FAQ

How do I prepare my cells?

1. Have a chat with FACS staff for the best tips regarding your cells.
2. Try to remove as much debris/ myelin/ unwanted cells from your prep as possible. This will reduce the amount of time that you need for sorting and give you a much better sort yield.
3. Ensure that your cells are in a single-cell suspension in medium that contains some protein. Typically samples are resuspended in DPBS without Ca++ or Mg++ containing 2% FCS or 0.5% BSA at a final cell concentration of 10-50million/ ml. More concentrated is better, as it is more efficient to dilute your sample rather than to concentrate it! Culture medium with phenol red is not recommended because this can interfere with some fluorophores.
4. Adding 1-5mM (final) EDTA is recommended if you are bringing adherent cells or cells from a solid organ. If you have a lot of dead cells in your prep, add 50ug/ml DNAse to mop up sticky DNA that is released from dead cells.
5. Add a viability dye!
6. Strain your sample through a 35-70um strainer prior to putting onto the cytometer.

What should I collect my sorted cells into?

If you require viable cells for further work, we recommend collecting your sorted cells into DPBS with some protein in it. Some people choose to collect into straight FCS. We do not recommend collecting into culture medium that is buffered with carbonate buffers.

Do I need a viability dye?

YES!! Dead/ dying cells are sticky and can give false positive results by binding fluorophores non-specifically. Fluorescent protein-expressing cells leak FP out when dying.

You have a few options for viability dyes:

- DNA-binding dyes: propidium iodide (PI), DAPI, DRAQ7 and 7aad enter the nucleus of dead/ dying cells. These dyes cannot cross the membrane of happy, live cells.
- Amine-binding dyes: bind any exposed amine groups on cells. Live cells will fluoresce less brightly as there are fewer amine groups exposed than dead/ dying cells that have the amine groups within the cytoplasm also exposed. These

dyes are great for use to distinguish live from dead when cells are fixed prior to conducting flow cytometric analysis. You can get these dyes from lots of manufacturers including Thermo Fisher and BioLegend.

What controls do I need to bring?

Your experiments are only as good as your controls. It is crucial that you bring:

1. *Negative control* – this is your untreated/ unstained/ untransfected sample that is equivalent to your experimental cells. Bring the parent line/ WT line. Ensure that if you are analysing monocytes in your experiment that you bring monocytes (not lymphocytes or other cell type) for your negative control. We use this control to set the baseline signals for your experiment.
2. *Single colour controls*

- only have one fluorescent parameter

- used for calculating fluorescence spillover to toher channels

- must be as bright or brighter than staining in your test sample

- with the exception of tandem dyes (which vary from batch to batch) you can use a different antibody for this control

- you can use antibody-capture beads for this control

Other useful controls:

- secondary only (where you are using two-step staining)

- isotype control (accounts for false positives due to antibody isotype)

- Fluorescence minus one, FMO, (for setting boundaries between positive and negative with certainty - very useful when sorting rare or dim populations.)