To Boldy 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, and is fully customizable, enabling you to choose the right fluorophore for your instrument.

Sample Preparation
Sample preparation is key to success, as poor samples give poor results. Looking after your cells during and after harvest is crucial. Consider cell concentration and storage temperature along with other parameters 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 considerations, including the instrument, marker expression, and 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.

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 and save you money by reducing the level of background staining while maintaining a bright, positive population.

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Controls

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

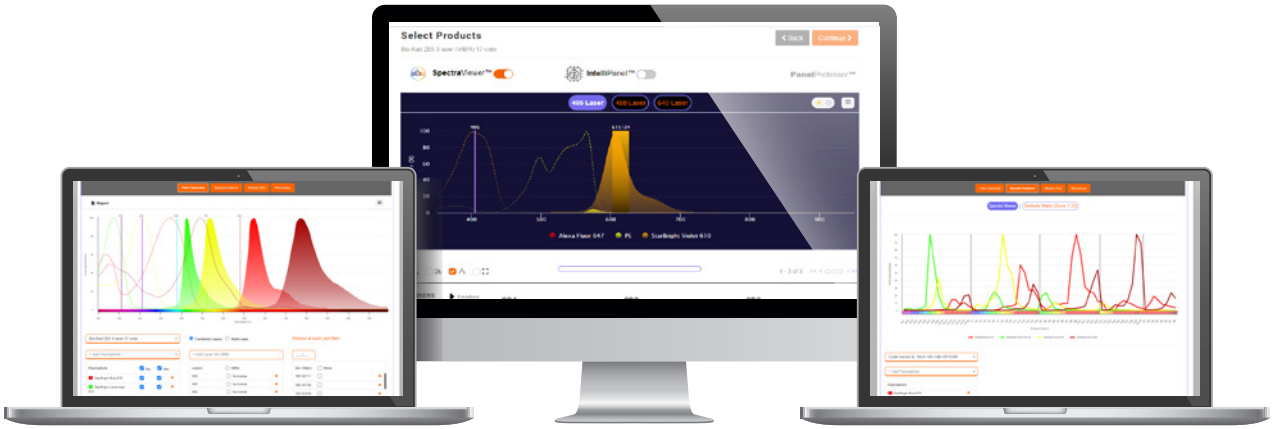
Staining Protocol

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 to avoid false positives is also an essential part of your analysis, particularly when looking at the cell cycle.

Visit **bio-rad-antibodies.com/flow-novice** for more information.



Visit **bio-rad-antibodies.com/spectraviewer** and **bio-rad-antibodies.com/flow-panel-builder** to see how our Fluorescence Spectraviewer and Multicolor Panel Builder tool can help you every step of the way in your experimental design.

StarBright Dyes

Visit **bio-rad-antibodies.com/StarBright** for more information about StarBright Dyes.

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