EMBL European Molecular Biology Laboratory

Sample Preparation for Cell Sorting

SAMPLE PREPARATION

IMPROVING THE QUALITY OF YOUR SORTS

Sample preparation

Successful sorting depends almost entirely on the quality of the input sample. Make sure your samples fulfil the following specifications:

1. Bring your cell suspension exclusively in FACS Buffer containing: PBS 1X + 2% FCS + 2,5 mM EDTA. EDTA stock solution at 0.5M pH8.0 is usually available at EMBL kitchen.

2. Filter your cells with a cell strainer of 40 µm pore size or using a tube with cell strainer snap cap. (BD Falcon® 5 mL Round Bottom Polystyrene Test Tube, reference: 352235). If you do not have the appropriate material, come to the facility and we will provide the strainer/tubes while you order them.

3. The sample should have a density of approximately 5-10 million/mL. If you have less than 5 x 10^6 cells put them into the minimal volume of 300 µL.

Bring to the sorting

- Negative controls and individual positive controls of the fluorochromes/dyes present in your sample in order to set -up the experiment and sorting gates appropriately.

- Extra 5mL of FACS Buffer in case further dilution of your sample is required.

- Collection tubes or plates with **fresh collection medium + antibiotics.**

Additional Tips:

- Make all cell preparations strictly on ice, unless otherwise stated in your protocol. Keeping the sample cool could in some cases improve the viability of the cells.

- **Collect your sorted cells in fresh complete medium with antibiotics**, especially if the cells will be culture after sort, to avoid risk of contamination.

- Dead cells can often be excluded by their light scatter characteristics. However this isn't always the case so we advise adding a **DNA-binding dye** that can be used as a marker to exclude dead cells from analysis and sort. The choice of dead cell exclusion dye depends on the colour combination of fluorochromes within the sample and the optical configuration of the sorter. Members of the facility could advise you on the appropriate DNA dye regarding your sample and sorter configuration.

- Dead cells can increase the incident of doublets and clumps due to release of DNA, which causes stickiness; Incubating the cells in the presence of DNAse I (100ug/mL with 5mM MgCl2) will help to reduce aggregates.