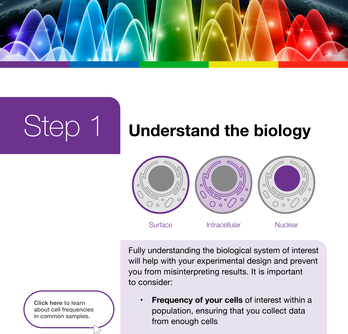
BIO-RAD

5 Steps to Flow Success

The route to successful flow cytometry that arrives at clean, easily analyzed data requires careful planning. Bio-Rad ensures you are equipped with the right tools for your journey.



Step 1

Understand the biology

Fully understanding the biological system of interest will help with your experimental design and prevent you from misinterpreting results. It is important to consider:

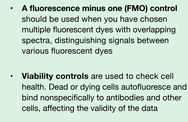
- **Frequency of your cells** of interest within a population, ensuring that you collect data from enough cells
- **Antigen expression**, checking that your detection method is able to identify rare or low-expression markers
- **Antigen location**, ensuring that you have planned your experiment with the ability to detect cell surface, intracellular, or nuclear markers

Click here to learn about cell frequencies in common samples.

Click here to learn about antigen density for human and murine surface markers.

Step 2

Choose antibodies and fluorescent dyes carefully



Check antibody datasheets to ensure they are supported in flow cytometry and match the target cell species. Ensure antibody-conjugated fluorescent dyes are compatible with the excitation lasers, filters, and detectors of your flow cytometer.

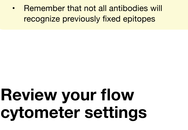
- If you are building a multicolor panel, it is best practice to separate your dyes across lasers and filters to minimize spectral overlap
- Always remember that for optimal detection, bright dyes should be reserved for rare markers, while less bright dyes can be used for abundant markers

The Bio-Rad Panel Builder tool can help you select compatible fluorescent dyes for your experiments. Click here to learn more.

Bio-Rad StarBright Dyes have been designed for flow cytometry with improved brightness and narrow excitation ranges. Click here to learn more.

Step 3

Consider controls



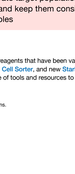
Suitable controls ensure that the experimental observations are genuine. They also reduce the need for burdensome troubleshooting and will give your data the validity it deserves. There are several types of control to consider:

- All experiments should include a **positive** and a **negative control**, demonstrating that your setup has the power to detect true positive and negative effects
- **A fluorescence minus one (FMO) control** should be used when you have chosen multiple fluorescent dyes with overlapping spectra, distinguishing signals between various fluorescent dyes
- **Viability controls** are used to check cell health. Dead or dying cells autofluoresce and bind nonspecifically to antibodies and other cells, affecting the validity of the data
- **Isotype controls** can help determine nonspecific binding

Click here to learn more about essential controls and when to use them to obtain publication-quality data.

Step 4

Plan your sample preparation



Sample preparation will depend on your cell type and source; for example, whether your cells are in suspension or from a primary tissue, and whether your cells will be fixed prior to, or after staining. For optimal sample preparation:

- Use membrane permeabilization for detecting intracellular antigens
- Remember to use an appropriate buffer for your experiment
- Filter cells before analyzing to create a single cell suspension and avoid clumps and aggregates. If you are sorting cells, perform this step immediately before the sort
- Remember that not all antibodies will recognize previously fixed epitopes

Click here to view a range of Bio-Rad's protocols that help with sample preparation.

Step 5

Review your flow cytometer settings

Before any experiment, you should check that the correct lasers and filters are selected to detect your fluorescent dyes:

- Ensure that voltages have been adjusted correctly, allowing you to see negative and positive populations on the same scale, and keep voltages consistent as you analyze different samples
- Check that the flow cytometer is running smoothly and that quality control steps have been completed without error
- Take time to set appropriate gates, allowing you to separate target populations from other cells, and keep them consistent across samples

Click here to learn how to identify different cell populations in a few simple steps using gates and regions.

Bio-Rad offers a variety of instruments, antibodies, kits, and reagents that have been validated for use in flow cytometry — including the ZE5 Cell Analyzer, the S3e Cell Sorter, and new StarBright Dyes. Visit bio-rad-antibodies.com/flow for a comprehensive range of tools and resources to ensure successful flow cytometry experiments.

BIO-RAD

Bulletin 3275 Ver B