AMREPFlow Facility Sample Preparation Guide for Sorting

Proper sample preparation for cell sorting (and for use on analysers) is paramount for downstream sorting quantity and quality as well as data analysis.

- Only samples free of debris or clumps with good viability will be accepted for sorting. If samples have poor viability clean them up with nycodenz or another density gradient before sorting.

- Resuspend your cells at 1x10^7/ml in FACS buffer, we recommend 1x mouse/human PBS, 0.5% (filtered) BSA/FCS, 0.5mM EDTA

- Use EDTA in FACS buffer to prevent clumping of cells, 0.5mM is usually adequate if the cells have been washed and are single cell suspension. For adherent cell lines or sticky cell types like Monocytes or Macrophages use 2mM EDTA.

- When processing tissue samples use DNase as there is almost invariably some release of DNA which will leads to clumping and blockages, 25ug/ml tissue culture grade DNAse is usually adequate.

- Filter all samples through a <70 um mesh just before brining your samples for sorting. This applies for the analysers as well. Un-filtered samples will not be accepted for sorting.
  If your sample blocks the instrument you will be charged for the time that it takes to re-align and QC the instrument, this will result in less time to sort your sample.

- Bring collection tubes (for your sorted population) with collection media. The collection media should be what the cells are usually maintained or grown in, including antibiotics.
  We recommend making a 2x concentration of this media and filling your collection tube about 10-20% of its volume ie; for a 5ml collection tube add 0.5-1ml of 2x concentrated collection media. Make sure to spin down and wash your cells post sort and resuspend in appropriate media for downstream use.
  You may need to bring more than one collection tube depending on how many cells you are expecting to get back, as a rough guide a 5ml tube holds about 3.5x10^6 cells on the 70um nozzle, 3x10^6 on the 86um nozzle and 2x10^6 on the 100um nozzle.

- When booking the Sorter please include your sorting details in the “special requirements” box antibody panel and fluorophores, cell type, filter size (Influx only), collection method (5ml, 15ml, 50ml, 96well plate)

- The ARIA has only the 70um nozzle size which is suitable for lymphocyte cell populations or smaller.
  The Influx has the option of the 86um nozzle, suitable for lymphocyte cell population or smaller and the 100um nozzle which is more suited for immortalised cell lines and larger cell populations such as Macrophages, Neutrophils, or single cell sorting into 96 well plates.

- All sorts are carried out at 4°C unless specified otherwise, we recommend you bring your samples in an ice bucket to minimise cellular activity while waiting to be sorted and post sorting. If sorting straight into plates then room temperature is recommended as not to temperature shock the cells from 37°C.

	ARIA	**INFLUX**
Sample delivery	5ml tube (polystyrene/polypropylene) 15ml tube	5ml polypropylene tubes
Sample collection	1.5ml eppies (1-4 populations) 5ml tubes (1-4 populations) 15ml tubes (1-2 populations) 96 well plate	1.5ml eppies (1-6 populations) 5ml tubes (1-6 populations) 15ml tubes (1-2 populations) 50ml tubes (1-2 populations) 6/24/48/96 well plate
Nozzle size	70um	86um or 100um