AMREPFlow Facility Sample Preparation Guide for Analysers

Proper sample preparation for use on flow analysis platforms is paramount for quality data generation and reproducibility.

Only samples which are in a single cell suspension, free of debris or clumps can be run on the analysis instruments.

Always use a Live/Dead stain in your sample preps - this is best practice for good data generation. There are Live/Dead dyes available for fixed samples.

If samples have poor viability, clean them up with nycodenz or another density gradient before acquisition (an additional slow spin ~200×g 10min can pellet your cells while leaving smaller particles and debris in the supernatant).

Wash your samples at least twice with FACS buffer (we recommend PBS/0.5-2% BSA/0.5mM EDTA), and then resuspend your cells at 1×10^6/mL (2×10^5/200µL) in FACS buffer.
This ensures removal of any unbound antibodies, residual fixative and debris using the slow spin mentioned above.

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

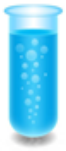
When processing tissue samples or high death assays use DNase as there will be some release of DNA, which will lead to cell clumping and blockages.
25µg/mL tissue culture grade DNAse is usually adequate.

Filter all samples through a 70µm mesh just before acquiring your samples on the instrument. Unfiltered samples are not acceptable for analysis.
Poor sample preparation will cause issues with the instrument which may include blockages, build up in the flow cell resulting in stream deflection and loss of signal and decrease in sensitivity. These will lead to compromised data generation.

Vortex your samples before acquisition, lab dancers are provided at each instrument. The instruments rely on an even distribution of cells in suspension to collect data accurately. If your sample is sensitive to vortexing resuspended with gentle flicking.

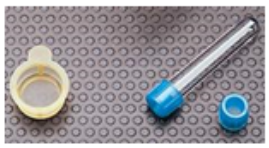
If your sample blocks the instrument due to non-compliance of the above guidelines, the time it takes to unblock and QC the instrument will be included in your booking time.

If your samples are unique and you are not confident they are in a single cell suspension please speak to your supervisor to ensure you can achieve this before running samples on the analysers, alternatively please contact AMREPFlow and we will assist.



Wash

- After staining – wash samples 2x with Facs Buffer and resuspend at 1x10^6/ml



Filter

- Filter samples though a 70um filter

Vortex

- Vortex each sample before acquisition for 2-5sec

Acquire

- Place sample on the instrument and acquire