MODE]** page 2/3).
3. With HT2000 inlet still connected to 1% bleach, select **[Perpetual Wash]** (**[SERVICE MODE]** page 3/3). Set a timer to run continuously with bleach for exactly 10min.

B. DI/Tween^2 prime and rinse

1. Connect HT2000 washer station to bottle containing DI/Tween^2 (manually or select **[Pri 2: Water]** channel if a buffer exchanger is installed).
2. Perform **[Standard Prime]** (**[OPERATION MODE]** page 2/3).
3. With HT2000 inlet still connected to DI/Tween^2, select **[Perpetual Wash]** (**[SERVICE MODE]** page 3/3). Set a timer to run continuously with DI/Tween^2 for a minimum of 20min.
4. Empty the waste containing bleach.

CAUTION: *Do not mix bleach and alcohols as the reaction produces chloroform, which is highly toxic.*

IMPORTANT*:* If an installed BX is intended for stowing, perform **[Flush BX]** first using 4-to-1 connectors (flush BX with DI/Tween^1, followed by Ethanol/Tween^2, and then Air). The BX is now clean and dry. Disconnect the BX from HT2000 and prime HT2000 manually for the remainder of the shutdown protocol.

C. Ethanol/Tween^1 prime

1. Connect HT2000 washer station to bottle containing Ethanol/Tween^1 manually or select **[Pri 3: EtOH]** channel if a buffer exchanger is installed).
2. Perform **[Standard Prime]** (**[SERVICE MODE]** page 2/3).

D. Air prime

1. Disconnect the inlet tubing to the HT2000 washer station or select **[Pri 1: Air]** channel if a buffer exchanger is installed).
2. Perform **[Standard Prime]** (**[SERVICE MODE]** page 2/3).
3. The HT2000 washing station is now decontaminated, dry and ready for storage.

Error Codes

The HT2000 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 HT2000.

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 HT2000 runs smoothly and efficiently.

Action	Daily	Weekly	Monthly	As Required
Perform priming of the machine with 1% Tween 20 in 70% Ethanol, followed by 1% Tween 20 in DI water and finally with Wash Assay Buffer at the start of the day's operation.	✓			
Perform priming of the machine with 1% Tween 20 in DI water, followed by 1% Tween 20 in 70% Ethanol and finally without fluid at the end of the day's operation.	✓			
Wipe all surfaces of the unit enclosure with a cloth moistened with 70% EtOH	✓			✓
Wash Filter under running DI water on a weekly basis.		✓		
Fluidic decontamination run for cleaning of internal fluidics: **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 components.**				✓
It is recommended to perform volume calibration during new machine installation, quarterly and after storage for long period. Refer to "Calibration" on page 28.				✓
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 HT2000 is not plugged into electricity supply, or wrong power adaptor is used.

- Ensure the jack is properly connected to the HT2000, 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.

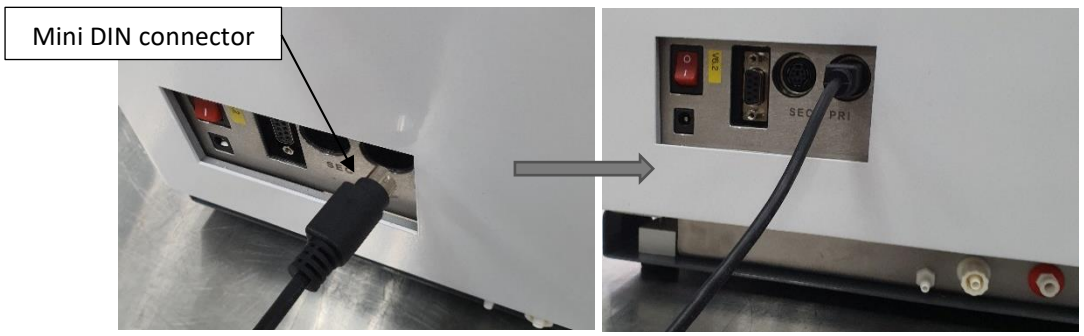


Figure 5-1: *Connecting Mini DIN connector between HT2000 and Buffer Exchanger*

- 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.

