Flow Post-its
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DAPI Permeability

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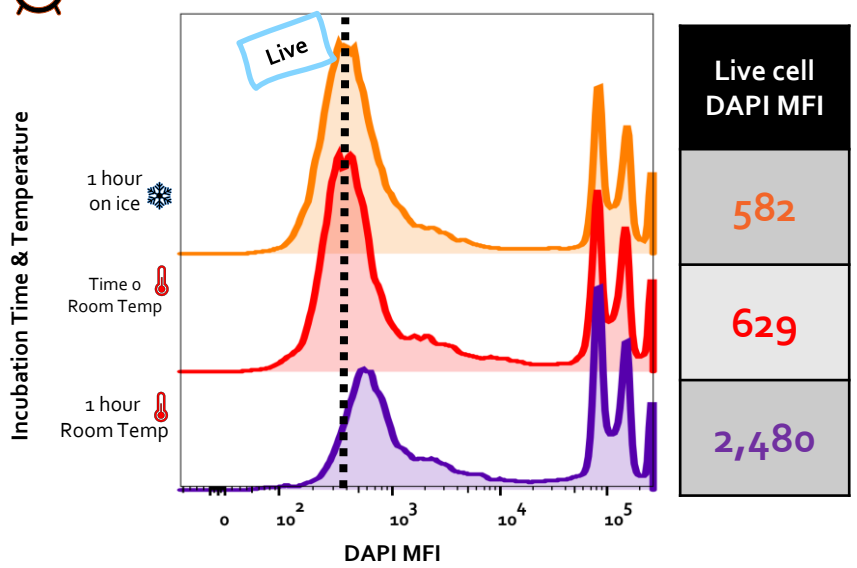
DAPI (4′,6-diamidino-2-phenylindole) is a fluorescent stain often used to differentiate between live and dead cells for **viability measurements** in flow cytometry. This reagent is a popular choice due to its short incubation time and high relative brightness. Similar to other reagents, DAPI staining conditions must be optimized for best experimental results.

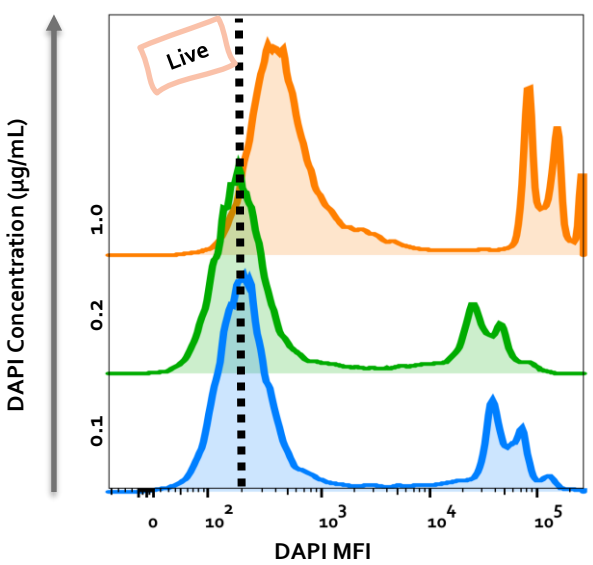
Incubation Conditions



Live cell DAPI MFI
582
629
2,480

Samples in figure above were all stained at a final concentration of 1.0 µg/mL. When left at room temperature for 1 h the live population experienced a shift in DAPI signal, whereas the sample kept on ice did not, indicating colder temperatures can be beneficial in preventing DAPI uptake in viable cells.

Concentration



Live cell DAPI MFI
582
241
259

Figure above shows the the impact of staining with increasing concentrations of DAPI. All samples were kept on ice and acquired immediately after DAPI was added. 1.0 µg/mL shows a shift of the live cell population, indicating the concentration is too high.

DAPI is a fast acting, bright fluorochrome which binds to the Adenine-Thymine regions in dsDNA. It is important to remember that DAPI can be permeable to live cells. **DAPI uptake by live cells can be prevented by optimizing concentration, adding it immediately prior to acquisition and controlling sample temperature,** understanding that ideal conditions can vary across cell types.

The FCCF thanks Sara Haddock for providing fcs files used in this Post-it