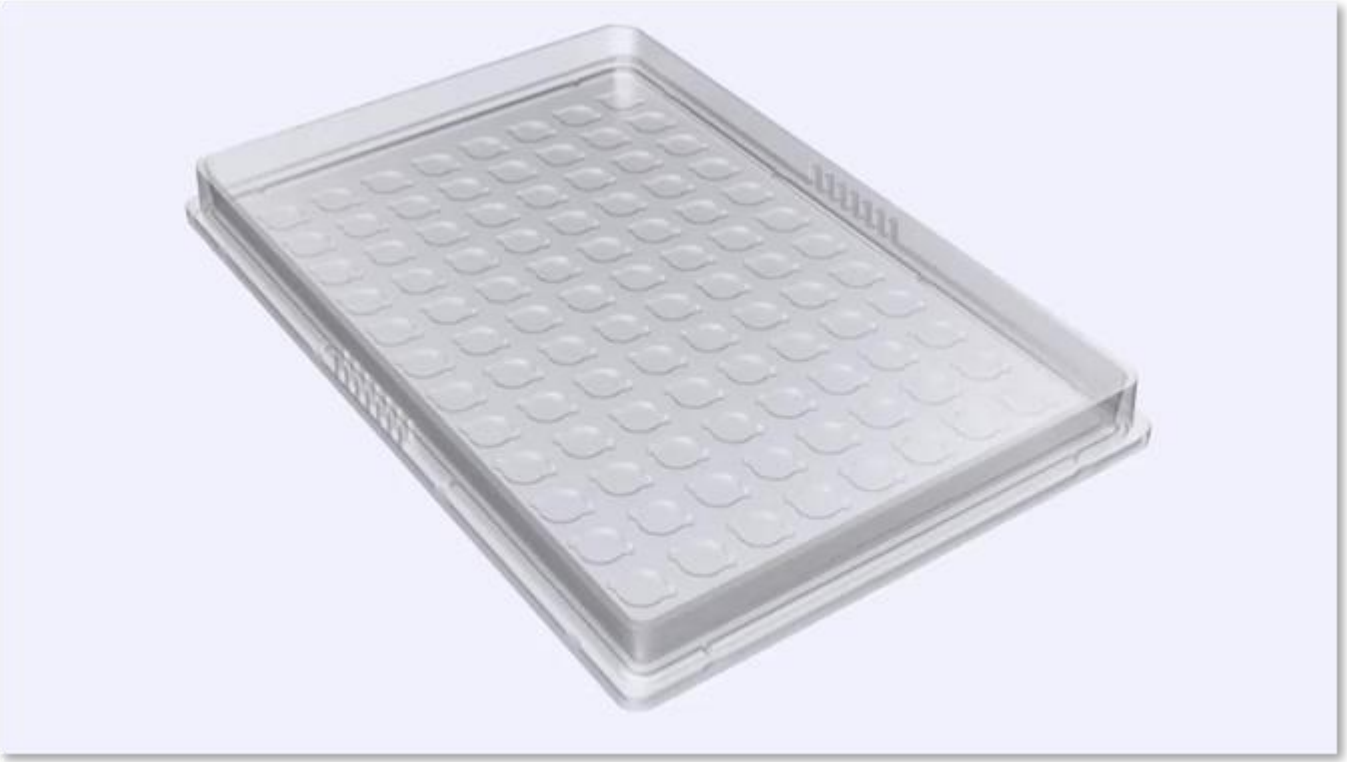
Congratulations on your purchase of a Laminar Wash™ HT2000!

How does Laminar Wash technology work?



