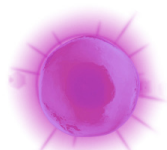


To Boldly Flow

Have confidence to start flow cytometry

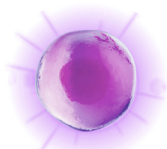


Flow cytometry is a powerful technique used in a wide variety of biological research, but if you are at the beginning of your flow journey it can be hard to know where to start. To help you with this, we have created a step-by-step guide to starting flow cytometry with useful tips and background knowledge to help plan, execute and interpret your experiment, ensuring you get the best results possible.



Know Your Instrument

If you don't know which lasers and filters are available, you are likely to get data you can't analyze. Our panel builder contains all the laser and filter settings of common instruments, as well as being fully customizable, enabling you to choose the right fluorophore for your instrument.



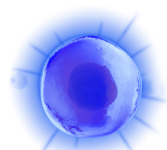
Sample Preparation

Sample preparation is key to success as poor samples can only give poor results. Looking after your cells during and after harvest is crucial, consider cell concentration and storage temperature amongst other things to keep your cells as healthy as possible.



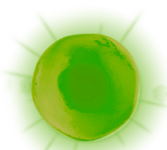
Marker Expression

Identification of cells through their marker expression is one of the key techniques in flow cytometry. To help you find the right marker, we have a marker selection tool, with maturation pathways for both human and mouse. Another important consideration is the level of marker expression or antigen density as this will influence your choice of fluorophore.



Cell Frequency

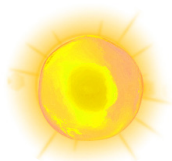
Cell frequency can influence both the choice of fluorophore and how many cells you need to collect to obtain meaningful data. If you are searching for rare cells such as stem cells, or have a complex gating strategy, you may have to collect many more cells compared to more common cell types such as T cells.



Fluorophore Choice

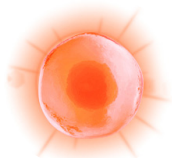
Choosing a fluorophore rapidly becomes complicated as you increase the number of fluorophores. Your fluorophore selection should take into account many things including the instrument, marker expression, excitation and emission wavelengths. The recommended way to avoid compensation and obtain the best resolution is to separate out fluorophores as much as possible across lasers and filters.

BIO-RAD



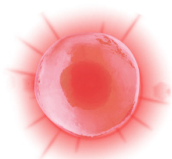
Antibodies

Finding the right antibody can be challenging. Search our website to find antibodies by marker, clone, isotype and target species, or use our panel builder to find antibodies with the right fluorophore. Titration can improve your data by reducing the level of background staining while maintaining a bright, positive population, and save you money!



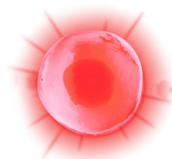
Controls

Controls are essential in any experiment to confirm positive results from background. In flow cytometry these can be biological, positive, negative, viability, isotypes, Fc blocking and fluorescence minus one controls depending upon your experiment. Dead cells bind antibodies non-specifically therefore it is essential to remove them from your analysis, using a viability dye. Easily fit controls to your experiment using our multicolor panel builder.



Staining Protocol

Depending on the location of your antigen on your cell, the staining protocol may change. Surface staining with antibodies can be relatively straightforward but intracellular staining requires fixation and permeabilization reagents, depending upon the antigen or technique being performed.



Data Analysis

Once you have collected your data, don't be afraid to play around with it and try different gates during analysis. Remember to remove dead cells using a viability dye rather than forward and side scatter. Removing doublets is also an essential part of your analysis to avoid false positives, particularly when looking at the cell cycle.

Visit bio-rad-antibodies.com/flow-panel-builder to see how our panel builder can help you every step of the way.



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