

<b>Bauer Core Standard Protocol</b>		
Title: Becton Dickinson LSR II/ LSR Fortessa		
Pages: 4	Revision: 1	Date: 03/24/2020
Author(s): Zachary Niziolek	Reviewers: Claire Reardon	
Contact: flowcore-list@fas.harvard.edu	Comment:	

## 1. Purpose

This protocol provides instructions for running samples on the BD LSR II and LSR Fortessa

## 2. Materials

Filtered samples

5mL polystyrene tubes or 96/384 round bottom plate

IsoFlow Sheath Fluid (Beckman Coulter #8546859)

Bleach

FACsRinse

Distilled Water

## PREPARING THE INSTRUMENT

1. Log on to computer with research computing account (account used to sign up for time on Spinal)
2. Turn on the Instrument by pressing green power button on the side.
3. Fill sheath tank with IsoFlow Sheath Fluid and empty waste in the sink, pouring 500mL bleach in the waste container after emptying.
  - a. Press “Restart” after changing fluidics
4. On the desktop find BD FACs Diva, double click to open. Login to the software with your username and password (typically first initial last name). The software will take a moment to connect to instrument. When it is completed the software will display a message reading “CST Mismatch” click the middle button “Use CST settings”.
5. Make a new dH<sub>2</sub>O tube by adding 3mL of water into polystyrene tube.
6. Remove tube of liquid (should be dH<sub>2</sub>O) from the sip of the instrument, while the lever is to the side, press “Prime” on the control panel
7. Once prime is finished replace with new dH<sub>2</sub>O tube, press “Run” to make sure the color of “Run” turns green
  - a. If it stays the yellow color, follow these steps
    - i. Check to ensure tube is not cracked
    - ii. Ensure bubble trap on computer desk is full of liquid
      1. If empty refill with IsoFlow by unscrewing cap
      2. Then drain the filter by moving roller towards you and letting liquid flow out
    - iii. Re-prime

## **RUNNING SAMPLES**

1. Create a new experiment from the drop-down menu or clicking the booklet icon
2. Add a new "Specimen"
  - a. Press the plus under the specimen to get access to the tubes
3. Remove unused parameters and add additional Height/Width parameters
4. Highlight the tube by double clicking or selecting the box to the left of the tube
5. Have the sample prepared, remove the tube of water from the sip and change the setting to "Run" and "Low", placing your sample on the sip
  - a. Double check to make sure the Run icon is green
6. Press "Acquire" you will begin to see events and you can begin to adjust the voltages to get the events into the appropriate scaling

## **CLEANING THE SYSTEM**

1. Place the new bleach tube onto the sip and run on High for 2 minutes
2. Run FACs Rinse on High for 2 minutes
3. Run dH<sub>2</sub>O on High for 2 minutes
4. Once finished leave the machine in "Standby"
5. Quit the software and log out of the computer
6. If last user, please shut off the instrument

## **HTS**

1. Remove the water tube from the SIP and keep the SIP to the side
2. Unscrew the SIP protector by turning gray connector to the left
  - a. Once loosened the metal protector will slide off
3. Change to Plate mode by switching lever down (will have a plate symbol)
4. Move the HTS assembly into the port, note the Fortessa is already connected, by placing metal rod into the anchor hole
5. Connect the fluid lines from the HTS to the instrument port.
  - a. The waste line is orange and the sheath lines are clear.
6. Connect the COM and power cord into the unit, they are to side by the power button
7. Slightly loosen the SIP connector on the HTS, this is a plastic part, this slides onto the SIP, tighten once it is fully attached.
  - a. Please note that this will need to be done on both the Fortessa and LSR
8. Turn on the Unit by flipping switch on the side, you may hear some initialization
9. You can leave the flow cytometer in "Run" and on "Low" during the entire usage
10. Replace the lid back onto the HTS if you previously removed it
11. Once the unit is on you can perform a prime on the unit by initiating it via the software under Cytometer
  - a. This freezes up the software so please wait until completed to perform any actions
  - b. Progress will show in the cytometer window in the software
12. You can add a plate under the experiment window
13. When selecting the wells note that there is 20uL of dead volume. Meaning if you want to run 100uL make sure there is at least 120uL in the well

- a. You do not need to select empty wells
14. When running the first criteria hit will finish the well. For example, if you want to run 100uL but have a stopping recording value of 10,000 events it will stop once those 10,000 events are recorded and throw away the rest of the sample.
15. Selecting run plate will overwrite any previous existing data, if need to rerun the plate you can duplicate without data to rerun the wells.
16. Once completed please run 2 wells of bleach and 2 wells of dH2O at 200ul and a speed of 3.0
  - a. You can decrease the record amount to 100 to ensure the wells do not take a long time
17. After cleaning disconnect the HTS and reattach the SIP protector and change back to "Tube" mode. Once toggle back replace the dH2O tube and briefly place in run to ensure the instrument is properly together, the run light will turn Green.

## **BIOSAFETY**

1. Closed toed shoes and clothing that goes below the knee is always required in the lab
2. Lab coat and gloves are required for BL2 samples and chemicals
3. Goggles must be worn in addition to a lab coat when pouring chemicals greater than 500mL
  - a. Chemicals cannot be disposed of down the sink
4. Instrument cleanup is required after running BL2 samples which consists of 10 minutes of bleach followed by 10 minutes of water
5. Spray down the workstation with 70% ethanol when finished with the machine