



## STANDARD OPERATING PROCEDURE: #APC008

**Title: FACSCalibur Start-up, Shut-Down and Cleaning**

**Effective Date:**

**Approvals (Signature and Date):**

Author : Panagiota Stamou

Technical Authority (Signature)

Date: 24/05/2017

### 1.0 PURPOSE

To correctly Book, start-up, shut-down and maintain the FACSCalibur Flow Cytometer.

### 2.0 SCOPE

Correct start-up and shut-down knowledge is required by all users. There is a cleaning procedure which is used after each run & a monthly cleaning procedure also.

### 3.0 RESPONSIBILITY

- Only registered researchers or students that have been trained by a member of facility staff, have signed the user licence agreement and instrument SOP may operate the instrumentation. Registered and trained researchers and students are allowed to operate the flow platform instrument unsupervised only after approval by the Flow platform manager.
- To register with APC Flow Cytometry platform you need to send your details: Name, Laboratory, Contact details (email address) to Dr. Panagiota Stamou, E-mail: [panagiota.stamou@ucc.ie](mailto:panagiota.stamou@ucc.ie), Phone: +35321490 1395
- All persons using the flow cytometer must follow the correct start-up and shut-down procedure as shown in this SOP.
- Exchanging sheath and waste canisters is part of operating a facility owned analyzer. Every user is expected to refresh sheath fluid if it is running empty and to exchange waste tanks either when they are 50% full or when they shut down the instrument as last user.
- Users should treat the analyzers with care when operating the system and loading samples. If you are in doubt of your operating procedures, please contact the staff and you will be shown proper use and procedure.
- Users should export and delete their experiment data from the Flow cytometer analyser equipment computer hard drive immediately after recording their experiments. Users can check the integrity of their data transfer before deleting the experiment from the database, but no more than a month must pass between recording and deletion.



- The facility takes no responsibility for the maintenance and physical integrity/storage of any user's data. Users are required to back up their data immediately after their analysis on their own groups secure server and to follow the above stated guidelines for data deletion and transfer at all times.
- Instrument can be booked on line via **iLab Operations Software** after user training. The instrument is user operated.
- There is a charging fee scheme for the machine as follows:

SERVICE	EQUIPMENT		APC Internal	UCC Departments Schools	Industry
FACS ANALYSIS (User Operated)	BD FACS Calibur ANALYSER	Usage Charge per hour*	No Charge <sup>#</sup>	€ 20	€ 40
* FACS SHEATH, CS&T calibration beads and monthly maintenance consumables (FACS Clean, FACS Rinse) are included in the defined charge					
<sup>#</sup> APC users are required to provide their own stock of SHEATH FLUID					

- Bookings of the instruments are not to be changed within a one working day period prior to the actual appointment. Booking durations are set within one working day period and can only be changed or modified in agreement with the Flow cytometry platform manager. Failure to show up for a booked appointment without cancelling (1 day earlier) 24 hours in advance will result in full charge for the duration of the booking.

#### 4.0 REFERENCES AND APPLICABLE DOCUMENTS

- There is a laminated user's guide located next to the flow cytometer lab, Room 5.19. as well as a typed version attached to the front panel of the FACSCalibur.
- There is BD\_FACSCalibur\_instructions pdf file on the desktop folder of BD\_FACSCalibur Computer

#### 5.0 MATERIALS AND EQUIPMENT

Materials & Equipment provided by the APC Flow Cytometry Platform:

FACSCalibur flow cytometer  
 BD FACS Rinse  
 BD FACS Clean  
 Distilled Water

Materials & Equipment provided by the registered users:

BD Falcon flow cytometry tubes  
 BD FACS SHEATH  
 PPE (Lab Coat, gloves, eye protection)



Instrument	Description/Availability
<b>BD FACSCalibur</b> FACS analyzer	<p>Software: Cell Quest</p> <p>Excitation lasers: 488nm, 635nm</p> <p>Emission Detection            4 colors, Standard set installed:            FL1: 530 nm (FITC),            FL2: 585 nm (PE/PI),            FL3 &gt;670 nm (PerCP)            FL4: 661 nm (APC) with FL4 option.</p> <p>Service: Instrument is cleaned thoroughly every month.</p> <p>Specialty: This instrument can be used for up to 4 fluorescent parameters plus FSC, SSC.</p> <p>Availability: Instrument can be booked on line via <b>iLab Operations Software</b> after user training. The instrument is user operated.</p>

## 6.0 HEALTH AND SAFETY CONSIDERATIONS

- Appropriate protective measures should be taken at all times (i.e. Gloves & lab coat). All used BD Falcon cytometry tubes and plates as well as gloves should be disposed of in an autoclave bag located in the main lab.
- All samples with biological safety category 2 or higher that have no approval from Health and Safety for live measurements must be fixed prior to entering the facility and running the analysis.
- All users must have prepared the samples for analysis at their laboratory bench outside the 5.19 room and all samples must be carried at the 5.19 laboratory in tubes with lid in an appropriate container.
- All spillages and contaminated areas must be disinfected with 1.0% Virkon and be reported to the flow cytometry platform manager.



