

PANEL DESIGN GUIDELINES

PROCEDURE

1.0 General Panel Design Guidelines

- 1.1 Make sure instrument can accommodate all fluorochromes in panel.
 - A. Equipment descriptions with available PMT detectors are on iLabs under schedule equipment tab. Expand description next to machine name. Flow Core instrument configurations are also uploaded in FluoroFinder.
- 1.2 As you add fluorochromes to the panel, choose fluorochromes excited by every laser on the chosen instrument. This will increase sensitivity.
- 1.3 Use the entire UV/visible/NIR spectrum for fluorescence emission. This will increase sensitivity.
- 1.4 Generally, only one marker should be used with each PMT detector channel.
 - A. Often, one PMT could be used for several dyes with similar spectral properties.
 - B. Example: GFP, FITC and Alexa Fluor 488 are all collected in the same channel. In most cases, you must pick only one of these in a panel.
- 1.5 Choose low expression/rare markers on brightest dyes and high expression markers on dimmer dyes. A few examples below:
 - A. Brighter Dyes: Brilliant Violet dyes, PE and APC.
 - B. Dimmer Dyes: FITC, PerCP-Cy5.5 and PE-Cy7.
 - C. High Expression: CD45 and CD8 in normal PBMC.
 - D. Low Expression: CTLA-4, PD-1, c-kit, and FoxP3 in normal, healthy tissue.
- 1.6 Use mutually exclusive markers or pairs in channels with high level of spillover.
 - A. Example: CD4 and CD8.
- 1.7 Include a viability dye to exclude dead cells. Choose an amine reactive dye for fixed samples.
- 1.8 When selecting reagents for a new panel, remember that commonly used markers will be available in a wider selection of fluorochrome conjugates. Select fluorochromes for less common markers first.
- 1.9 As the number of colors increases in panel the more important it is in designing panel. Contact the flow core to confirm your choices before purchasing reagents.

RELATED PROCEDURES

This handout is related to ACSF SOP EX005. It is a handout only.