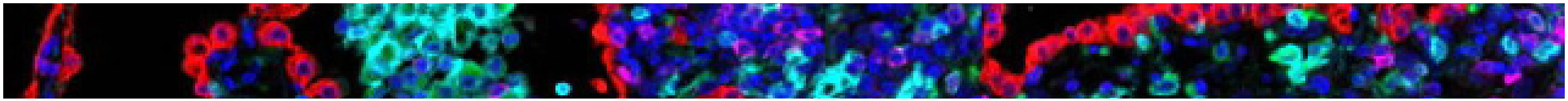
Panel Design Consideration



Pairing Opal Fluorophores to Primary Antibodies

Opal fluorophores need to be paired to each marker. To help in your selection process, we suggest considering the following:

1. Rare vs. Abundant Markers

- Low expressors should be assigned to brighter fluorophores, while more abundant markers should be allotted to dimmer fluorophores (see table below)

Opal Brightness Rankings	
Fluorophore	**Vectra Polaris**
Opal Polaris 480	Highest
Opal 520	Highest
Opal 540	Medium
Opal 570	Medium
Opal 620	Medium
Opal 650	Highest
Opal 690	Low
Opal Polaris 780	Lowest

Staining Order

Choose a staining sequence for each marker based on the the amount of antigen retrieval required, its biology, and co-localization.

2. Antigen Retrieval

- The order of immunostaining can change signal intensity! Some epitopes become more exposed after successive rounds of HIER and result in greater signal when detected later in a multiplex protocol. Some rare epitopes may be degraded by multiple exposures to heat and should be placed early in the staining order.

3. Biology

- Some Opal fluorophore signal intensity can also be affected by HIER (i.e., attenuation of Opal 520 and Opal 570). Designing your monoplexes with the correct number of HIER can help determine any corrections needed on Opal concentration and assay order prior to running the multiplex.

4. Co-localization

- When possible, arrange the order so sequential antibodies do not co-localize in the same cellular compartments within the same cells.

RESOURCES—For additional resources and information, please see our updated Antibody-Opal pairing guide on our website under **Phenoptics Reagent Support.**

*Opal Polaris 780 should be allocated for the last position in the staining order sequence and as it is the dimmer dye, it should be paired with the most abundant marker.