

Flow Cytometry Panel Design Cheat Sheet

1. Define the Biological Question

- Identify primary population
- List essential markers
- List optional markers
- Identify expected low-expression markers
- Identify co-expression patterns

2. Confirm Instrument Configuration

- List available lasers
- Confirm detector/filter configuration

3. Rank Markers by Antigen Density

Expression Level	Fluorophore Strategy
Very Low	Assign brightest dyes
Moderate	Assign moderate dyes
High	Assign moderate/dim dyes

4. Evaluate Spectral Overlap

- Avoid overlapping dyes on co-expressed markers
- Distribute markers across laser lines
- Review emission spectra before finalizing

5. Controls Checklist

- Unstained control
- Single-stained compensation controls
- FMO controls for dim markers
- Isotype controls for nonspecific binding
- Biological negative control

6. Final Review

- Are brightest dyes reserved for dim markers?
- Are co-expressed markers spectrally separated?
- Are essential markers prioritized?
- Are controls planned?

ESSENTIAL TOOLS

Spectra Viewer

innocyto.com/web/fluorescence-spectra-viewer.php



Cell Marker Expression Tool

innocyto.com/web/cell-maker-expression-tool.php



Fluorophore Brightness Tool

innocyto.com/web/fluorophore-brightness-tool.php



Panel Builder

innocyto.com/web/panel-builder-tool.php

