



## STANDARD OPERATING PROCEDURE: #APC032, Version 1

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

**Effective Date:**

**Approvals (Signature and Date):**

Author : Panagiota Stamou

Technical Authority (Signature)

Date: 22/05/2017

### 1.0 PURPOSE

To correctly Book, start-up, shut-down and maintain the FACS Celesta 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 machine *will not be available for use during non-working hours* (6.00pm-9.00am Monday to Friday, 24 hours during weekend) that the platform staff is not around. The platform staff will be turning on the machine and operating CS&T calibration for the particular configuration only if a booking has been made and the user has informed the platform staff by email for the booking and the particular configuration required.
- 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 Celesta ANALYSER	Usage Charge per hour*	No Charge#	€ 30	€ 60
* FACS SHEATH, CS&T calibration beads and monthly maintenance consumables (FACS Clean, FACS Rinse) are included in the defined charge					
# 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 BD\_FACSCelesta\_instructions pdf file on the desktop folder of BD\_FACSCelesta\_Computer

#### 5.0 MATERIALS AND EQUIPMENT

Materials & Equipment provided by the APC Flow Cytometry Platform:

- BD FACS Celesta flow cytometer
- BD FACS Rinse
- BD FACS Clean
- Distilled Water

Materials & Equipment provided by the registered users:

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

Instrument	Description/Availability																				
<b>BD FACS CELESTA</b> FACS analyzer	<p>Software: BD DiVa 8.0.1.1</p> <p>Excitation lasers: 405nm, 488nm, 640nm</p> <p>Emission parameters:</p> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th colspan="2" style="text-align: center;">Blue/Violet/Red</th> </tr> <tr> <th style="text-align: center;">Laser</th> <th style="text-align: center;">Fluorochromes</th> </tr> </thead> <tbody> <tr> <td rowspan="5" style="text-align: center; vertical-align: middle;">405 nm</td> <td>BD Horizon BV421, V450, Pacific Blue</td> </tr> <tr> <td>BD Horizon BV510, V500</td> </tr> <tr> <td>BD Horizon BV605</td> </tr> <tr> <td>BD Horizon BV650</td> </tr> <tr> <td>BD Horizon BV786</td> </tr> <tr> <td rowspan="3" style="text-align: center; vertical-align: middle;">488 nm</td> <td>BD Horizon BB515, FITC, Alexa Fluor® 488</td> </tr> <tr> <td>PE</td> </tr> <tr> <td>BD Horizon PE-CF594, PI</td> </tr> <tr> <td rowspan="3" style="text-align: center; vertical-align: middle;">640 nm</td> <td>PerCP, PerCP-Cy5.5, 7-AAD</td> </tr> <tr> <td>APC, Alexa Fluor® 647</td> </tr> <tr> <td>BD Horizon™ APC-R700, Alexa Fluor® 700</td> </tr> <tr> <td></td> <td>APC-H7</td> </tr> </tbody> </table> <p>Service: Instrument cleaned thoroughly every second Monday, CST run prior to user bookings in the morning by staff.</p> <p>Availability: Instrument can be booked via booking calendar after user training. The instrument is user operated.</p>	Blue/Violet/Red		Laser	Fluorochromes	405 nm	BD Horizon BV421, V450, Pacific Blue	BD Horizon BV510, V500	BD Horizon BV605	BD Horizon BV650	BD Horizon BV786	488 nm	BD Horizon BB515, FITC, Alexa Fluor® 488	PE	BD Horizon PE-CF594, PI	640 nm	PerCP, PerCP-Cy5.5, 7-AAD	APC, Alexa Fluor® 647	BD Horizon™ APC-R700, Alexa Fluor® 700		APC-H7
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## 6.0 PROCEDURE

### Daily FACS Celesta Start-up Procedure Performed by APC Flow platform staff:

1. Fluidics system must always be Switched on. If not switch on Fluidics system.
2. Make sure sheath and waste tank levels are correct and no indication or alarm from the fluidics system is on.
3. Switch on flow cytometer and the computer.
4. Perform three primes so that the flow chamber correctly fills and no air bubbles remain.
5. Use cleaning procedure to remove any air bubbles in the tubings.
6. Allow 15 minutes for lasers to warm up.
7. Log in as operator. And turn on Diva software.
8. Perform CS&T performance test, on the configuration required.
9. Log off Diva software

### Daily FACS Celesta Start-up Procedure Performed by user:

1. Log on Diva software as instructed during training.
2. Create new Diva experiment.
3. Select fluorochromes and detectors.
4. Create Application settings
5. Calculate compensation
6. Run samples.

### Daily FACSCelesta 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. Export experiment (select delete after export).
10. Switch off the computer if no other booking after you on the calendar.
11. Note – for samples that may contain large particles e.g. tissue samples, ir use DNA dyes Propidium Iodine or 7AAD it is strongly recommended that the cleaning procedure be performed twice.



## 7.0 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.

## 8.0 DOCUMENTATION REQUIREMENTS

- All users must e-mail APC FACS Platform manager **Pepi Stamou** [panagiota.stamou@ucc.ie](mailto:panagiota.stamou@ucc.ie) to inform the Configuration they will use (standard configuration or PECY7 configuration).
- All users must document usage time in the FACS Celesta 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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