Dyes for Paraformaldehyde Fixed Samples

There are many more dead-cell discrimination dyes available with varied excitations and emissions for use in immunophenotyping and other assays. These include the amine reactive dye sets (made by all major suppliers) that can be used in either live or fixed cell assays.

Amine Reactive Dyes:

Amine reactive dyes are another class of molecules that selectively label dead versus live cells. Like classical DNA binding dyes, they are similarly reliant on cell permeability to enter the cell. Unlike DNA stains, these dyes irreversibly react with free amine groups of proteins both on the surface of or inside cells. The availability of the free amine groups on the cell's interior versus exterior is much greater and therefore dead cells stain much more brightly in comparison to healthy cells. These dyes can also withstand harsh cross-linking reactions, making them very useful to measure viability at the time of fixation.

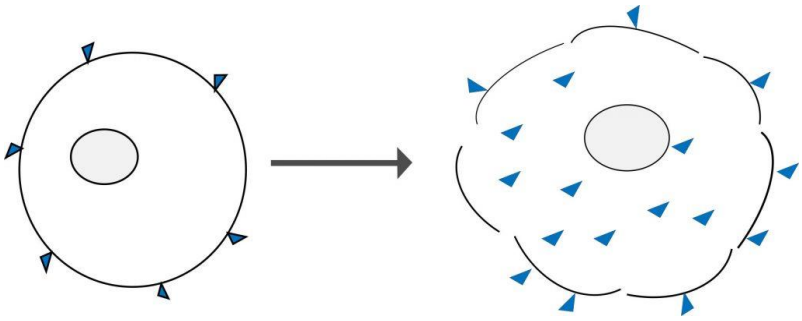

Figure 1: Amine reactive dyes bind only to the surface of live cells (left) and in lesser amounts when compared to cells that have lost membrane integrity (right). The porous membrane allows greater dye access to the free amines group of intracellular proteins which yields a brighter fluorescent signal when viewed on a flow cytometer.

These dyes have become more popular since first coming onto the market about approximately 10 years ago and are available in an array of colours allowing for easy incorporation into any staining panel.

EMA (Ethidium Monoazide) Bromide:

Ethidium monoazide bromide is a fluorescent nucleic acid stain that covalently binds DNA after photolysis when exposed to UV light. Because this probe is relatively impermeant to live cells, it selectively labels DNA in dead vs. live cells making it useful in complex staining protocols involving fixation and further staining. This method is less widely used simply due to attirition due to the popularity of the amine reactive dyes, but remains a cheap alternative for dead cell staining and offers an additional emission band over not available in with the amine reactive dyes i.e. PE-Texas red emission wavelengths.

Preparation:

- Reagents: EMA powder Molecular Probes: E1374 or Sigma-Aldrich: E2028
- Prepare EMA stock solution to 5mg/mL in DMSO and store as single use aliquots in a desiccator at -20 °C under foil or in light resistant tubes.
- Depending on the optimal working concentration for your current lot, add enough EMA (dilute accordingly) to achieve a final concentration of 0.5 – 5 µg/mL
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    - Titration will be necessary - Too much EMA can lead to extremely high background fluorescence leading to an underestimation of live cells….
- If using EMA at 0.5 µg/mL, add 10 µL of stock solution to 990 µl of FACS staining buffer.
- Add 1 µL of working solution/ 100 µL of final staining volume/ sample (stain cells in ~ 100-200 µL)
- Incubate cells from 10-15 minutes under a bright fluorescent lamp at distance of approximately 18 cm.
- Wash cells 2x with PBS
- Stain with extracellular flours and then fix as desired.

Spectral Properties:

- EMA is very light sensitive to care must be taken to minimize exposure to light when making the stock solution, storage and up until ready to add to samples.
- EMA can be excited with 488 nm or 561 nm laser light (excitation max = 510 nm) with detection in the PECy5 (670nm) or PE-Texas (610) red channels (emission max = 600 nm).
- Include an EMA compensation control when using in multicolour flow cytometry applications
    - EMA emits across a wide range of wavelengths (see spectrum below) and can potentially have large spillover values with fluors like PE-Cy5

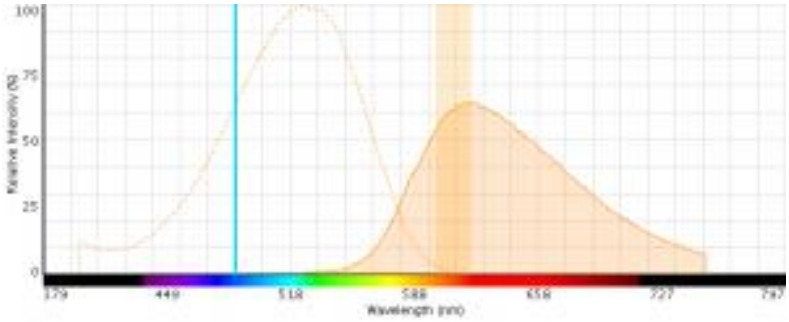

Figure2: EMA excitation at 488nm with an emission bandpass filter typical for PE-Texas Red centred at 610nm.

Useful Links:

BD Biosciences: BD Horizon Fixable Viability (FVS) Reagents

Thermo Fisher: Fixable Viability Dyes for Flow Cytometry

Biolegend: All New Zombie Dyes (Blog Post)

ThermoFisher: Ethidium Monoazide Bromide

ThermoFishser: Checking Vital Signs: Don't Let Dead Cells Mislead You

Biolegend: Live Cell/Dead Cell Discrimination

R&D Systems: Flow Cytometry Protocol for Analysis of Cell Viability using Propidium Iodide

Expert Cytometry: 3 Reagents for Identifying Live, Dead, And Apoptotic Cells by Flow Cytometry

BitesizeBio.com: Viability Dyes for Flow Cytometry: It's Not Just a Matter of Life and Death

References:

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Perfetto S.P, et al. (2006) Amine reactive dyes: An effective tool to discriminate live and dead cells in polychromatic flow cytometery. *Journal of Immunological Methods*, **313,** Issue 1-2, 30, pp 199-208. https://doi.org/10.1016/j.jim.2006.04.007

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