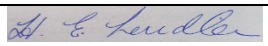
Standard Operating Procedure for: SOM Flow Cytometry using BD FACSCanto II or Fortessa X20 Analysers				
Prepared by	Bing-Ru Wu	Date	11/01/2024	
Approved by	Elizabeth Laidlaw	Date	23/5/2024	
Reviews made by	Elizabeth Laidlaw	Date	22/5/2024	
Signed by		Date	23/5/2024	

1. **Activity: Quick guide: start up, experimental setup, and shut down.**

2. **Introduction:** This BD FACSCanto II flow cytometer / Fortessa X20 SOP should help you in operating and cleaning the flow cytometer to ensure its optimal working condition. Please read these instructions carefully and apply them appropriately. Maintenance, long cleaning procedures, and calibration of the flow cytometer will be done by Dr. Bing-Ru Wu. If you have any questions regarding the flow cytometer, your experimental setup or when the flow cytometer is not working as it should, please contact Bing-Ru Wu (Ph.no: +61 3 5227 8593, Room No: KA4.222)

3. **Authorisation**

All personnel must undergo training and induction before using/operating the flow cytometer. A use and training request form should be submitted prior to use and training for the facility. For training to become a licensed user please e-mail Bing-Ru Wu (or contact on +61 3 5227 8593).

4. **Hazards associated with equipment /machinery /technique /process**

All samples involving the below mentioned require a Work Safety Assessment and/or risk assessment to assess and identify the potential hazards.

- Hazardous chemicals – Category 4 or 5
- Radiation
- Biological materials – Risk Class 2 Pathogens
- Nanoparticles or nanomaterials

5. **Before starting**

- Familiarise yourself with the procedure.
- Experimental design: – determine the appropriate controls, cell strainers, nozzle size, fluorochromes, the cell numbers and concentration of the antibody required.
- Location of further information about the hazards, e.g. material safety data sheets, radiation safety manual or laboratory safety manual if required
- Proper sample preparation and FACS tubes are required before commencing task.
- It is essential to filter all samples before acquisition on the flow cytometers to avoid damage to the equipment and decrease the need for service callouts.

- All samples are required to be fixed to eliminate biohazard risks before analysis on the Canto and X-20, e.g. using paraformaldehyde as the fixative, noting that it is a hazardous chemical and further SOPs/risk assessments may be required.
- If you have questions regarding filtering of samples or fixation (standard practices in flow cytometry facilities) please contact the Flow Cytometry manager to discuss.

6. Tools, equipment, and consumables

- FACSCanto II flow cytometer
- FACS Fortessa X20 cytometer
- BD Falcon flow cytometry tubes
- Flow cytometry reagents

7. PPE

- Safety glasses, nitrile gloves, and white laboratory gown with back-opening, long-sleeves, and cuffs.

8. Emergency procedures

If an accident/incident or other unexpected event occurs, immediately cease work, and contact a member of SoM Technical Team.
If you identify a hazard or the risk of a hazard, please contact via e-mail Bing-Ru Wu (or phone +61 3 5227 8593) and contact a member of SoM Technical Team.

9. Procedure

A. START-UP - The first user of the day.

A.1. Check that the MilliQ cube and sheath tank are full.

A.2. Empty the waste tank and add 250 ml of bleach to the empty waste tank for both cytometers.

A.3. Turn on the instrument system power by pressing the green button on the left side of the FACSCanto II or the right side of Fortessa X20.

A.4. Check that the FACS Flow Cart is on and remove any air bubbles in the filter.

A.5. Let both cytometers warm up for 30 mins (this is very important)

A.6. Turn on the computer after 30 minutes warm-up. Log in to Windows as the Operator.

A.7. Double click the Tera term icon to run cytometer self-diagnosis. Once the procedure is completed, click FACSDiva icon to open the instrument acquisition software. Log in to the FACSDiva software.

A.8. For FACSCanto II, select **Cytometer > Fluidics Startup** and follow the prompts from the software.

A.9. For Fortessa X20, find 3 tubes with 4 mL of 50% bleach, 4 mL of FACS Rinse, and 4 mL of MillQ water. Install each type of tube on the SIP arm (tube holder) centered in order and select run on the green control panel for 5 mins on **HIGH** flow rate (15 minutes total).

A.10. On the bottom of the Cytometer window, the time is indicated for remaining warm-up time for the laser, and there are four indicators. Three green indicators for FACSFlow, FACSClean, and ShutDown Solution (full = green, red = level too low, change fluids) and one black indicator for Waste (empty = black, red = full, empty Waste fluid tank).

A.11. Both cytometers are now ready to run samples, start cleaning procedures, and for calibration.

B. CLEANING AFTER EACH RUN – ALL USERS

B.1. Find 3 tubes with 4 mL of 50% bleach, 4 mL of FACS Rinse, and 4 mL of MillQ. Install each type of tube on the SIP arm (tube holder) centered in order and click Acquire for 5 mins on **HIGH** flow rate (15 minutes total).

B.2. For FACSCanto II, remove your tube while holding the SIT all the way to the left. Let the SIT go back to the middle position, and the needle is cleaned automatically.

B.3. For Fortessa X20, place a tube of MillQ on the SIT.

B.4. Export your data to your group folder and transfer it to your own hard drive ASAP.

**DATA THAT IS OLDER THAN 6 MONTHS WILL BE DELETED.

B.5. Log out or close software.

B.6. Check the instrument schedule to determine if you are the last user of the day. If you are the last user of the day, continue with fluidics shutdown procedure.

**Deviations from SOP (INCLUDING FAILURE TO RECORD CLEANING) that result in instrument downtime or inhibit the next user from typical use will incur extra charges.

C. SHUT DOWN – The last user of the day.

C.1. For FACSCanto II, click on "**Instrument>Fluidics Shutdown**" this will open a small text box where you should confirm the Fluidics Shutdown by clicking OK.

C.2. For Fortessa X20, find 3 tubes with 4 mL of 50% bleach, 4 mL of FACS Rinse, and 4 mL of MillQ. Install each type of tube on the SIP arm (tube holder) centered in order and click Acquire for 5 mins on HIGH flow rate (15 minutes total).

C.3. When fluidics shutdown is complete, shut down the FACSDiva software, shut down the computer.

C.4. Shut down the flow cytometer by pushing the green button on the left side of FACS Canto or right side of Fortessa X20. When the flow cytometer shuts down it will make some noise (depressurizing the system), this is normal.

10. Clean up

Clean up any spills, throw away any garbage and take your belongings with you.

11. Waste disposal

Empty flow cytometer waste tank and fill it with 10% volume of household bleach prior to starting instrument. Once full, empty the waste tank down the sink with running water. Unused samples of <1mL are capped and disposed in biohazard containers. Larger volumes should be treated with 10% bleach for 30 minutes prior to disposal in the sink which is then rinsed for 1 minute. Solid waste is disposed of in biohazard bags. Refer to the SOM waste disposal guide for samples involving

- Hazardous chemical waste

12. Record keeping

Bookings can be made via online Outlook calendar using these instructions. All the data from the experiments need to be exported to your own hard drive as the data will be cleared from the system every 6 months.