One of the Flow Cytometry Facility's goals is to help investigators learning the skills of flow cytometry.

To help you preparing your samples, you will find below the basics of sample preparation, safety factors related to sample preparation, and the choice of control samples.

FLOW CYTOMETYRY ONLY WORKS with SINGLE CELL SUSPENSIONS

1. Sample tubes

a) Analysis

You can use tubes available at service

- Tubes 5ml non-sterile reference 352008
- Microtubes reference 3492 from ThermoFisher scientific

b) Cell sorting

You can use any kind of tubes.

- 5 ml tubes, Eppendorf, 15 ml conic tubes, etc….
- Steriles tubes reference 352054 (tubes with cap)

Cells suspension should be brought in in the appropriate tubes.

2. Cell concentration

a) Analysis

Phenotyping, cell cycle, apoptosis, GFP or similar experiments

The cells should be resuspended at a minimum final concentration of $1x10^6$ cells/ml and in a minimal volume of 300 µl.

b) Cell sorting

- For cell lines, the sample should be resuspended to $5\text{-}10x10^{6\ cells}$ / ml and minimal volume of 300µl
- For lymphocytes, the suggested concentration is $20\text{-}30\ x10^{6\ cells}$ / ml and and minimal volume of 300µl

3. Sample Media

For analysis and cell sorting, the most common preparation buffer is
PBS 1x, 0.5% BSA, 2mM EDTA.
However, if, after sorting, you put your cells back in culture or for other functional assays,
DO NOT ADD EDTA into your buffer.
You can also leave your cells in their culture medium with less than 5% FCS but supplemented with HEPES to maintain the pH.

4. Controls

Negative controls are necessary because they help to distinguish positive from negative!

For each sample, a minimum of $5x10^5$ cells is needed to perform a correct analysis.

The following controls are also required (minimum $5x10^5$ cells per control).

a) Unstained cells

b) Cells stained only with the secondary antibody in case of indirect staining

c) Single color positive controls for multicolor analyses
(one tube per color in order to set the compensation on the machine)

d) Negative control for each cell type if you have different cell types
(in order to set the level of autofluorescence of your cells)

5. Collecting Media

- Cell culture medium supplemented with FCS and Penicillin Streptomycin or
- PBS/0.5% BSA

Sterile or non-sterile sorts

- Coat your tubes with the desired collecting medium
- Fill the collecting tubes to the top and leave them at +4°C ON or
at least 3 hours at room temperature and leave the coated tubes on ice until use

6. Information about the samples

You must supply the following information when you reserve a sorting time

- Cell type with approximate Cell size
- Number of cells to be sorted and desired number of cells after sort
- Fluorochromes used
- Percentage of target Cells in the sample
- Sterile or non-sterile
- desired temperature for the sort (4° or 37°)

- Please be on time and respect your reservation slots.

- If you are late, you may not be able to finish your sort before the next scheduled person.

7. Filter your samples

- For the analyzers, filter the samples with nylon mesh (80µm or 50 µm)

- For cell sorting, filter your samples just before your sort with the 50µm filcons (sterile or non-sterile depending on your sort)

All the filters are available at the facility.

In order to get good analysis or good sorts, thanks to follow all these instructions!