Sample Preparation Guidelines: Cell Sorting

An outcome of the cell sorting experiment is largely affected by cell viability, auto-fluorescence, and cell aggregation. Good practice in cell preparation is crucial and will result in a better sort purity and efficiency. Please read through the following guidelines for cell preparations intended for cell sorting.

Bio-safety consideration

Whenever the new sorting experiment is planned, all sorting experiments must be described in the sorting setup form and sent to the flow lab along with its Biological Safety Protocol and BUA. The approval from the flow lab is required before setting up the appointment.

When transporting living cells between labs, researchers must use a secondary container.

Basic Buffer suggestion

- ✓ PBS (-) or HBBS, no CA^{2+}/Mg^{2+} to suppress cell-cell adhesion
- ✓ 0.1-1 % BSA or 1-5 % FBS (dialyzed or heat inactivated)
- ✓ 1-5 mM EDTA
- ✓ 25 mM HEPES
- ✓ Filter with 0.2um filter, store at 4C

Buffer Considerations:

- ✓ The % of BSA/FBS should kept minimum since they increase auto-fluorescence. Non-dialyzed FBS should be avoided as they promote cell-cell adhesion.
- ✓ If the sample is pure lymphocyte population, EDTA might not be necessary. 1 mM works for most cells but it needs to be increased when cells are stickier (5mM).
- ✓ HEPES helps to keep the pH of buffer stable during the high pressure sorting.
- ✓ If high dead cell population is expected, it's recommended to wash cells with a buffer containing DNaseI and include DNaseI in a final suspension. See below for the protocol.

DNaseI treatment to reduce cell-cell aggregation:

1) Treat cells for 15-30 minutes in a sterile HBSS containing 100ug/mL (or 10U/mL) of DNase and 5mM $MgCl_2$, room temperature
2) Wash cells with excess amount of sterile HBSS containing 5mM MgCl2, centrifuge and remove supernatant
3) Re-suspend the cells in a sterile HBSS containing 25-50ug/mL of DNase and 1-5mM $MgCl_2$

Filtration policy: Aggregation is an enemy of the cell sorting

Filtration of the sample is MANDATORY. Filter immediately before sorting. If your cells are sticky, we recommend to filter in our lab right before putting them in the sorter. We can sell 40 um filters (flowmi) per count.

Filter information

Item	Good for volume	Strainer size	Ordering information (vwr link)
Flowmi cell strainer	~1mL	40um, 70um	https://us.vwr.com/store/product?keyword=10032-802
Falcon FACS tube with strainer cap	1~4 mL	35um	https://us.vwr.com/store/product?keyword=21008-948
Strainer cap only	1~5 mL	35um	https://us.vwr.com/store/product?keyword=76449-662

Dead cell discrimination:

We strongly suggest including dead cell discrimination method using viability dyes. This helps to reduce autofluorescence and lower non-specific baseline.

Cell concentration:

Avoid keeping cells at unnecessarily high density. Keep the cell suspension at 1-10 million/mL. Cell density impacts on the followings, 1) the formation of aggregation, 2) sorting quality, and 3) total sorting time.

Collection device:

- It's advised to pre-fill the collection device with small amount of media with higher content of FBS (~50%) to protect cells from the shock of sorting.
    - 2-3mL for 15mL tubes
    - 750~1000 uL for 5ml tubes
    - 100 uL for 96 well plate
- The cells recover better when collected to a smaller tubes when cell number is low
- The collection tube should be polypropylene

Controls:

- Proper controls are essential to place accurate gates.
- Unstain, mock transfected control, isotype control: to draw baseline
- Compensation control: When cells are stained with multi-color panel
- FMO control: when tricky gating is expected due to the antigen expression level and fluorescence spread/spillover
- Positive control: When antibody is not well validated, ←Recommended to run a pilot experiment with regular flow cytometer before scheduling the sorting experiment
- Treated/untreated/activated: When the antigen expression depends on the treatment/activation status