

JMJ-Group – Microbiology – BMB – SDU

Title: Sorting setup FACS Aria II

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Version number: 01

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1. Purpose

To set the FACS up for sorting

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) – V16-501f-2	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 	

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

Name	Components	Supplier / Cat. #	Room	Safety considerations
Accudrop beads	This product contains a single population of 6- μ m 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 ₂ O 8 g NaCl 0.2 g KCl 1.44 g Na ₂ HPO ₄	Sigma-Aldrich / 31434N J. T. Baker / 3040-01 MERCK / 1.06580.1000 MERCK / 1.04873.1000	V18-405-0 V18-405-0 V18-405-0 V18-405-0	
FACS Flow sheet fluid	See paragraph 12. Appendix	BD / 342003	Refrigerator V16-501b-2	

5. QC – Quality Control

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

When sorting large or fragile cells, use a larger nozzle size and lower pressure:
Nozzle 85 microns and pressure on 45 psi or
Nozzle 100 microns and pressure on 20 psi

When sorting smaller or less fragile cells, use a smaller nozzle size and higher pressure:
Nozzle 70 microns and pressure on 70 psi

Flow rate (events/sec) according to nozzle size for autodrop delay:

70 micron = 1.000 – 3.000
85 micron = 800 – 2.000
100 micron = 600 – 1.500
130 micron = 400 – 1.200

Sort precision modes:

Purity mode (Yield Mask 32, Purity Mask 32, Phase Mask 0):

Gives a very pure sample, but a low yield and lots of cells which are discarded because of conflicts.

4-Way Purity (Yield Mask 0, Purity Mask 32, Phase Mask 0):

Recommended for four-way sorting where precise deflection is required

Yield (Yield Mask 32, Purity Mask 0, Phase Mask 0):

Low purity - could be used as a first round sort for enrichment of target particles, followed by a sort for purity.

Single cell mode (Yield Mask 0, Purity Mask 32, Phase Mask 16):

Used for plate sorting or situations where precise counting is required.

Initial (Yield Mask 32, Purity Mask 0, Phase Mask 0):

As Yield (used during sort Setup, automatic)

Fine Tune mode (Yield Mask 0, Purity Mask 0, Phase Mask 0):

Give the maximum number of drops. Are used to fine-tune the drop delay value.

6. List of other SOPs relevant to this SOP

JMJ_SOPFACS0001_v01_TK_Daily_use_of_FACS_Aria_II

7. Environmental conditions required

8. Procedure

- 8.1 Start up the FACS as described in JMJ_SOPFACS0001_v01_TK_Daily_use_of_FACS_Aria_II
- 8.2 Make sure that the steam is stable and set according to nozzle size
- 8.3 Turn on sweat spot
- 8.4 Mix 1 ml PBS with 4 drops of accudrop beads in a FACS tube (remember to vigorously shake the beads before mixing with PBS). Can be used until empty
- 8.5 Open a new experiment – accudrop delay
- 8.6 Mark the tube and open the sort layout
- 8.7 Load the accudrop beads and adjust the steam according to nozzle size, see in paragraph 5
The flow rate settings shall be between 1 and 5 else adjust the beads concentration
- 8.8 Turn on the voltage in the side stream window
- 8.9 Click sort in the sort layout window
- 8.10 Click cancel in the confirm dialog according the waste drawer
- 8.11 Adjust the micrometer dial to obtain the brightest bead spot on the center stream
- 8.12 Click the auto delay button in the side stream window
- 8.13 The two boxes representing the optical filters, indicate the region of the image where the left and center stream intensities are calculated during image processing
- 8.14 If the left side stream is not completely contained in the left region, adjust the voltage slider to place the stream in the center of the region
- 8.15 When the auto drop delay dialog is open click start run
- 8.16 Don't turn off sweat spot after the run is finished, the sweat spot must be on during the whole sort
- 8.17 If the steam are turned off during the sort, the drop delay must be re-runned
- 8.18 The drop delay is set manually by the arrows at the drop delay in top of the side stream window.
- 8.19 Click the optical filter button in the side stream window, so you can see the accudrop beads in front of the lower camera
- 8.20 It take few seconds to register the change so be patient 😊
- 8.21 Unload the accudrop beads
- 8.22 Open the experiment in question
- 8.23 Adjust the gates to define the population(s) of interest
- 8.24 Mangler indstilling af stream ned i rør

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
ON Culture		Liquid bacterial waste	
Once use plastic		GMO yellow waste	

10. Time consumption

- Start up: 10 min
- CST:
- Drop delay:
- Baseline:
- Clean after run:
- Shut down: 8 min

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.01.16 / TK	01	The SOP has been written
13.01.02 / JR	01	The SOP has been approved

12. Appendsixes

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 H₂O.
 - 400 g of NaCl
 - 10 g of KCl
 - 72 g of Na₂HPO₄
 - 12 g of KH₂PO₄
2. Adjust pH to 7.4.
3. Adjust volume to 5 L with additional distilled H₂O.
4. Sterilize by autoclaving