Flow Cytometry Panel Design Journey

**Waterfall of Discovery**
There are hundreds of research applications for flow cytometry.

**Define your experimental hypothesis**

**Step 1:** Defining your experimental hypothesis is the first step in panel design. Start with identifying:

- The biological information you are trying to achieve
- The population(s) of cells you wish to interrogate
- Whether targets are found on the cell surface or intracellularly

**Marker selection**

**Step 2:** During the second step of the panel design process, you will need to identify which and how many markers you need to identify the population of interest.

Pay attention to:

- Marker expression levels
- Primary antigen: Expressed at high density, often defining lineages
- Secondary antigen: Often expressed over a continuum
- Tertiary antigen: Critical markers expressed at low density
- Marker coexpression, especially of dim markers
- The gating strategy needed to identify the population(s) of cells you wish to interrogate

**Fluorochrome assignment**

**Step 4:** Carefully select fluorochromes to resolve markers at all expression levels and minimize spectral overlap. Consider using tools like a fluorochrome resolution ranking and a spectrum viewer to help assess:

- Cross laser excitation
- Fluorochrome spillover

Remember to pair bright fluorochromes with low expressing antigens and dim fluorochromes with high expressors. Keep in mind that spread only impacts the resolution of coexpressed markers.

**Review panel**

**Step 5:** Review your panel design and begin ordering your reagents.

Remember to titrate your mass size reagents and optimize your staining protocol. Include proper controls for compensation, FMO and biological controls to help ensure optimal panel performance.

To learn more about flow cytometry panel design resources or for support, please contact your BD Sales Representative.

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