Sorting Preparation Guide

This document is intended to be an introductory guide through the sample preparation required for sorting. However due to sample variability it may be necessary to modify sample preparation further in order to fully optimise your sorting experiment.

What you need to bring with you for sorting.

- Sample (filtered with a 30-40um filter prior to sorting)
- Extra Sorting buffer
- Extra Collection media
- Relevant controls (discussed later in this guide)
- Collection tubes containing collection media.

Samples should be kept on ice at all times to preserve the viability of the cells before and during sorting.

Sample preparation

Basic Sort Buffer

Ca/Mg++ free PBS

25mM HEPES pH7.0

1%FBS

*For adherent cells 2mM – 5mM EDTA can be added to prevent clumping. Higher concentrations of EDTA can result in compromised cell viability

**For samples with a high percentage of dead cells DNAase at a concentration ranging from 10U/ml or 1ug/ml to prevent DNA clumping

Sample concentration

Suspension cells - 10-15 million/ml

Adherent cells - 8-12 million/ml

If there is a low cell number re-suspend your cells in 500ul and the volume can be increased if required.

Controls

The controls for each sorting experiment need to be carefully chosen in order to ensure that the sorted cells are the correct population.

In General every experiment requires:

- Unstained Cells
- Positive and negative controls
- Single colour compensation controls.
  - You can use either cells or compensation beads.
  - When using beads you have to make sure you use the same Ab for the beads as you do with the cells.

Further control considerations:

Isotype – You can buy an Isotype control of your antibody to assess the background staining of the Fc proportion of your Antibody. This control is best used when you are dealing with new antibodies or cell types that have not been fully characterised.

FMO (Fluorescence Minus One control) - All antibodies and dyes are added to the sample apart from the one under investigation. This allows the operator to set an accurate gate for a population that is not clearly defined.

Biological - If you are working with a sample that you are activating in some way it is helpful to have an unstimulated and/or stimulated control.

Collection

- Can sort 4 separated populations into 1.5ml Eppendorf tubes or 5ml FACS tubes
- Can sort 2 separate populations into 15ml Falcon tube

If you have any further queries regarding experimental design or sample preparation please contact a member of the Flow Cytometry facility.