JMJ-Group – Microbiology – BMB – SDU

Title: Daily use of FACS Aria II	**Date issued:** 2013.01.16
SOP number: SOPFACS0042_v01	**Review date:** 2014.12.01
Version number: 01	**Written by:** Tina Kronborg and Michelle Madelung

1. Purpose

To start up, perform CST, perform drop delay and shut down the FACS Aria II

2. Area of application

This procedure is valid for the FACS Aria II placed in class II laboratory V16-501f-2

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
FACS	Laboratory (class 2) – V16-501b-2	• Size of the neutral density filter • Nozzle size • Waste bin empty • Sheath fluid above minimum	Both tubes are connected to the sheath fluid tank
Refrigerator	Laboratory (class 2) – V16-501b-2	•	

4. Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room	Safety considerations
CST beads	Cytometer Setup and Tracking beads consist of bright (3 µm), mid (3 µm), and dim (2 µm) beads dyed with a mixture of fluorochromes that are excited by the lasers used in BD digital flow cytometers. See paragraph 12. Appendix	BD / 642412	Refrigerator V16-501b-2	
Accudrop beads	This product contains a single population of 6-µm particles. Every particle contains a fluorophore that is excited at 670 nm and emits at 750 nm. The particles are supplied in 1.5 mL of water containing 0.05% Tween® 20 and 2 mM sodium azide. See paragraph 12. Appendix	BD / 345249	Refrigerator V16-501b-2	
PBS	1x Phosphate Buffered Saline (PBS Buffer): 1 L distilled H_2O 8 g NaCl 0.2 g KCl	 Sigma-Aldrich / 31434N J. T. Baker / 3040-01	 V18-405-0 V18-405-0 V18-405-0	Autoclave before use

	1.44 g Na_2HPO_4 0.24 g KH_2PO_4	MERCK / 1.06580.1000 MERCK / 1.04873.1000	V18-405-0
FACS Flow sheet fluid	See paragraph 12. Appendix	BD / 342003	Refrigerator V16-501b-2
FACS Clean	See paragraph 12. Appendix	BD / 340345	Refrigerator V16-501b-2
FACS Rinse	See paragraph 12. Appendix	BS / 340346	Refrigerator V16-501b-2
Deionized H_2O		V18-405-0 or Hallway storage (1. Floor)	
FACS tube		BD / 352054	V16-501b-2
15 ml tube		Sarstedt	BMB storage

5. QC – Quality Control

- Check expiry date on the beads
- Make sure the beads are dissolved before use

Settings	70 micron	85 micron	100 micron
Cell type	Bacteria	Can be used for yeast, eukaryotic cells	Eukaryotic, yeast
Sheath pressure	70	45	20
Amplitude	60	32	12
Frequency	87	47	30
Drop 1	150	150	150
Gap (upper limit)	6 (14)	7 (17)	10 (21)

Table 1

Neutral density filter (ND)	0.5	1.0	1.5	2.0
Cell type	Bacteria		Yeast, Filaments	Eukaryotic cells

Table 2

6. List of other SOPs relevant to this SOP

JMJ_SOPFACS0002_v01_TK_Sorting_setup_FACS_Aria_II

7. Environmental conditions required

8. Procedure

8.1 Write all decisions into Scheme_for_daily_run. xlsx which can be found in S:\999_Public\!SOPs_and_Protocols\FACS\FACS_Aria_II\SOPs_for_daily_runs

8.2 **DAY 1:** If possible check PBS/FACS flow level in sheet tank, add so it contains about 4 liters, leave to next day so air bobbles can disappear.