Click on the icon
to watch video

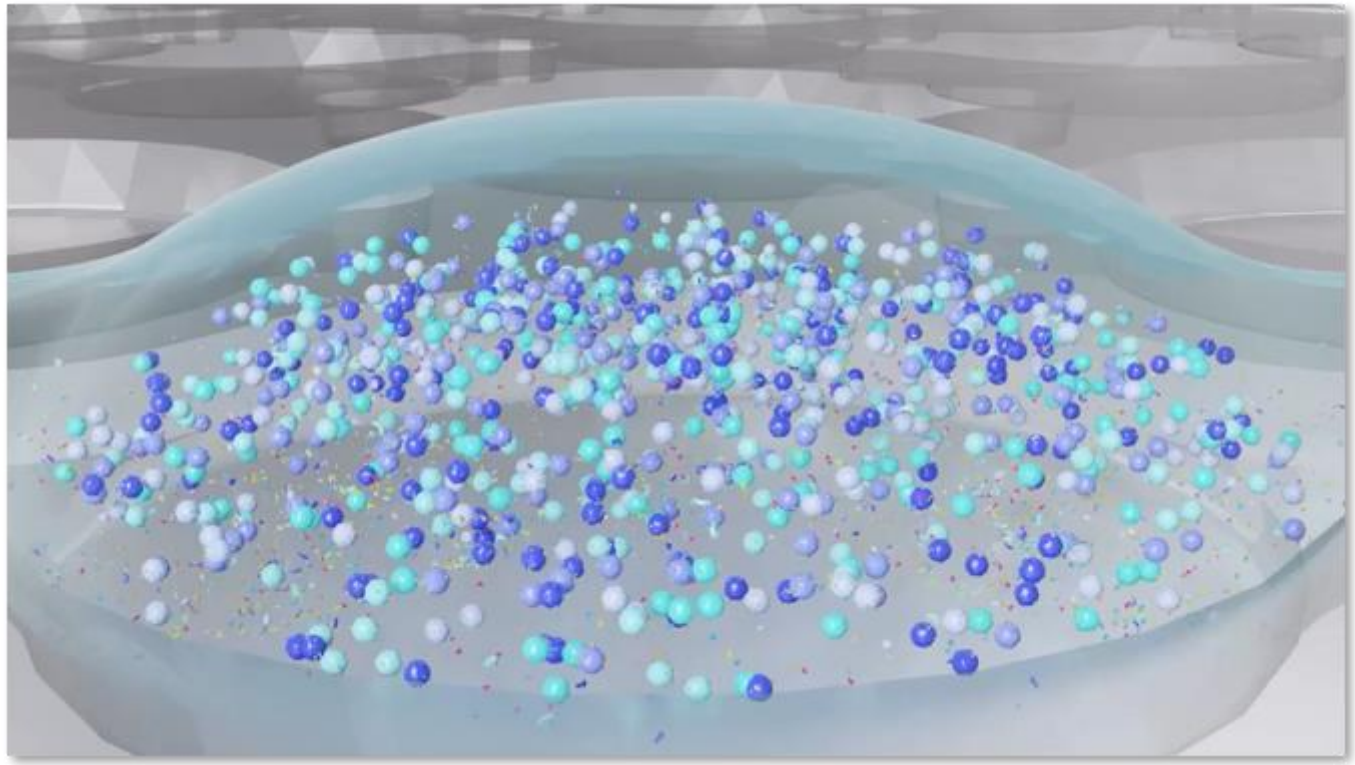
UNIQUE PLATE DESIGN

- Hydrophilic surface accommodates buffer and cells.
- Hydrophobic surface separates wells and enables droplet formation.
- Wells accommodate ≤80 µL (≤150 µL large volume adaptor).

CELLS GENTLY SETTLE BY GRAVITY

DOUBLE NOZZLES INITIATE LAMINAR FLOW

How does Laminar Wash technology work?



Click on the icon
to watch video

UNIQUE PLATE DESIGN

CELLS GENTLY SETTLE BY GRAVITY

- Physiology undisturbed by centrifugal forces.
- Cell settling and reagent binding are combined in a single step.
- Unbound reagent and cell debris diffuse into bulk buffer solution.

DOUBLE NOZZLES INITIATE LAMINAR FLOW

How does Laminar Wash technology work?