## 7.0 PROCEDURE

### Daily FACSCalibur Start-up Procedure

1. Switch on flow cytometer followed by the computer 15 seconds later.
2. Remove the distilled water from the SIP holder.
3. Remove the Sheath container from the FACSCalibur and fill with FACS Sheath until it is three quarters full. \*\*\*Note- FACSClean is corrosive and as such will cause damage to the FACS panel which is costly to replace. Do not fill the sheath container in place on the FACSCalibur with a measuring cylinder. Please ensure the sheath container is removed before filling.
4. Empty the waste container and add 200 ml of FACSClean.
5. Pressurize the sheath container, using the VENT VALVE.
6. Allow 20 seconds to pressurize the sheath container. Verify that pressurization is achieved by touching both sheath and waste container.
7. Clear any air from the sheath filter by using the bleed tube.
8. Perform three primes so that the flow chamber correctly fills and no air bubbles remain.
9. Pipette 4 ml of distilled water in a flow cytometer tube. Install the tube and run for 2 minutes.

### Daily FACSCalibur Shut-Down Procedure

1. Fill and label three flow cytometry tubes with 4 ml of FACSClean, 4 ml of FACS Rinse and 4 ml of distilled water.
2. Install the tube with 4 ml of FACSClean with the SIP arm centered, select RUN mode and HI flow rate and wash for 3 minutes.
3. Place the SIP arm to the right and allow the flow cytometer to aspirate 2 ml of FACSClean.
4. Wash until 1 ml of FACSClean is left in the tube.
5. Repeat steps 2, 3, and 4 using FACS Rinse and distilled water.
6. Record 3 minutes a Water tube sample after the cleaning procedure
7. When washing is completed ensure that the sipper is centered.
8. Turn to STANDBY mode.
9. Depressurize the sheath container using the VENT VALVE-FAILURE TO DO SO WILL RESULT IN WET FILTERS AND POOR PERFORMANCE OF THE FACSCALIBUR.
10. Switch off the computer. Allow the lasers to cool for 10 minutes then switch off the flow cytometer.
11. Note – for samples that may contain large particles e.g. tissue samples, it is strongly recommended that the cleaning procedure be performed twice.



### FACSCalibur Monthly Cleaning Procedure (Long Clean)

This procedure will be run once a month by the APC Platform staff. However in the case where there is too high background a user can run Long Clean protocol.

1. Switch on the flow cytometer.
2. Remove the sheath container and bypass the filter – Disconnect the upper tubing of filter from the Saline filter port, connect the sheath tube (white) to the port labeled as Saline Filter (refer to Fig 1-4, pg 25 in FACSCalibur manual).
3. Install a spare container with 1-2 L of BD FACSClean solution or bleach (10%).
4. Install a flow cytometer tube with 3 ml of FACSClean or 10 % bleach on SIP.
5. Select RUN mode and HI flow rate.
6. RUN for 20-30 minutes.
7. Remove flow tube from SIP.
8. Repeat steps 3-8 using:
  - 8.1 BD FACSRinse solution.
  - 8.2 Distilled water.
9. Replace the original sheath container.
10. Connect the sheath filter tubes.
11. Place a flow cytometer tube with 1 ml of distilled water on SIP.
12. Press STANDBY, depressurize and turn of cytometer.

Note: If Cytometer has not been used for three weeks, only use distilled water for maintenance to get rid of crystallization.

### **8.0 DOCUMENTATION REQUIREMENTS**

- All users must make a booking via **iLab Operations Software** to use FacsCalibur. All users must ALSO document usage time in the FACS Calibur LogBook (located within the flow cytometry room 5.19).
- Users must also document the performance of the equipment after each run taking care to record any problems. Please inform **Pepi Stamou** [panagiota.stamou@ucc.ie](mailto:panagiota.stamou@ucc.ie) of any problems.

Date:

User Signature:

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