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| **Standard Operating Procedure for: SOM Flow Cytometer****BD FACSCanto II analyser Standard Operating Procedure** |  |
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| Approved by |  | Date |   |
| Reviews made by |  | Date |  |
| Signed by |  | Date |  |

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

# Introduction: This BD FACSCanto II flow cytometer 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. Faiza Basheer. If you have any questions regarding the flow cytometer or your experimental setup or when the flow cytometer is not working as it should, please contact Faiza Basheer (Ph.no: +61 3 522 73448, Room No: Ka4.222)

# Authorisation

All personnel need to undergo training before they can operate the flow cytometer. A [use and training request form](SOM%20Flow%20Cytometry%20Use%20and%20Training%20Request%20Form%20-%20FB%2022.10.2018.pdf) should be submitted prior to use and training for the facility. For training to become a licensed user please e-mail Faiza Basheer (or contact on +61 3 522 73448).

# Hazards associated with equipment /machinery /technique /process

All samples involving the below mentioned require a [Work Safety Assessment](http://www.deakin.edu.au/students/health-and-wellbeing/occupational-health-and-safety/health-and-wellbeing/project-safety-plans) and/or risk assessment to assess and identify the potential hazards.

* Hazardous chemicals – Category 4 or 5
* Radiation
* Biological materials – Risk Class2 Pathogens
* Nanoparticles or nanomaterials

# Before starting

* Familiarise yourself with the procedure.
* Design your experiments – decide on the getting the appropriate controls, cell strainers, nozzle size, fluorochromes, the cell numbers and concentration of the antibody.
* Location of further information about the hazards, e.g. material safety data sheets, radiation safety manual or laboratory safety manual if required
* Preparation of samples and FACS tubes are required before commencing task.

# Tools, equipment and consumables

* FACSCanto II flow cytometer
* BD Falcon flow cytometry tubes
* Flow cytometry reagents

# PPE

* Gloves and laboratory coat/back-opening gown.

# Emergency procedures

If an accident or other unexpected event occurs, immediately contact technical officers.

# Procedure

1. **START-UP - The first user** **of the day.**
	1. Check that the MilliQ cube and sheath cube are full
	2. Check the waste tank is empty. Add 250 ml of bleach to the empty waste tank.
	3. Turn on the computer. Log in to Windows as the Operator. The password is “BDIS”.
	4. Double click the FACSDiva icon to open the instrument acquisition software. Log in to the software.
	5. Turn on the instrument system power by pressing the green button on the left side of the instrument.
	6. Check that the FACS Flow Cart is on and remove any air bubbles in the filter.
	7. On the main tool bar select **Cytometer > Fluidics Startup** and follow the prompts from the software.
	8. 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 Shut Down Solution (full = green, red = level too low, change fluids) and one black indicator for Waste (empty = black, red = full, empty Waste fluid tank).
	9. The flow cytometer is now ready to run samples, to start cleaning procedures, and for calibration.
2. **CLEANING AFTER EACH RUN – ALL USERS**
	1. Place a tube of 5 mL of 50% bleach (WHITE KING) on the SIT (tube holder). ACQUIRE for 5 minutes on **HIGH** flow rate.
	2. Place a tube of a tube of 5 mL of Milliq water on the SIT. ACQUIRE for 5 minutes on **HIGH** flow rate.
	3. 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. Export your data to your group folder.
	4. Export your data to your group folder and transfer to your own hard drive ASAP.

\*\*DATA THAT IS OLDER THAN 1 MONTH WILL BE DELETED.

* 1. Log out or close software.
	2. **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.

1. **SHUT DOWN – The last user of the day**
	1. Click on “**Instrument>Fluidics Shutdown**” this will open a small text box where you should confirm the Fluidics Shutdown by clicking OK.
	2. When fluidics shutdown is complete, shut down the FACSDiva software, shut down the computer.
	3. Shut down the flow cytometer by pushing the green button on the left side of the flow cytometer. When the flow cytometer shuts down it will make some noise (depressurizing the system), this is normal.

# Clean up

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

# 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 in biohazard bags. Refer to the SOM waste disposal guide for samples involving

* Hazardous chemical waste

# Record keeping

 Bookings can be made via online Outlook calendar using [these instructions](http://www.deakin.edu.au/__data/assets/pdf_file/0011/864380/How-to-book-SOMFLow-in-Outlook-v2.pdf). All the data from the experiments need to be exported to your own hard drive as the data will be cleared from the system on a monthly basis.