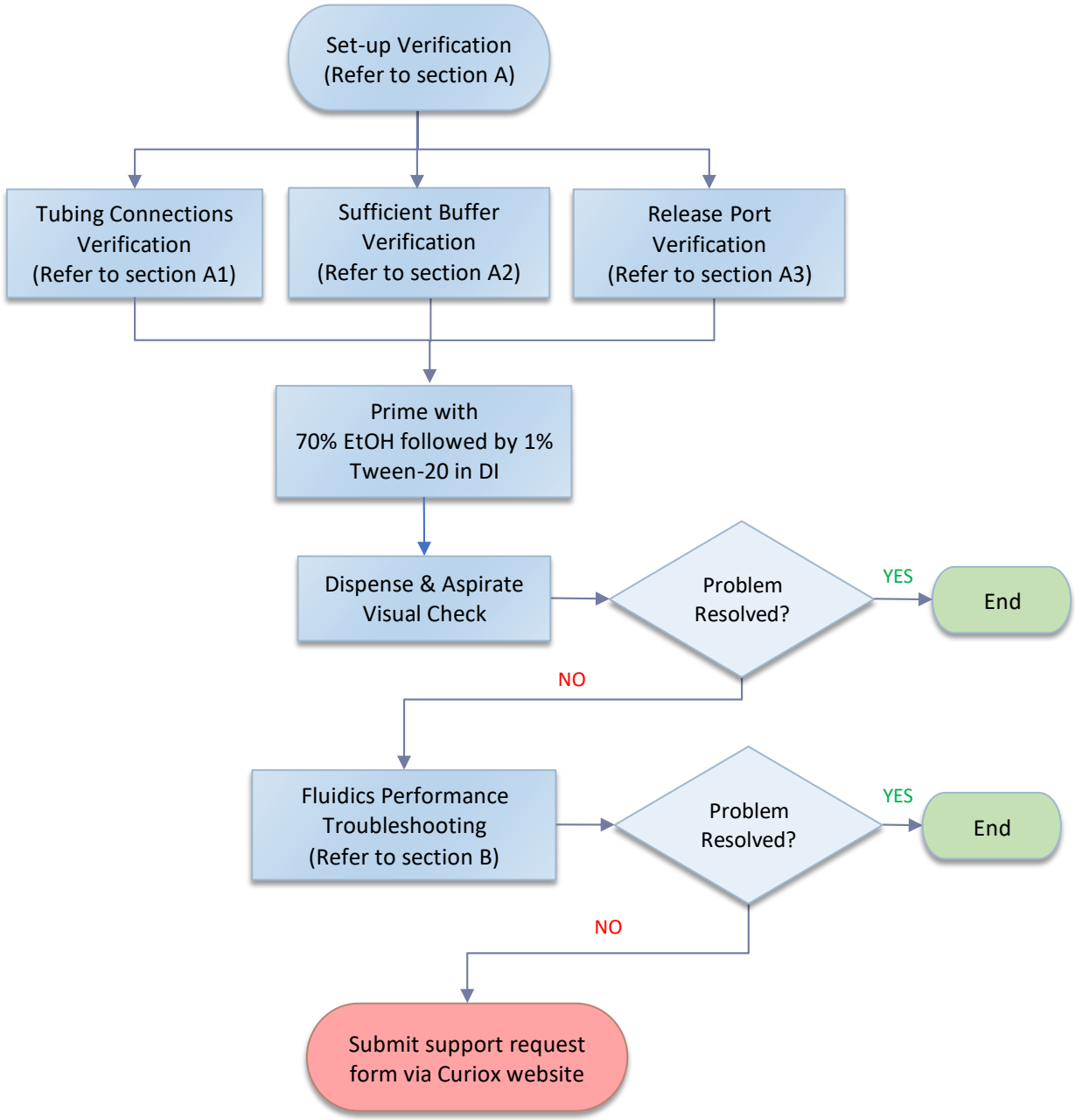


Figure 5-2: *Overall flow chart on troubleshooting HT2000*

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.