UNIQUE PLATE DESIGN

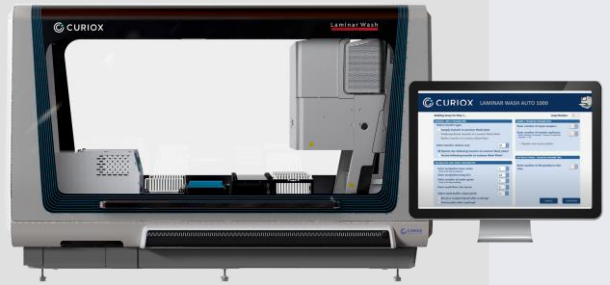
CELLS GENTLY SETTLE BY GRAVITY

DOUBLE NOZZLES INITIATE LAMINAR FLOW

- Customize flow rate, # of washes.
- Laminar flow velocity greatest at top, almost stationary around cells.
- Subsequent washes serially remove unbound reagent and debris.

Click on the icon to watch video

Laminar Wash system for every laboratory

	MINI1000 Low Throughput	HT2000 Customizable	AUTO1000 Turnkey
			
Laminar Wash technology	☑	☑	☑
Automated pipetting			☑
Fits in biosafety cabinet	☑	☑	
Touchscreen		☑	☑ +GUI
Number of wells	16 (1–8 simultaneous)	1–96	1–96
Max volume (µL)	80	150 (with adaptor)	150 (with adaptor)
Dimensions (cm)	23 x 28 x 18	51 x 25 x 30	137 x 28 x 89

What is possible using the Laminar Wash system?

What *projects* are applicable?

- HLA crossmatching
- Cellular phenotype identification
- Single-cell genomics/transcriptomics
- Antibody discovery
- Compound screening/profiling
- And more!

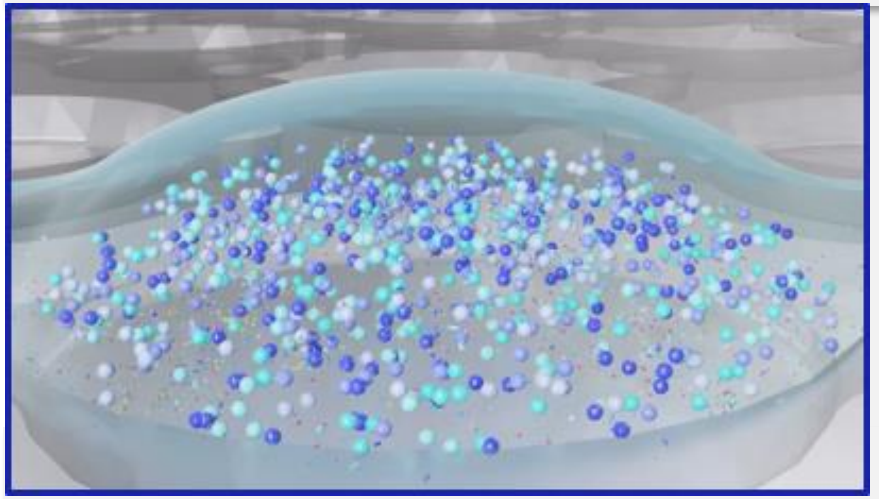
What *samples* are applicable?

- Anything with a density greater than that of the fluid medium
  - PBMCs
  - Lymphocytes, CAR-products, monocytes
  - Mammalian cell lines (CHO, HEK, etc.)
  - Bacteria
  - Nuclei
  - And more!

