Core Facility Flow Cytometry

- CELL SORTING -

Sample preparation

Test your expression level or transfection efficiency prior to sorting (highly recommended)

You should test your transfection efficiency or expression on an analyzer first and please have in mind that fluorescence microscopy is often not comparable to flow cytometry. It takes a lot of time to set up the sorter and it's a waste of your time and our time and your money if you realize on the sorter that your transfection did not work out.

Please avoid any mechanical detachment. If possible, use an enzymatic detachment method or incubate in Calcium/Magnesium-free media. It is essential that the cells do not clump, as this will immediately clog the cell sorter. Therefore, be sure to add EDTA to your sorting buffer as mentioned below. A **single cell suspension** is crucial for sorting. The cells need to be **filtered** according to the size of the cells. Bring your cells in 15 ml tubes or 12x75 mm standard FACS tubes (preferably polypropylene tubes if your cells attach easily).

Cell concentration

- Adaption of cell concentration is essential for sortings into tubes and if you aim for higher cell numbers. Otherwise, the sorting will take significantly longer than necessary
- <15 µm in diameter: Lymphocytes, small cells 10-15 x 10^6 cells/ml
- >15 µm in diameter: cell lines, pressure-sensitive cells 5-10 x 10^6 cells/ml
- Minimum volume 500µl / max. volume 2.5ml

Sorting buffer

Our basic buffer is:
1x PBS (Ca2+/Mg2+ free), 2 mM EDTA, 0.5% BSA, 0.2 µm filtered, store at 4 °C
The buffer might need further optimization dependent on the cell type (see table below).

- Usage of 1x PBS alone is not recommended
- If **pH**-stability is crucial, supplement the PBS with HEPES
- You could also use **cell culture media**, but it should not contain any phenol red
- The sample solution should also contain some kind of **protein** like BSA or heat-inactivated fetal calf serum (FCS)

- If you have extensive **cell death** in the sample, add DNase. It will not only decrease clump formation in the sample, but also will decrease viscosity of the single cell suspension buffer, by digestion of the free DNA. Sort purity and yield could go substantially up after DNase treatment.

Purpose and Details	Components	Basic Buffer	Sticky Cells	Adherent Cells	Dead cells	Clean lymphoid cells
Increase buffer capacity and stability	1x PBS (DPBS, without Ca^{2+}/Mg^{2+}), pH 7.0-7.4	✓	✓	✓	✓	
	HEPES pH 7.0 (10-25mM)		✓	✓	✓	
	HBSS without $Ca^{2+}Mg^{2+}$, no Phenol Red)					✓
Chelating agent to prevent aggregation	EDTA 2mM (0.5-5mM)	✓	✓	✓		
Maintain cell viability	BSA 0.5% (0.1-1%)	✓	✓	✓	✓	
	FBS/FCS (heat inactivated) 1-5%					✓
Free DNA	DNaseI 25µg/ml (RNase free, 10U/ml or 25-50 µg/ml) Add $MgCl_2$ 1-5mM		✓		✓	
Sterility	0.2µm filter; store at 4°C	✓	✓	✓	✓	✓
Notes			If cells are sticky, due to a high number of dead cells, add DNaseI	use Cation-free FBS to stop trypsin	keep DNaseI in all buffers	

Controls

Negative control, mandatory

- Please always bring along cells that do not express your antigen, e.g. non-transfected or better mock-transfected cells
- Concerning surface stainings, always bring along unstained cells otherwise we are not able to set the cutoff appropriately

Compensation controls for multicolor setup (if you use your panel the first time)

- Unstained cells
- Single stainings for every antibody
- If the expression level of the epitope is low, use a surrogate marker or compensation beads
- Use fixed/permeabilized cells mixed with living cells for live/dead staining or use ARC-beads

Gating controls

- Fluorescent Minus One (FMO) controls, as proper way to evaluate the background in certain channel and set sorting gates appropriately
- Full staining

Collection tubes

- If possible, use polypropylene tubes rather than polystyrene tubes
- Your tubes or plates should contain some media containing 10-20% FCS in order to keep the cells vital. You can also use 1x PBS supplemented with FCS
- Concentration of post-sorted cells is between 0.3 - 1.0 x 10^6 cells/ml and depends on the nozzle size
- In order to prevent cells sticking to the sides of the tubes, pre-coat the tubes, filling them with 1% BSA solution

Your cells can be sorted in

Tube type	Medium	Sort options
15 ml Tubes	5-7 ml	2 way
5 ml Tubes	2-3 ml	2 – 6 way
1.5-2 ml Tubes	0.5 -1 ml (for subsequent RNA isolation, lysis buffer can be used here)	2 – 6 way
0.2 ml Tubes	10 µl (single cell / single nuclei analysis)	1 way
Multi-Well Plates	Dependent on Well Plate	1 way

Time considerations (at max. flow rate)

Required number of particles	Desired particles as % of total									
	0.1%		1%		5%		10%		50%	
10^3	3min	4min	15s	20s	3s	4s	1s	2s	0.2s	0.5s
10^4	25min	40min	3min	4min	30s	40s	15s	20s	3s	4s
10^5	60min	90min	25min	40min	5min	8min	3min	4min	30s	40s
10^6	35h	55h	60min	90min	45min	60min	25min	40min	5min	8min
10^7	15d	22d	35h	55h	7h	10h	60min	90min	45min	60min
10^8	150d	200d	15d	22d	3d	4d	35h	55h	7h	10h
Nozzle size	**85µm**	**100µm**	**85µm**	**100µm**	**85µm**	**100µm**	**85µm**	**100µm**	**85µm**	**100µm**

Maximum throughput of particles:

85 µm Nozzle: 12 000 – drop size ~3 nl
100 µm Nozzle: 8 000 – drop size ~5 nl