Microscopy and Cytometry Facility

Sample Preparation Guidelines

(Flow Cytometry Analysis and Cell Sorting)

Sample

- Prepare single cell suspension.
- Re-suspend cells in filtered PBS buffer (Ca^{2+}/Mg^{2+} free) with 0.1-1% BSA + 2.5 mM EDTA) but, if necessary, in any other medium without phenol red and serum (FCS, FBS).
- Remove cells and tissue aggregates by filtering the sample with 30-40 µm mesh filters (cell strainer). MCF can provide cell strainers.
- The sample should have particles density of 5-10 million/ml. If less cells are available, put then in a minimum volume of 500 µl.
- Bring the samples in any leak-proof tubes or in a 5ml round-bottom polypropylene tube. MCF can provide 5ml round-bottom tubes.
- Provide additional buffer in case the sample for sorting needs to be diluted.
- Provide collection tubes/plates one-fifth filled with collection buffer or medium. Note that the collected sample will be diluted with PBS from the instrument sheath fluid.

Tips

- To improve cells viability, all preparation steps should be performed on ice unless otherwise specified in your protocol.
- Use minimal speed to sediment cells (start with 300xg). Avoid vigorous vortexing, drying of the cell pellet and introduction of air bubbles during sample preparation.
- To exclude dead cells from the analysis or sorting it is HIGHLY recommended to use the so called live/dead dyes (DNA-binding or amine-reactive dyes) as markers of dead cells.
- DNA released from dead cells during cells dissociation causes stickiness. To reduce cells aggregation in your samples, incubate the cells in the presence of DNase I (100 µg/ml with 5 mM $MgCl_2$).

Gaiting and Compensation controls

- Each single-color or multi-color experiment requires appropriate staining controls to properly set the population gates. Please provide:
  - Negative control - unstained sample (to detect autofluorescent background),
  - Positive controls - single stained samples (to measure positive signals and eliminate spillover to other detectors),
  - FMO controls (Fluorescence Minus One) for multi-color experiments,

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