Basic Overview of Laminar Wash Use

General Procedure

1. Obtain desired cells and calculate cell number and viability
2. Concentrate samples to dispense up to $1x10^6$ per well in 25µL-75µL volumes
3. Add 25µL-75µL of sample to each well with the desired number of replicates
4. Allow 20-30 minutes for samples to settle to the bottom of the well



Samples settle via gravity due to their density relative the medium

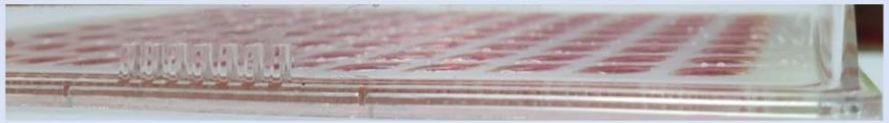
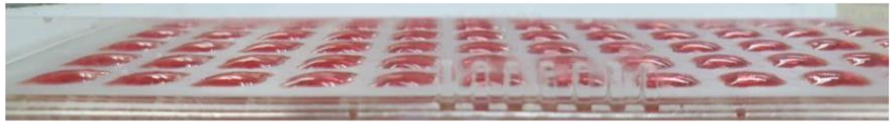
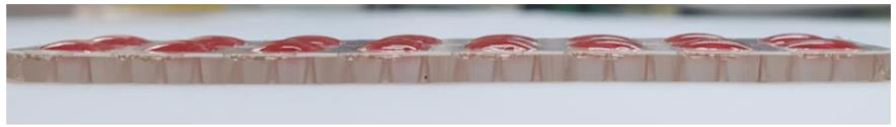
- Smaller volumes per well correlate with less time required to settle
- Samples with little more density relative to their fluid mediums (i.e., methanol fixed cells) will require additional settling time up to 1 hour

5. Set wash parameters using the HT2000's touchscreen.
6. Place the laminar wash plate on the instrument's plate feeder
7. Begin the wash using the HT2000's touchscreen
   - The entire wash step is automated by repeated dispensing and aspiration of wash buffer

Plate Handling

- Wells can accommodate up to 80µl/well. However, for experimental use we recommend not exceeding **75µl/well** to ensure easier handling.
- Plates must be incubated on a flat, non-vibrating surface.
- Plates can be shaken using the Big Bear Shaker if the samples volumes are ≤ 50µl.
- Plates can be incubated at 0˚C to 80˚C

Plate Handling Sectional View of Buffer Level on a Curiox Laminar Wash Plate

25 µL	Buffer level barely above the hydrophobic barrier 	Closer look when the wall was removed 
35 µL	Buffer level slightly bulged above the hydrophobic barrier 	Closer look when the wall was removed 
80 µL	Buffer level largely bulged above the hydrophobic barrier 	Closer look when the wall was removed 

Helpful Tips

To resuspend cells after wash:

- Use the Curiox Big Bear Shaker and/or pipette mixing to ensure sufficient sample mixing with antibodies/reagents

If samples are typically processed at cold temperatures:

- Set-up the laminar wash system in a cold room

-OR-

- Keep wash buffers on ice during the wash process.
- Place laminar wash plate in a refrigerator during incubations.

Compatibility with buffers and solutions:

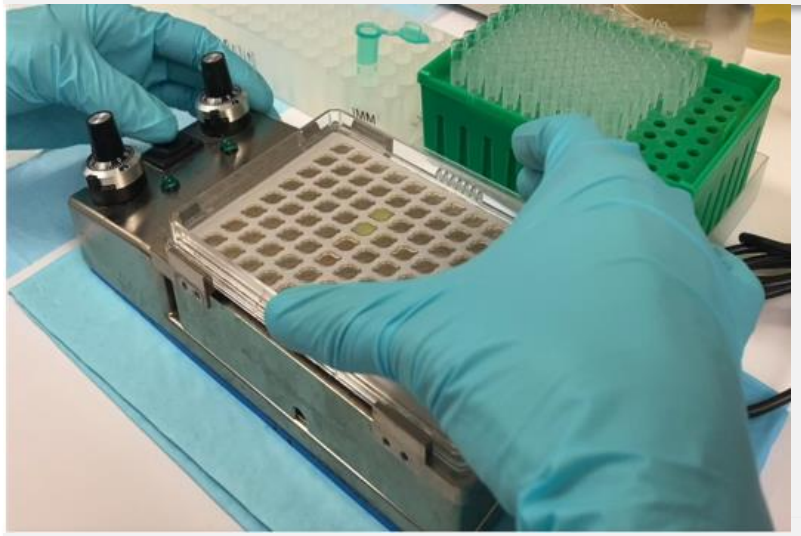
- The HT2000 fluidics are compatible with most reagents. For specific chemical compatibility consult the HT2000 User Manual or **CONTACT US**

