How should I prepare my samples?

**How should I bring my samples?**

Samples for flow cytometry must be in a single-cell suspension. The optimal cell concentration depends on how likely your cells are to clump together in the tube.

* **Flow Cytometer Analyzers**: 1 million cells/mL, minimum volume 250 µL.
* [**Amnis ImageStreamX**](https://imm.medicina.ulisboa.pt/facility/flowcytometry/doku.php?id=imagestreamx): 20 million cells/ mL in a volume range of 15-200 µL.
* **Sorters**: up to 50 million cells/mL (70 µm nozzle), 20 million cells/mL (85 µm nozzle) and 5 million cells/mL (100 µm nozzle).

**Suspend cells in the right buffer to avoid cell clumps:** If you are working with non-adherent cells, a buffer of **PBS + 2% FBS/BSA** is a good basic buffer. Adding **25 mM HEPES buffer (pH 7.0)** is a good idea as well, as HEPES has better buffering properties at high pressure than PBS does.

You may need to add **1mM EDTA**, especially if you have adherent cells, as it helps chelate divalent cations that are often required for the formation of cell aggregates. In addition, if you have a high percentage of dead cells, adding **DNase** is strongly recommended, as it reduces clumping caused by free DNA.

**Should I filter my samples?**

* Yes! Filter the cells through a nylon mesh. For the analyzers, you can use up to 70 um mesh (BD Falcon™ cell strainers ref. 352350). For the sorters, The mesh size should be inferior to the size of the nozzle: 40µm mesh for 70µm nozzle (BD Falcon™ cell strainers ref. 352340) and 70µm mesh for 100µm nozzle (BD Falcon™ cell strainers ref. 352350).

**What controls do I need?**

Every experiment needs controls, every time. Bring **unlabelled cells (unstained)** as a negative control. If you are using more than one color, you need **single-color compensation controls**. You may need or want other controls for your experiment.

**For sorting, which collection devices can I use?**

* Collect up to four different populations simultaneously into tubes of your choice, 1.5 mL, 5 mL tubes, or up to two population for 15 mL tubes.
* Sort into any kind of plate. 6-well,12-well, 24-well,36-well, 96-well, 384-well, PCR plate, etc.

The most recommended collection buffer is your **cell culture medium with 10% FBS** or some other serum. Collection tubes should be about 1/3 full of collection media.

We try to keep everything as clean as possible, but our sorters are not in cell culture hoods, so if you want to culture your sorted cells it’s a good idea to add antibiotics Pen-strep or gentamycin, and antifungal agents to the collection media.

**How can I fix my cells?**

* **With paraformaldehyde (PFA)**: Add PFA 2% to your cells, on ice for 20 min, wash twice with PBS. Samples should not be left in PFA overnight, this may increase the autofluorescence.
* **With high grade Ethanol (EtOH)**: Resuspend cell pellet in 300ul of PBS and add 700 ul of 100% EtOH pre-chilled at -20°C, mix thoroughly. Store at –20°C until required.