**Preparing Cells for Sorting in Flow Cytometry**

Written by: Anni Skovbo

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**This guideline works in connection with the FACS Core Facility Guidelines**

* Panel Design in Flow Cytometry (1)
* Titration in Flow Cytometry (2)
* Controls in Flow Cytometry (3)
* Compensation in Flow Cytometry (4)

**For sorting you must consider the same conditions as for analysing cells (1-4)**

Furthermore, you might want to keep your cells aseptic for further culture.

You need to consider, which temperature is best for your cells before, during and after sorting.

These aspects and more are shown in the Application Form you fill out when requesting the sort in iLab.

**For proper analysis and sorting, the cells have to be in a single cell suspension.**Worthington describes various methods for tissue dissociation (5)
If your cells tend to clump while waiting for the sort, resuspending your single cells in this EDTA containing buffer immediately after bringing the cells to single cells might help:

Non-stick cell sorting buffer

* HBSS or PBS supplemented with
* 1% BSA (bovine serum albumin)
* 2,5 mM EDTA
* 25 mM HEPES buffer

 Keeping your cells on ice will also minimize clumping tendency.

If the cells still tend to clump, we will filter the sample immediately before sorting (causing some cell loss).

**The cell density for sorting is important for the performance of the sort.**For small cells (< 9µm), adjust the cell concentration to approximately 15 x 106 cells/ml.
For larger cells, adjust the cell concentration to approximately 10 x 106 cells/ml.
However, the volume of each sample should not be lower than 300 ul.

**What to bring**

* Negative control.
The first time we need enough negative cells to set up the sorting experiment/PMT values for your sort
* Other relevant controls, e.g compensation controls, mock transfected cells, positive controls
* Your cells to be sorted
* Collection tubes/plates and medium for the sorted cells

**Data transfer**

When the sort is finished, we will transfer your data from the Aria computer to your personal folder on the AU server.

If you do not have server access, you can buy a USB stick from us for the transfer (cost 60 DKK). This stick can only be used once.

**References**

1. [FACS Core Facility Guidelines, Panel Design in Flow Cytometry](https://facs.au.dk/fileadmin/www.facs.au.dk/files/Panel_Design_2020-09-16.pdf)
2. [FACS Core Facility Guidelines, Titation in Flow Cytometry](https://facs.au.dk/fileadmin/ingen_mappe_valgt/Titration.pdf)
3. [FACS Core Facility Guidelines, Controls in Flow Cytometry](https://facs.au.dk/fileadmin/ingen_mappe_valgt/Controls.pdf)
4. [FACS Core Facility Guidelines, Compensation in Flow Cytometry](https://facs.au.dk/fileadmin/ingen_mappe_valgt/Compensation_FACSCore.pdf)
5. Single cells suspension: <http://www.worthington-biochem.com/tissueDissociation/default.html?mc_cid=0231483b66&mc_eid=b2d66a7f68>