Helpful Tips (continued)

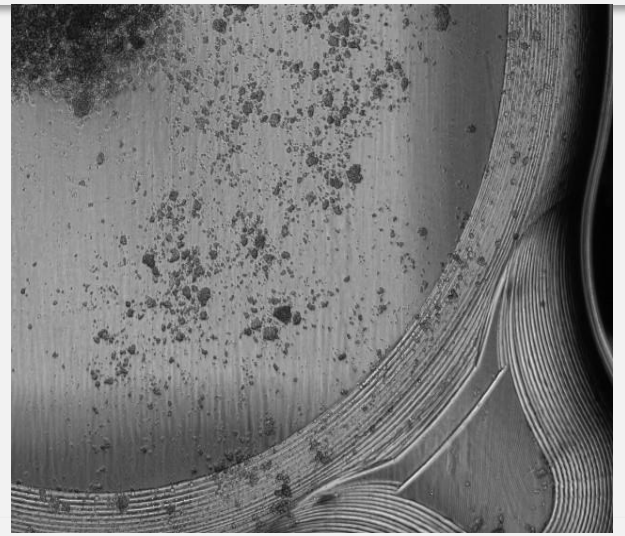
Post-Wash Sample Acquisition

There are 2 options for sample acquisition:

- Transfer the samples to a conventional plate or tube:



- Rinse wells 2-3 times with buffer, pipetting in a circular motion around the edges of the well



- Observe under a microscope if most of the cells have been remove. If not, repeat the rinsing process

- Directly acquire samples from the Laminar Wash Plate
  - Most high throughput systems on analytical equipment like flow cytometers can acquire directly from the Laminar Wash 96-well plates. Some might require extra accessories. **For suggested parameters for a specific cytometer please contact us**.

Caution!!!

Please keep in mind that:

- The HT2000 must be kept on a **flat surface (avoid vibration and strong air currents)** and in a lab environment with temperature (4 - 28 °C) and humidity conditions (< 80%, non-condensing).
- If the machine installation was carried out in a cold room, or moved into a cold room, it needs to be acclimatized in the cold room for at least 3 hours before operation

If you need to move the washer to a new location, **PLEASE CONTACT US** or send a **direct email** beforehand so we can assist you.

Please keep the bag with the accessories and tools

- They are not needed for everyday use. However, if repair is needed or the instrument is going to be moved, the Curiox field engineer or scientist will need to locate some of these tools.

