

Cell sorting guidelines: planning a cell sorting experiment

Steps:

- 1) Department investigators, research fellows and external users who wish to start using the FACS-service should contact Dr.ssa Solito Samantha (<u>citometria.cpt@ateneo.univr.it</u>) to discuss their use of the equipment already before planning the experiment.
- 2) Consult the "Cell sorting guidelines" and "Cell sorting FAQ" that we provide.
- 3) Provide your flow cytometry method. First, we strongly recommend that you do some preliminary experiments on the BD LSRFortessa X-20 flow cytometer analyzer and on BD FACSAria Fusion sorter to work out the staining and cell preparation procedures rather than doing this for the final experiment.
- 4) Fill out and sign the "Flow Cytometry Request Form"
- 5) Reserve a sorter with an operator. Many factors (including sample stability, necessity of extra cleaning pre-sort and/or post-sort, etc.) can affect how much time will be required. We will decide together on how much time to reserve to ensure there is ample time to sort your sample. The BD FACSAria Fusion usage will be scheduled through the Google Calendar BD FACSAria Fusion scheduling system.
- 6) At least three/two working days before the sorting experiment, contact the operator to discuss all the details of your experiments
- 7) At the time of your appointment, please, be in time and bring the following items to the cell sorter:
 - 1. Cell suspensions and controls into the FACS sample tubes (5 mL Polypropylene Round-Bottom Tubes, sterile, with caps or other sample collection devices, if applicable) resuspended in **SORTING BUFFER.** It is mandatory to disaggregate samples immediately before sorting with 20–50 µm pore size sample filters or cell strainers.
 - Labeled collection tubes containing (5 mL Polypropylene Round-Bottom Tubes, sterile, with caps or other sample collection devices, if applicable) with 1-2 ml (on the basis of the sample collection devices) COLLECTION BUFFER.
 - 3. 10 to 50 mL of sterile (0.2 µm-filtered) of sorting buffer and collection buffer for diluting the cells if needed.
- 8) Always check with the operator of the cell sorter to make sure that all gates are properly set and populations to be sorted are clearly identified.