Set Up a Flow Experiment

10 Tips for Flow Cytometry Success

Flow Cytometry

Presented here are some tips to keep in mind when starting a flow cytometry experiment. They will help you maximize cell separation and isolation and set you up for downstream processes, including cell sorting.

1 **Use a proper cell dissociation method.** Single-cell suspensions are critical to the success of a flow cytometry experiment. Use gentle mechanical dissociation, or nonenzymatic or trypsin alternatives, as these are better for preserving cell viability while keeping surface markers unchanged.

2 **Use the right temperature.** Live cells are not static; therefore, keep them cold if they can tolerate it. However, if you're using a mechanistic cell probe, you'll be better off keeping the cells warm.

3 **Filter your samples.** Nothing is worse than clogging the nozzle tip. Make sure you filter your cells to remove clumps.

4 **Use EDTA to avoid clumps.** If your cells are sticky and filtering doesn't solve the problem, use EDTA to help reduce clumps.

5 **Include serum or protein in your buffers.** This will increase the viability of your cells in all phases of the experiment, from staining and washing to sorting. Using 1–3% BSA or FBS in your buffers helps without making the cells too sticky. Some protocols don't work with protein, so make sure you know your protocol.

6 **Titrate your antibodies.** This improves the signal-to-noise ratio, reduces nonspecific binding, and increases sensitivity. Make sure you're using the right concentration of every reagent.

7 **Use a viability dye.** Antibodies bind to dead cells indiscriminately, so eliminating dead cells is a must. This needs to be carried out with a viability dye in each sample.

8 **Use compensation controls.** In order to use fluorophores that have overlapping emission spectra, single-stained controls must be used. The software can calculate the correct spillover values to ensure good data.

9 **Use FMOs.** Fluorescence minus one controls are excellent gating controls. If you're using multiple colors and the division between positive and negative isn't clear, these are a must.

10 **Know your cytometer.** Make sure you understand the excitation and emission wavelengths your cytometer can support. This determines the dyes you can use. Not every four-color instrument can utilize the same four dyes.

Visit **bio-rad.com/N/FlowSuccess** for more information.

BIO-RAD

Bulletin N6724 Ver A US/EG 15-0963 0316