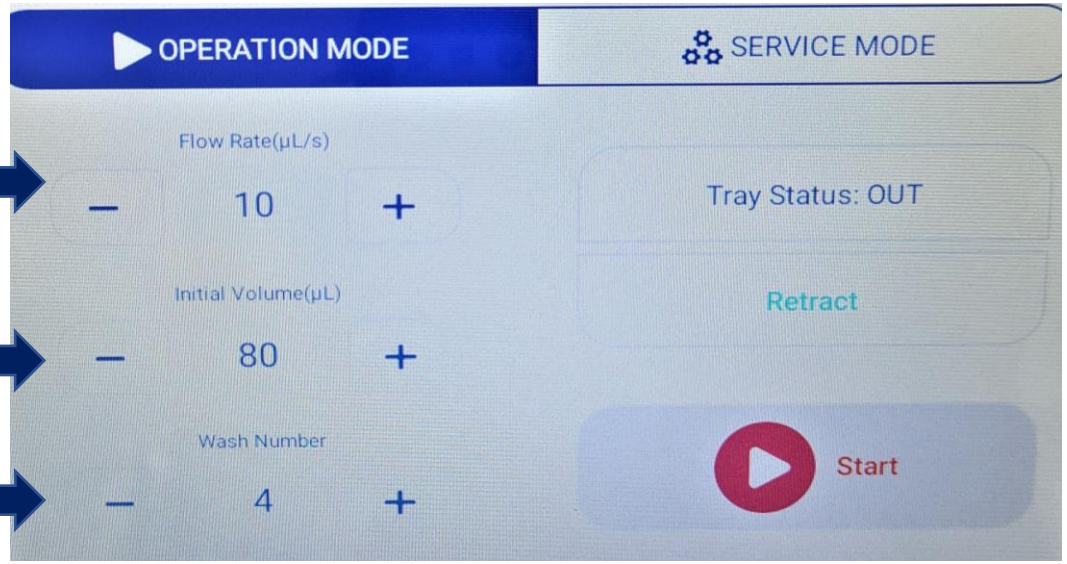
Plate storage

- Keep the boxes of plates facing up, in a lab environment with temperature (4 - 28 °C) and humidity conditions (< 80%, non-condensing).

Frequently Asked Questions - Wash Settings

What do the various wash settings mean?

- Flow rate is the velocity at which buffer is removed from the top of the droplet by the aspiration nozzle (5µl/s - 20µl/s)
- Initial wash volume is the volume per well on the laminar wash plate before a wash starts (1-100µl)
- Wash number is the total number of cycles the instrument will perform (1-50)



What settings are best for my application?

- Higher flow rates finish the job quicker, but result in slightly more sample loss than lower flow rates
- More wash cycles will increase the dilution factor, but will also take longer for the process to complete

How long does will a wash cycle take?

- At a flow rate of 10µL/sec, one wash cycle will finish in about 22 seconds, with higher or lower flow rates taking less or more time, respectively

Suggested Settings based on Application

Wash Number Input:

- If looking to only **exchange buffer within the well** of the Laminar Wash plate : **7 cycles**
- If washing during **flow cytometry (post antibody staining)** sample prep:
  - Post surface-stain (Normal 2x-3x wash) : **9 cycles**
  - Post intracellular-stain or secondary antibody (If more thorough wash is necessary) : **12 cycles**
- If washing **tumor samples for TIL analysis** or other **debris-laden samples**: **18 cycles**
- If washing during **post oligo-tagging during CITE-seq or similar sequencing** sample prep: **25 cycles**
- If washing for the purpose of **HLA Crossmatching**: **40 cycles**

❖ *These settings are intended to serve only as a guideline, actual assays may require additional optimization of wash settings*

Frequently Asked Questions (continued)

Are the plates tissue culture treated?

- The laminar wash plates are not tissue culture treated

Can plates be used for microscopy?

- Yes, laminar wash plates are compatible for light microscopy using a 20X or less objective
- If using plates fluorescent microscopy, some laser voltages may display some autofluorescence of the plates

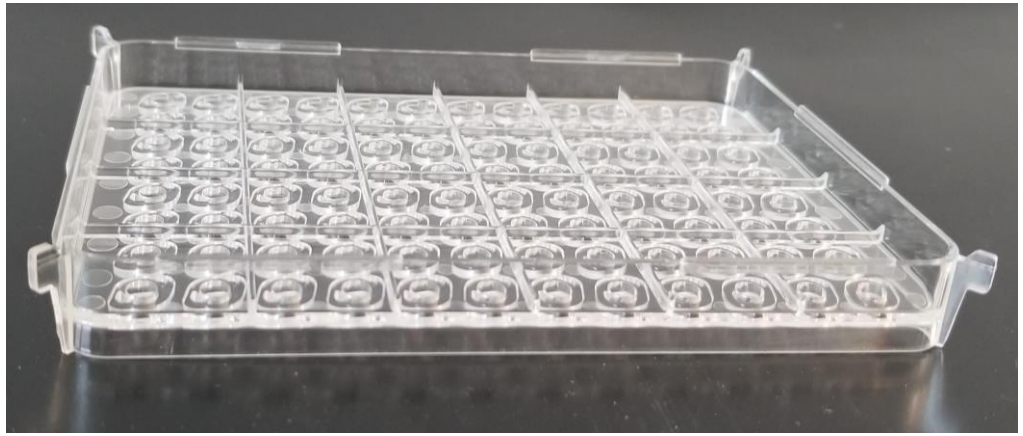
Are there any limitations to the sample type?