Figure 5-3: *Release port on bottle cap*

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

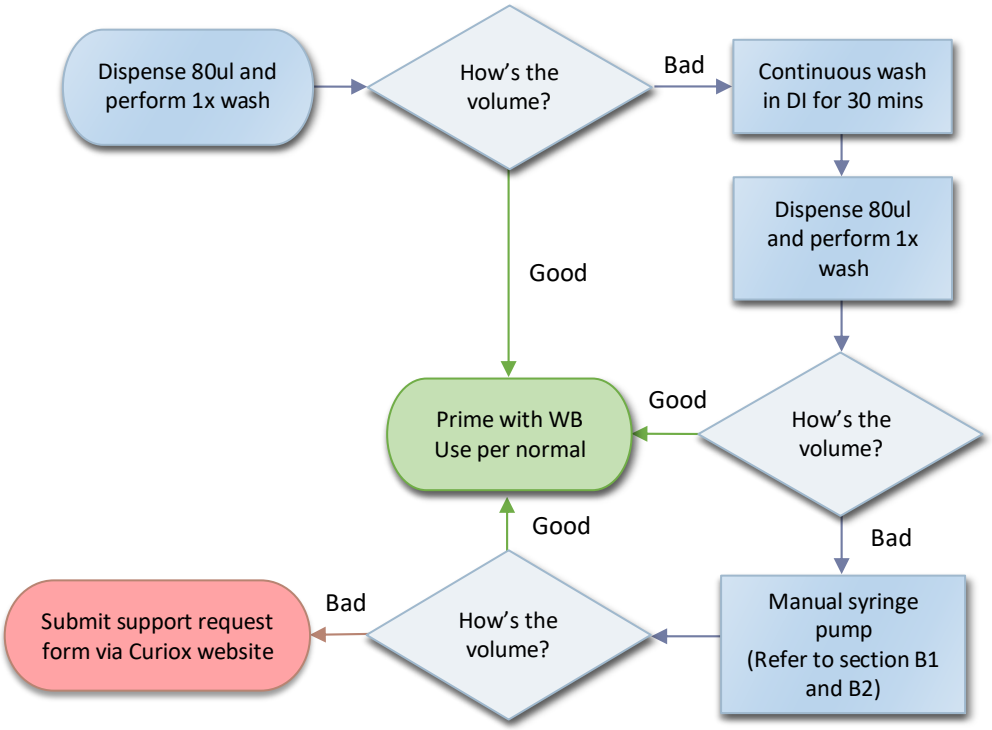


Figure 5-4: *Flowchart to troubleshoot fluidics performance*

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

B1.1. Plate mapping:

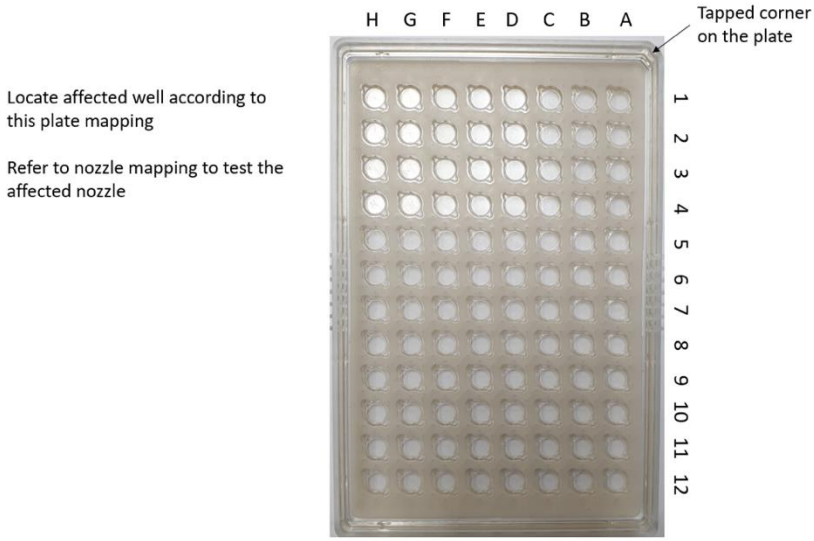


Figure 5-5: *Plate map of LW96 plate*

B1.2. Nozzle mapping:

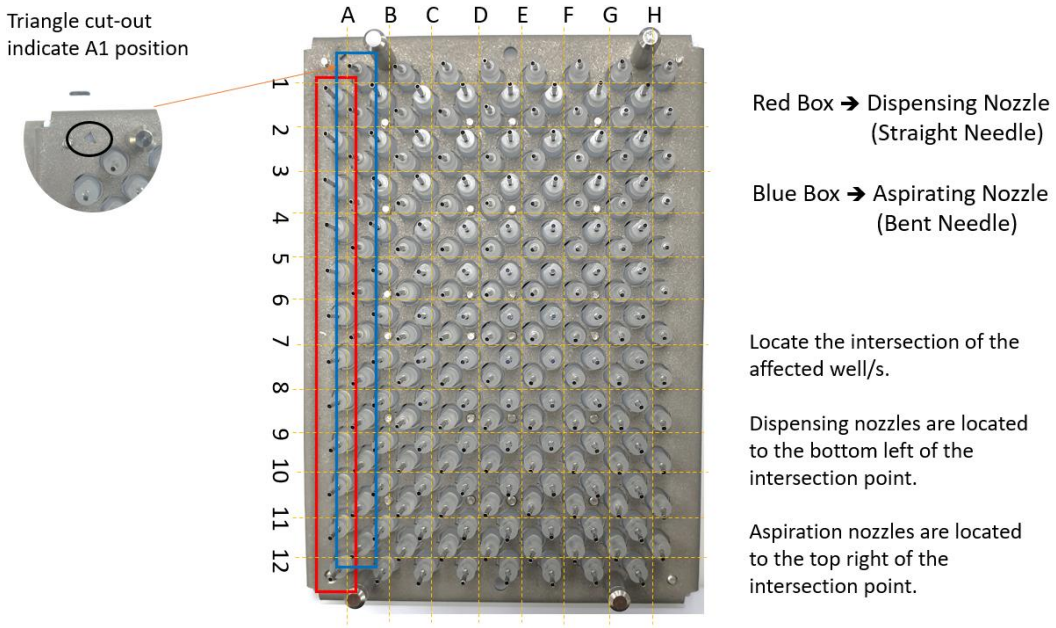


Figure 5-4: *Map of nozzles showing corresponding plate well positions*

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.

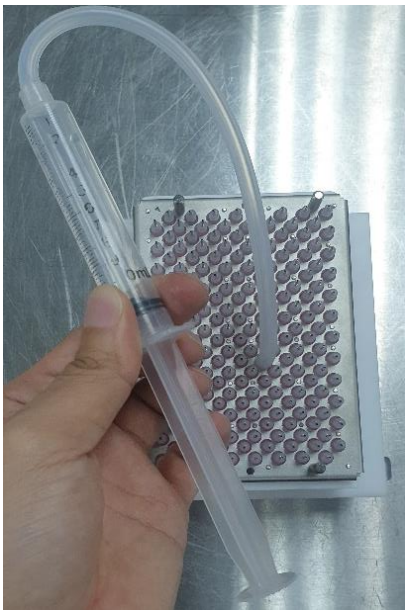


Figure 5-4: *Manual syringe resetting of valve membrane*

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 Perform 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 HT2000 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.

1. Remove all biological contents from the HT2000. Turn off and unplug the HT2000 from the power supply and disconnect the power jack.
2. Wearing gloves, clean all surfaces of the HT2000 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.
3. Moisten (do not soak) a cloth with 70-85% EtOH solution. Wipe all surfaces of the HT2000 enclosure. Wait for 20 minutes.
4. Ensure all used cloths are disposed of in a bio- hazard bag/container.
5. **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 HT2000 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™ HT2000? If so, please let us know below

End of Document

Revision History

Rev No	Date	Change Description	Author
10	31 MAY 2023	Addition of Ethanol Storage, Long Term Storage Procedure, use of sodium azide (Addendum_2 and Addendum_3). Addition of GUI version 2.0 operation instructions	Chyan Ying Ke
11	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