# Flow cytometry workflow journey

We're with you every step of the way

After designing a flow cytometry panel, it is important to keep the following concepts in mind to help ensure optimal resolution of your populations of interest. **Protocol Jungle** Have questions about specific protocols? View our online Potocol Library here.

Tips and Tricks:

Frozen cells undergo

stress from freezing

and thawing. Resting

cells in culture may help

restore surface

phenotype or

biological function.

Staining Your Panel For best performance, be sure to:

- Keep tubes protected from light, and wash samples 2–3 times post staining to minimize background
- Adhere to incubation time and temperature requirements.
- Learn some practical strategies for conducting surface and intracellular staining. Watch now!

## Sample Acquisition

Compensation is a critial step in the flow cytometry workflow process as it can help correct fluorochrome

- Quality control your instrument and set up your settings to ensure optimal and consistent assay performance.
- Make sure all the positive signals are on scale before acquiring single-stained controls or samples.
- Run single-stain controls and calculate compensation.
- Determine the amounts of events to be recorded for robust analysis of cells of interest.
- Be sure to maintain acquisition speed constant across samples.
- Remember to clean your instrument after acquiring your samples.

Tips and Tricks: Do not change fluorescence PMTV during acquisition of compensation controls or after calculating compensation.

Check out some practical tips and tricks for conducting



Ready for Data Analysis

### **Secure Materials**

Start

Here

Before starting your experiment ensure you have all the reagents and products you need.

Products may include:

- Single-color reagents: BD offers over 18,000 singlevial reagents to meet your research needs
- Staining buffers such as BD Horizon™ Brilliant Stain Buffers and BD Pharmingen™ Stain Buffer (FBS or BSA)
- Permeabilization buffers
  - Lysis buffers
  - Fixation buffers
- Viability dyes

Before using any product, be sure to check the

Consider aliquoting viability in single-use amounts to prevent repeated freezethaw cycles.

### Tips and Tricks:

expiration date

dyes and nucleic acid stains

### Titration

For best resolution, identify the optimal staining concentration for your specific cell of interest by performing an antibody titration.

Tips and Tricks: Keep an eye on the sample volume the tube dry.

Watch our Scientist-to-Scientist Video to learn some useful strategies for cell preparation.

Feasibility Forest

To ensure experimental performance, it's important to

• Low frequency cell analysis may require an isolation

Protein transport inhibitors, such as BD GolgiStop™

(Monensin) and BD GolgiPlug™ (Brefeldin A), are

Pay attention to incubation time to avoid toxicity.

clumping to prevent high background staining or

used to trap cytokines/proteins inside the cells

Frozen and cultured cells, as well as tissue-derived cells,

often have many dead cells that can demonstrate

unusual autofluorescence and nonspecific staining.

Filtration with a cell strainer can remove cell

Further cell death can be prevented

**Cell Preparation Methods** 

use appropriate cell preparation methods.

and enrichment step

instrument clogging.

your analysis.

by maintaining cells at the

appropriate temperature.

dead cells out can improve

Using a viability marker to gate

Cell activation

**Controls** 

Appropriate controls are critical for accurate interpretation of results.

Controls	Use
Single-color controls	Either cells or beads used to calculate compensation and eliminate spillover artifacts
Biological controls	Resting vs activated or healthy vs disease cells used to increase confidence in data by confirming exclusive expression patterns in specific samples
Fluorescence minus one (FMO) controls	Stained cells in cocktails containing all fluorochromes but one at a time to help set gates and allow the identification of fluorochromes that introduce spread into given detectors

Watch our Scientist-to-Scientist Video to learn how choosing right controls can help improve the resolution of your experiment.

To access more flow cytometry resources, contact your BD Sales Representative.



Videos



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