8.3 **DAY 2:** Turn on the FACS, wait one minute

8.4 Turn on the computer

8.5 Log in on the computer

8.6 Start “BD FACSDiva software”

8.7 Log in with own user name and password
8.8 "CST Mismatch" popup, look at "Details" if not much difference say "Use CST settings", if big difference press "Keep CST settings"
8.9 Turn down the flow level to 1 evt/sec
8.10 Choose "Cytometer" and in the dropdown menu choose "Fluidics startup" - do as instructed
8.11 Change nozzle depending on cell type, see Table 1
8.12 Check for the right filter according to FITC 530/30 or GFP 525/30
8.13 Choose "cytometer" and in the dropdown menu choose " view configuration"
8.14 Under OU P17700039 or GFPP17700039 depending on FITC or GFP filter respectively, choose program after nozzle size, press "set configuration" and press ok to popup window
8.15 Press OK in the cytometer configuration window and close the cytometer setup and tracking window which have opened
8.16 Set amplitude and frequency depending on nozzle size, see Table 1
8.17 Turn on the stream
8.18 Alter frequency to get a proper drop pattern
8.19 Turn on sweet spot and wait for the FACS to adjust Amp to get the right gap
8.20 Turn off sweet spot
8.21 It is possible to set the gap manual by changing the amplitude, the drop pattern shall show at least 6 drops with a satellite which is “caught” before the last drop
8.22 Choose "cytometer" and in the dropdown menu choose "CST"
8.23 Mix 1 ml PBS with 3 drops of CST beads in a FACS tube (remember to vigorously shake the beads before mixing with PBS). Can be used until empty.
8.24 Check that Lot ID corresponds
8.25 Place the mixture in the FACS, without a lid
8.26 Press "Run" and then "OK"
8.27 When the performance is finished press "View report"
8.28 Check that the % Difference Target Value are below ?? and that the ΔPMTV is below 10. Afterwards choose the performance tracking tab and look at the graphs, check that all are within normal range
8.29 Close the Cytometer Setup and Tracking window
8.30 "CST Mismatch" popup, look at "Details" and press "Use CST settings"
8.31 Turn off stream
8.32 Change the neutral density filter according to Table 2
8.33 Create a new folder, name it
8.34 Create a new experiment, name it
8.35 create a new specimen, name it
8.36 At add as many new specimens and tubes as needed
8.37 Choose a tube
8.38 Choose needed parameters and threshold
8.39 Set up a FSC vs. SSC dot plot
8.40 Turn on stream, flow rate = 11
8.41 Run a sterile filtered PBS sample for 5 min
8.42 Turn down flow rate to 1 before unloading sample, check evt/s, this should be 0, if not run cleaning programs.
8.43 Run samples and analyze or sort see SOP for sort
8.44 Closing down - Turn on stream
8.45 Set flow rate to 11
8.46 Load 12 ml FACS clean in a 15 ml tube run for 5 minutes
8.47 Unload tube
8.48 Load 12 ml water in a 15 ml tube and run for 5 minutes

8.49 unload tube
8.50 Load 12 ml FACS Rinse in a 15 ml tube and run for 5 minutes
8.51 Unload tube
8.52 Load 12 ml water in a 15 ml tube and run for 5 minutes
8.53 Unload tube
8.54 Turn off stream
8.55 Choose "Cytometer" and in the dropdown menu choose "Cleaning Modes" and clean flow cell - do as instructed
8.56 Add 70 % ethanol to ethanol tank
8.57 Choose "cytometer" and in the dropdown menu choose "fluidics shutdown" - do as instructed "Cleaning solution" = sterile demineralized water
8.58 Turn off the FACS
8.59 Right click in the experiment, choose export experiment, press ok
8.60 Close the FACS diva software
8.61 Move exported experiment from BDExport to BMB-data S-drive
8.62 Turn of the computer

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
Once use plastic		GMO yellow waste	

10. Time consumption

- Start up: 45 minutes
- Shut down: 40 min

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.01.16 / TK and MM	01	The SOP has been written
16.18.10 / JR		The SOP has been reviewed

12. Appendixes

Data sheet for CST Beads: **23-9141-01_CS&T Beads_PI_RUO** is found in:

S:\999 Public\!SOPs and Protecols\FACS\FACS Aria II\SOPs for daily runs\Data sheets

Data sheet for Accudrop Beads: **23-8292-01_AccudropBeads** is found in:

S:\999 Public\!SOPs and Protecols\FACS\FACS Aria II\SOPs for daily runs\Data sheets

Data sheet for: FACS Flow sheet fluid: **342003-MSDS-EUEN-01 FACS Flow** is found in:

S:\999 Public\!SOPs and Protecols\FACS\FACS Aria II\SOPs for daily runs\Data sheets

Data sheet for: FACS Clean: **340345-MSDS-EUEN-00 FACS Clean** is found in:

S:\999 Public\!SOPs and Protecols\FACS\FACS Aria II\SOPs for daily runs\Data sheets

Data sheet for: FACS Rinse: **340346-MSDS-EUEN-00 FACS Rinse** is found in:

S:\999 Public\!SOPs and Protecols\FACS\FACS Aria II\SOPs for daily runs\Data sheets

PBS:

10 x concentration: 5 L

1. Dissolve the following in 3.5 L distilled H_2O.
    - 400 g of NaCl
    - 10 g of KCl
    - 72 g of Na_2HPO_4
    - 12 g of KH_2PO_4
2. Adjust pH to 7.4.
3. Adjust volume to 5 L with additional distilled H_2O.
4. Sterilize by autoclaving