Flow Post-its
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Vital Dyes in Flow Cytometry

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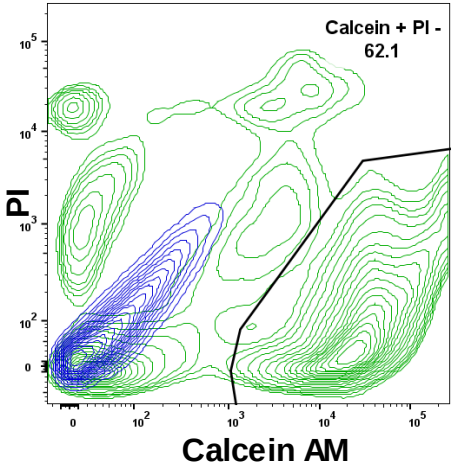
Why should we use vital dyes?

- Distinguishing debris from small cells in tissue preps for Flow Cytometry can often be difficult. Dead cell removal (through nuclear or amine reactive viability dyes) and scatter gating alone ***cannot*** be used in scenarios such as these to pull out live cells for analysis or sorting. **Remember that debris will not always be stained by your viability dye**.
- The use of a vital dye in conjunction with a dead cell exclusion dye allows researchers to eliminate dead cells and debris through clear visualization of metabolically active live cells.
- Vital dyes are available in a variety of wavelengths for excitation and emission, making them compatible for multicolor panel design.

Helpful Tips

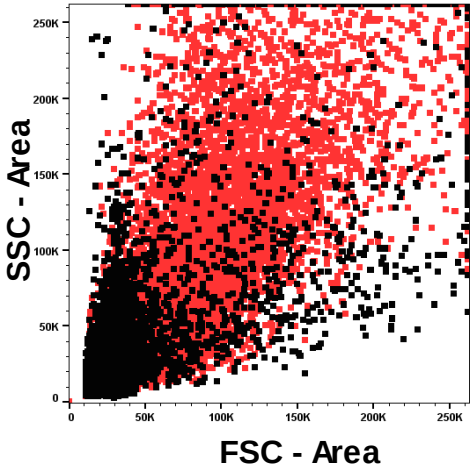
- Calcein can enter through the membrane of live cells. Esterases present in these metabolically active cells cleave the acetoxymethyl ester group of calcein, resulting in fluorescence and subsequent live cell identification by flow cytometry/microscopy.
- Titration of vital dyes is highly suggested. Excess dye can result in cytotoxicity.
- Adding vital dyes is recommended when removal of debris is essential for accurate analysis or successful downstream applications after sorting (96 or 384 well plate sorting, sequencing, etc.)
- DNA binding Draq5 and Hoechst dyes can also be used to identify live cells.



Sample **(+)** PI/Calcein AM

Sample **(-)** PI/Calcein AM

Overlay above shows the same sample. The addition of Calcein AM and PI showed that only 62% of these events were live cells excluding debris.
Note: Calcein AM and PI double positive events indicate cells that are in the process of dying.



Calcein AM **Positive**

Calcein AM **Negative**

Sample above pre gated to exclude dead cells and small debris as seen by scatter. Red events are Calcein-AM positive, whereas black events were Calcein-AM negative. Overlapping events show that the vital dye is necessary for live cell identification