# **FACS Aria III with 4 lasers**

**Relevant and important information for publication of flow cytometric data**

Remember to acknowledge the FACS Core Facility, Aarhus University in your publication and presentations (oral presentations and posters).

Send your paper to [*facs@au.dk*](mailto:facs@au.dk) for review and we will give you feedback within 2 workdays.

**Method and materials:**

Instrument and software

* FACSAria III with 4 lasers (405 nm, 488 nm, 561 nm, 633 nm) and 12 fluorescence detectors (BD Biosciences, San Jose, CA).
* Software: BD FACSDiva Software version 8.0.2 (BD Biosciences, San Jose, CA)

Instrument performance is checked on a daily basis by CS&T research beads (BD Biosciences) and Accudrop Beads (BD Biosciences).

|  |  |
| --- | --- |
| Treatment, utensils and hardware | |
| Nozzle size |  |
| Sample temperature |  |
| Collection temperature |  |
| Collection medium |  |
| Type of sort1 |  |
| Collection type2 |  |
| Sort mask3 |  |
| Prior cleaning of the instrument4 |  |
| ND filter | 1.5 |
| Date of sort |  |

1 1-way, 2-way, plate etc.

2 5 ml tube, 96 well NUNC plate, etc.

3 Single cell, purity, yield or 4-way purity.

4 Aseptic sampleline (20 minutes with 70% ethanol followed by 10 minutes UF H2O) Cleaning before RNA sort (5 minutes of FACS Clean, followed by 1 minutes of Backflush and 1 minutes of UF H2O). Enzyme test before sorting single cells into PCR tubes (single HRP-coated bead sorted into 2 µl of TMB).

*Experimental*

* Antibodies (clone, isotype, manufacturer, fluorochrome) or fluorescent proteins used and in which filters they were detected.
* Antibody concentrations, staining conditions (incubation time, temperature, buffers, blocking etc.). State if antibody titration was performed.
* Controls included: E.g. compensation controls, biological controls, FMO controls, stimulation controls, mock controls.
* For compensation, state if you used beads or cells.
* Stop conditions: E.g. number of events in a specific gate or a sample volume.
* Amount of cells sorted and how many events were collected for later analysis.
* Reanalysis: Note the purity of the sorted populations.

**Abbreviations:**

|  |  |
| --- | --- |
| Forward Scatter | FSC |
| Side Scatter | SSC |

The table below is an example of how to provide information about your experiment.

Enter the fluorochrome, fluorescent protein or DNA dye you used into the filter you used.

|  |  |  |  |
| --- | --- | --- | --- |
| Laser & power | Wavelength detected | Fluorochrome/  fluorescent protein | Antigen |
| 405 nm | 450/40 |  |  |
| 50 mW | 510/50 |  |  |
|  | 605/30 |  |  |
| 488 nm | 488/10 | FSC |  |
| 20 mW | 488/10 | SSC |  |
|  | 530/30 |  |  |
|  | 695/40 |  |  |
| 561 nm | 582/15 |  |  |
| 50 mW | 610/20 |  |  |
|  | 670/14 |  |  |
|  | 780/60 |  |  |
| 633 nm | 660/20 |  |  |
| 20 mW | 730/45 |  |  |
|  | 780/60 |  |  |