**Bigfoot 6 laser**

**V01**

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

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

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

Having used the 6-laser Bigfoot in the Skou building in the period 1 February 2022 to 31 January 2027, the Carlsberg foundation must be acknowledged:

The 6-laser Bigfoot cell sorter is a generous gift from the Carlsberg Foundation, grant number CF21-0363.

**Methods and materials:**

*Instrument*

* Bigfoot cell sorter equipped with six lasers (349 nm, 405 nm, 455 nm, 488 nm, 561 nm and 640 nm) and 52 fluorescence detectors (Thermo Fisher, Fort Collins, CO).
* Software: SQS (v. 1.9.4, Thermo Fisher, Fort Collins, CO).

Instrument performance is automatically checked by the its system. Bigfoot Calibration Beads (Thermo Fisher, Fort Collins, CO) are used daily to perform alignment of the stream and lasers.

|  |  |
| --- | --- |
| Treatment, utensils and hardware | |
| Nozzle size |  |
| Temperature |  |
| Conventional or spectral |  |
| Collection medium |  |
| Type of sort1 |  |
| Collection type2 |  |
| Sort mask3 |  |
| Prior cleaning of the instrument4 |  |
| Date of sort |  |

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

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

3 Single cell, purity or enrich.

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 UF H2O). Enzyme test before sorting single cells into PCR tubes (single HRP-coated bead sorted into 2 µl of TMB).

*Experimental*

1. Antibodies (clone, isotype, manufacturer, fluorochrome) or fluorescent proteins used and in which filters they were detected.
2. Antibody concentrations, staining conditions (incubation time, temperature, buffers, blocking etc.). State if antibody titration was performed.
3. Controls included: E.g. compensation controls, biological controls, FMO controls, stimulation controls, mock controls.
4. For compensation, state if you used beads or cells.
5. Stop conditions: E.g. number of events in a specific gate or a sample volume.
6. Amount of cells sorted and how many events were collected for later analysis.
7. 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.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Filter** | **349 nm** | **405 nm** | **455 nm** | **488 nm** | **561 nm** | **640 nm** |
|  | **100 mw** | **100 mw** | **200 mw** | **125 mw** | **120 mw** | **100 mw** |
| 387/11 |  |  |  |  |  |  |
| 420/10 |  |  |  |  |  |  |
| 434/17 |  |  |  |  |  |  |
| 455/14 |  |  |  |  |  |  |
| 465/22 |  |  |  |  |  |  |
| 473/15 |  |  |  |  |  |  |
| 507/19 |  |  |  |  |  |  |
| 525/35 |  |  |  |  |  |  |
| 549/15 |  |  |  |  |  |  |
| 575/15 |  |  |  |  |  |  |
| 583/30 |  |  |  |  |  |  |
| 589/15 |  |  |  |  |  |  |
| 605/15 |  |  |  |  |  |  |
| 615/24 |  |  |  |  |  |  |
| 622/15 |  |  |  |  |  |  |
| 650 LP |  |  |  |  |  |  |
| 661/20 |  |  |  |  |  |  |
| 670/30 |  |  |  |  |  |  |
| 685/15 |  |  |  |  |  |  |
| 700/13 |  |  |  |  |  |  |
| 710/20 |  |  |  |  |  |  |
| 720/60 |  |  |  |  |  |  |
| 720/24 |  |  |  |  |  |  |
| 728/40 |  |  |  |  |  |  |
| 747/33 |  |  |  |  |  |  |
| 750 LP |  |  |  |  |  |  |
| 760/50 |  |  |  |  |  |  |
| 770/LP |  |  |  |  |  |  |
| 800/12 |  |  |  |  |  |  |
| 832/37 |  |  |  |  |  |  |
| 860 LP |  |  |  |  |  |  |