- Samples that are less dense than their fluid medium will not settle and will therefore be aspirated away
- Viscous samples may require dilution to decrease viscosity since very viscous samples are difficult to aspirate effectively

Frequently Asked Questions (continued)

Why is the plate volume limited to 80µL and is there a way to increase that volume?

- 80µL is the maximum volume because the surface tension at the top of the droplet becomes unstable at higher volumes and may result in cell merging.
- There is an adaptor called Large Volume Adaptor (LVA) to increase volumes



What is the Large Volume Adaptor?

- It increases droplet size up to 150µL
- It is best used for providing additional volumes of antibodies or reagents for incubation
- It must be removed before washing (with it will take some of the supernatant out, leaving ~80µL.)
- It is a consumable – should not be reused

HT2000Daily Operation – detailed instructions

Start-up procedure

- Power on the HT2000 and ensure all tubes are in the correct configuration and that there is at least 500ml of each maintenance solution.
- Switch to **Service Mode**, Connect the inlet tube to 70% Ethanol+1%Tween, place the dummy plate on the feeder and press **STANDARD PRIME** (page 2/3).
- Connect the inlet tube to DI+1%Tween, place the dummy plate on the feeder and press **STANDARD PRIME** (page 2/3).

Calibration

- Switch to **Service Mode**, In Service Mode, place the calibration plate on the plate feeder, and press **DISPENSE 80µl** (page 2/3). Visually check that volumes are even across the plate (see next page for details). Place the plate back in the plate feeder.
- In **Operation Mode**, set the parameters to: **Flow rate 10µl/s, Initial volume 80µl, Wash number 4**, and press START. Visually check that volumes are even across the plate (see next page for details).

Operation

- **Prime HT2000 with your buffer of choice**: Connect the inlet tube to your buffer of choice, place the dummy plate on the feeder and press **STANDARD PRIME** (page 2/3).
- **Set the right parameters for washing**: In **Operation Mode**, set the appropriate flow rate, wash volume and wash number for your assay. Place the assay plate on the feeder and press **START**.
- **Buffer change**: Connect the inlet tube to the new buffer, switch to **Service Mode**, place the dummy plate on the feeder and press **STANDARD PRIME** (page 2/3).

Shutdown procedure

- Switch to **Service Mode**, Connect the inlet tube to DI+1%Tween, place the dummy plate on the feeder and press **STANDARD PRIME** (page 2/3).
- Connect the inlet tube to 70% Ethanol +1%Tween, place the dummy plate on the feeder and press **STANDARD PRIME** (page 2/3).
- Disconnect the inlet tube (so air will come through), place the dummy plate on the feeder and press **STANDARD PRIME** (page 2/3).
- Power off the HT2000.

How to maintain the Laminar Wash instrument?

- Run the daily maintenance (start-up, calibration, buffer exchanger maintenance and shutdown) **every day that the machine is going to be in use** (Refer to "Daily Operation" Section)
- If the HT2000 isn't used regularly, please run the daily maintenance procedure (start up, calibration, and shutdown) **at least once every 2 weeks**.
- On a weekly basis, wash the filter under running DI H_2O

In case of a problem, e.g.,

- Uneven volumes during calibration or washing
- Possible clogging
- Bubbles on the inlet tubing or washing plate
- Need of decontamination of the instrument
- Problems with the plates or accessories
- Other

PLEASE CONTACT US or send a **direct email** beforehand so we can assist you.

Need Assistance?