Flow Cytometry & FACS
Core Facility

Frequently Asked Questions

Which cell types and microorganisms can be analyzed and sorted?
Biological material, that meets the requirements of protection level S1, can be analyzed or sorted. Working with higher S-level material in the facility is not possible.
For detailed information on the cell types and microorganisms accepted please contact the facility.

Which minimal and maximal particle sizes can be analyzed and sorted?
In general, the facility instruments can detect particles with a dimension between 1-50 µm.

Which information about the applied fluorophores is required to setup the instrument?
You need to know the excitation wavelength and emission wavelength maximum of the fluorochrome in order to select the appropriate laser and detector.

Which controls do I have to prepare for setting up the instrument?
A negative control without fluorescence is essential for any experiment. This can be an unstained control or a biological negative control without expression of the reporter or antigen, but with the same labeled antibody applied. Positive controls are useful to confirm staining or expression in a single-color-experiment. If you use multiple colors with overlapping emission spectra in one experiment, positive controls (each color alone) are obligate to calculate compensation values. Using compensation beads stained with the same antibodies as used for the cells is possible as well. Other controls may be included depending on your experiment, e.g., Isotype-Controls to identify unspecific binding of the antibody, FMO-Controls to set the positive gate for weakly expressed antigens.

What kind of buffer should I use to suspend my cells for flow cytometric analysis and sorting?
Cell analysis buffer may contain 1x PBS without Ca^{2+}/Mg^{2+} and 0.5-1% BSA or 5-10% FCS. To extend storage time it is possible to fix cells with 1-4% paraformaldehyde.
Cell sorting buffer may contain 1x PBS without Ca^{2+}/Mg^{2+} and 0.1% BSA or 0.5% FCS. Using a low serum concentration is necessary to avoid clogging of the sorter. Optionally HEPES buffer 25 mM can be added to keep pH7-8 during sorting.

How do I remove cell clumps from my sample?
Please filter the samples using a cell strainer (35-50 µm), e.g., available as round bottom tubes with cell strainer cap (BD #352235). For easily clumping cells add EDTA at 1-5 mM to the buffer.

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Which particle concentration should samples have?
Samples should be single particle suspensions at a concentration of 10^6 to 10^7 per ml.

What is the minimum sample volume required?
Sample tubes should contain at least 100 µl of sample fluid. For setup controls a higher volume is recommended (300-500 µl). The more different colors you use in one experiment, the more control sample fluid is needed to setup the instrument before running the real samples.
Minimum well volume for plates is 50 µl. In order to avoid running out of sample fluid you should always prepare at least 2 wells as setup controls.

Which device should I use to bring my samples and how should I store them before the slot?
Samples should be brought in round bottom tubes (PS 12x75 mm) suitable for BD FACSCanto™/FACSCanto™ II or BD FACSAria™ III, respectively (e.g., BD #352054). For cell sorting it is also possible to bring the samples in 15 ml conical tubes. Plate-based acquisition using the HTS on FACSCanto is possible from 96-well (U/V/Flat bottom) or 384-well plates. Other plate formats are not compatible with the machine.
It is recommended to store the stained samples on ice protected from light.

Which collection devices can be used for cell sorting and should I add some medium?
Cells can be sorted into round bottom tubes (PS 12x75 mm), 15 ml conical tubes or cell culture plates (6-, 12-, 24-, 48-, 96-, 384-well). During the sorting cells are enclosed into droplets of sterile PBS. Adding some sample medium into the collection vessel is recommended, if sorted cells are taken into culture. It is also useful to add antibiotics to the medium, because sorting is not a completely sterile process.

How can I get my data?
Please export your data after the measurement either as FCS file (Flow Cytometry Standard file format) or as FACSDiva Experiment file to your own storage device (virus free). Additionally, you can create a PDF. The facility is not responsible to backup or store any user data.

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