## Fixation Protocol

As with all new SOP's please make sure the fixation protocol you select is appropriate for your cells and the antibodies you are using.

We recommend staining your cells with your antibodies first and then follow by fixation. This is because the fixation process can alter the epitopes of particular antigens which can affect antibody binding.

1. After antibody staining, wash the cells 3x with FACS buffer and spin down at 400 g for 5 min.
2. Resuspend the cells in 500 µL to 1 mL of fixative (1% paraformaldehyde for 30mins. For known infectious samples, use 3-4% paraformaldehyde for 2-3 hours)
3. After fixation period, wash the cells with FACS Buffer and spin down at 400 g for 5 min and resuspend cells @1x10^6/ml with FACS Buffer

   Always wash out the fixative before running on the analysis instruments as extended exposure can affect fluorophore stability and fluorescence intensity.

For human cells and general immunophenotyping purposes if you're using AMREPFlow instruments you **MUST fix your sample**. If you require to perform functional studies on live human samples, you must provide a Risk Assessment Document which will be reviewed by the Flow OHS Committee before work can commence.