**LSR II HTS Users Guide**

**Stanford Shared FACS Facility**

**Samples**
- Minimum of 50μl sample per well
- Compatible with V-, U-, Flat bottom plates
- Unstained and compensation samples should be in tubes

**Before Running Your Plate**
- Start your experiment as usual with tubes running the 197 beads
- Set the **Window Extension to 10**
- Set up all your settings/voltages using TUBES, record the tubes, and calculating any necessary compensation.
- Put the LSRII on **STANDBY**

**Install the Plate Reader**
1. Remove any tubes from the SIP
2. Put plate reader metal mounting plate into connecting slot, not all the way yet
3. Connect the sheath/waste lines
4. Connect the sample tube
5. Connect the power and data cables
   (be careful, power cable has electricity on, don’t touch metal surfaces!!!)
6. Switch on the **power**
7. Switch the machine from tubes to plates. This switch is on the front upper right corner of the LSRII.
8. Push plate reader/mount all the way in so that it is safely connected
9. Put LSRII in **RUN** (lo/med/hi doesn’t matter)

**Setting up Plates and Running Samples**
1. Go **HTS→Prime**
2. Click new plate button and the arrow next to it to choose the plate type.
3. Use the plate window to select the throughput mode. (Standard mode is recommended) This is also where you can adjust the loader setting for the wells.
4. Highlight wells that you would like to run and click ‘add specimen’:
   a. Setup Controls (Not recommended)
   b. Specimen (samples)

5. Highlight the assigned wells or groups and assign the proper loader settings.

6. Ensure that each well contains sufficient sample for the entered volume plus dead volume.
   **Not having enough volume or having a mixing volume that is larger than the available volume will introduce air bubbles intro the sample and will result in poor, fluctuating or no signals.**

**Plate Loader Settings Information**
*Make sure you are choosing the right settings for your cells.*

<table>
<thead>
<tr>
<th>Setting</th>
<th>High-Throughput Mode</th>
<th>Standard</th>
<th>User defined (Ranges)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Throughput Mode</td>
<td>High</td>
<td>Standard</td>
</tr>
<tr>
<td>Sample Flow Rate (µl/sec)</td>
<td>1</td>
<td>1</td>
<td>0.5 – 3.0</td>
</tr>
<tr>
<td>Sample Volume (µl)</td>
<td>2-10 (+20µl)</td>
<td>2-200 (+20µl)</td>
<td>2 – 200µl (+20µl dead vol.) (limited by max. capacity of well)</td>
</tr>
<tr>
<td>Mixing Volume (µl)</td>
<td>50</td>
<td>100</td>
<td>Recommend ≤ ½ the volume contained in well</td>
</tr>
<tr>
<td>Mixing Speed (µl/sec)</td>
<td>200</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Number of Mixes (cycles)</td>
<td>2</td>
<td>2</td>
<td>1 – 5 (recommend ≥3)</td>
</tr>
<tr>
<td>Wash Volume (µl)</td>
<td>200</td>
<td>400</td>
<td>200 – 800 (recommend ≥400)</td>
</tr>
<tr>
<td>Approx. Acquisition Time (mins)</td>
<td>15</td>
<td>44</td>
<td>Depends entirely on settings chosen</td>
</tr>
</tbody>
</table>

*You may choose to Enable BLR. This will cut the first 5 seconds of flow off. This is useful since it normally takes time for the flow to become consistent on the HTS.*

**Running and Recording your plate**
1. Open the HTS lid and place your plate (A1 direction is marked), close HTS lid
2. Go to Experiment  \rightarrow Experiment layout  \rightarrow Click on the **Acquisition Tab**
3. Set the number events to record you would like to record.
   **The machine will record either your sample volume or your desired event number and stop at whatever comes first (ex: to run 150 µl, set it up to 10^6 per well)**
4. Close experiment layout window
5. Select **RUN PLATE** in acquisition dashboard
6. Make sure you are monitoring the **TIME** parameter
7. When plate is done, Diva will tell you, click ok

**Cleaning and Disconnecting the HTS**
1. Export/check-in your DATA.
2. Remove the HTS lid and your plate
3. Disconnect the HTS sheath line (white connector)
4. Connect the water line to the HTS sheath port (single white male connector, open on one end)
5. Place the open end of the water line into a beaker of ddH2O
   a. This will replace the sheath with water during the clean cycle
   b. Make sure to use water from the 20L carboy rather than from the sink.
6. Set up and run the **clean plate**. This can be done through **HTS > Clean**.
   a. A1-A4 should have 250uL of **10% BLEACH**
   b. B1-B4 should have 250ul of **WATER**
   c. The cleaning takes about 15min, and tells you when it’s done
7. Remove the cleaning plate, rinse with super hot water from sink
8. Switch **OFF** the HTS power
9. **Switch** the machine from plates to **TUBES**.
10. Put the LSRII in **STANDBY**
11. Pull out HTS a little so that you can access all connecting cables
12. **REMOVE** all connections in reverse order (again, be careful with power cable!)
13. Take out the HTS completely; put it back on cart
14. Put the lid back on and the dust cover

---

**Don’t Forget to Export and Check-IN your data**
Basic Troubleshooting

Always tell the FACS Facility if there are ever any problems with the analyzer, even if these steps resolve it.

The HTS is clogged!
1. Stop the plate.
2. Remove your plate from the HTS.
3. Leaving everything connected run the clean plate program. This can be done through HTS\rightarrow Clean.
   a. A1-A4 should have 250\mu L of 10\% BLEACH
   b. B1-B4 should have 250\mu L of WATER
4. Try rerunning 197 beads in a clean plate. If that still doesn’t work continue with the following steps
5. Disconnect the HTS, and check the 197 beads at the tube level:
   a. If the beads don’t look good then there is a clog in the SIP follow the declogging steps for the tubes.
   b. If the beads look fine, disconnect the HTS sample line from the cytometer and run the clean plate. Is cleaning sample coming out of the sample line?
      a. If yes, then reseat the sample line on the SIP and complete cleaning.
      b. If no, you will need to continue to run the clean plate program till the clog passes and you see drops coming out of the sample line. If no drops appear after a clean cycle, SSFF staff may need to change the clogged line – please ask for help.
6. Try the 197 beads again using the bead settings
7. If that still does not work, ask for help!
I FORGOT TO TAKE THE LID OFF THE PLATE!!!
1. Quickly lift the lid on the HTS, this will stop the probe immediately.

Rules

Data Rules
1. Do NOT use USB sticks, thumb drives, external hard drivers or any other form of USB with the computer.
2. Do NOT connect to the internet from this computer.
3. You MUST export your experiment.

Running the Machine Rules
4. You should FILTER your cells before running.
5. You MUST follow the cleaning procedure.
6. Leave the area neat and tidy.

Notes on Data
- Check in your data to protect it
- Data is deleted monthly. If it is not checked in, it is gone.
- USB and personal back up devices are not allowed on our machines.
- A .jo file for MAC (legacy Flowjo 9.75 and older) and a .wsp (version 10 both Mac and PC). Will be sent to you through the data archive once your data is checked in
- Currently we are recommending FlowJo version 10.4
- Start FlowJo and from the FileMenu select Open and then open your .wsp file.
- You will be prompted for Username: ‘flowjo’ Password: ‘314159’.