

STANDARD OPERATING PROCEDURE

Procedure:	Use of BD Accuri™ C6 Flow Cytometer
School/Department:	School of Molecular Bioscience
SOP prepared by:	So Ri Jung & Markus Hofer
Version:	SMB063.1

Section 1 - Personal Protective Equipment (PPE)

1. Lab coat or lab gown (buttoned up)
2. Proper enclosed shoes
3. Gloves
4. Safety goggles (For the dilution of concentrated Decontamination Solution and Bacteriostatic Concentrate Solution)
- 5.

Section 2 – Potential Hazards + Safety precautions

1. Concentrated Decontamination Solution (#653154) contains 5.25 % sodium hypochloride solution. It is an irritant and corrosive. Avoid skin and eye contact, inhalation, and swallowing. PPE to be worn at all times. Wear PPE including safety glasses when handling it.
2. Bacteriostatic Concentrate Solution (#653156) contains 7.0 % EDTA, 5.0 % sodium fluoride and 2.0 % sodium hydroxide. It is corrosive. Avoid skin and eye contact and swallowing. PPE including safety glasses to be worn at all times.
3. Instrument may have been used for infectious samples. Wear PPE including gloves at all times. NOTE: Infectious samples or human samples must not be analysed unless properly fixed in paraformaldehyde.
4. Workers with pre-existing medical conditions (e.g. allergy, immunocompromised state, chemical sensitivity) and workers who are pregnant or expecting pregnancy must consult with their supervisor AND medical specialist AND the university's WHS services before performing this procedure. If there are any serious concerns expressed by any of these individuals, this task must not be performed.

Section 3 – Procedure

NOTE: Infectious samples or human samples must not be analysed unless properly fixed in paraformaldehyde.

NOTE: You require induction by the custodian of the instrument before being permitted to use it.

Before running samples:

1. Check on the Quality Control Sheet that appropriate validation has been conducted in the month that you are performing your run. If not, please contact Dr. Markus Hofer (Rm 704).
2. Check levels of Sheath fluid (MiliQ water), waste, diluted Decontamination Solution and Cleaning Solution. Waste container must be empty.
3. If the level of Sheath fluid is below minimal level, it must be filled with MiliQ water (Bacteriostatic Concentrate can be added according to the manufacturer's instruction if your application requires). Concentrated Decontamination Solution must be diluted according to the manufacturer's instruction if the level of diluted Decontamination Solution is below minimal level. Cleaning Concentrate Solution must be diluted according to the manufacturer's instruction if the level of diluted Cleaning Solution is below minimal level.
4. Ensure that a 12x75 mm tube (FACS tube) containing Sheath fluid (MiliQ water) is placed on the Sample Introduction Probe (SIP).
5. Turn on BD Accuri™ C6 Flow Cytometer and allow 5 minutes for the machine to warm up
6. Remove the FACS tube containing Sheath fluid and replace it with an empty FACS tube.
7. Open BD Accuri™ C6 Software on the desktop computer and click BACKFLUSH.
8. Place a fresh FACS tube with 2 mL of MiliQ water on the SIP.
9. Set time limit for 15 minutes and Fluidics speed to Fast. Click RUN.
10. Once time limit is reached, click DELETE SAMPLE DATA.
11. Remove the tube and run samples.

While running samples:

1. During the run, if one finds the sample collection rate lower than expected, place an empty FACS tube on the SIP.
2. Click BACKFLUSH.
3. Once finished, empty the FACS tube and place it on the SIP.
4. Click UNCLOG.
5. Proceed to running samples, ensuring that the samples are not run dry

After running samples:

1. Place a FACS tube with 2 mL of diluted Decontamination Solution on the SIP.
2. Select an empty data well.
3. Set time limit for 5 minutes and Fluidics speed to Fast. Click RUN.
4. Once time limit is reached, remove the tube of Decontamination Solution and replace it with a FACS tube of 2 mL MiliQ water.
5. Set time limit for 5 minutes and Fluidics speed to Fast.
6. Click RUN. The cytometer will stop automatically when the time limit is reached.
7. Leave the tube on the SIP until the cytometer is used again.
8. Turn off BD Accuri™ C6 Flow Cytometer if it is not to be used again on the same day.

Section 4 – Disposal / Spills / Incidents

1. Dispose the waste into the sink in the room.
2. Remove spills with paper towel and dispose of it in autoclave waste.
3. Report any accidents/incidents on Riskware.
4. If there is skin contact with Concentrated Decontamination Solution or Bacteriostatic Concentrate Solution, wash under running water in the sink.

Section 5 – Certification / Training

1. Handling of chemicals requires completion of the Working with Chemicals training.
2. Using this machine requires in-house Flow cytometry training. Contact Dr Markus Hofer.

Section 6 – Relevant Material safety data sheets

1. Concentrated Decontamination Solution (BD Biosciences #653154)
2. Bacteriostatic Concentrate Solution (BD Biosciences #653156)

Section 7 - References

1. BD Biosciences webpage (<http://www.bdbiosciences.com>)

SOP Consultation, Training and Approval

Print names and enter signatures and dates to certify that the persons named in this section have been consulted/trained in relation to the development and implementation of this Standard Operating Procedure. WHS Representative (WHS Committee) certifies that consultation has taken place.

Position	Name	Signature	Date
Supervisor			
employee / student			
employee / student			
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