

## Dead Cell Discrimination in Unfixed Samples

### Classical Dyes for Dead Cell Discrimination in Unfixed Samples

When live cell analysis is required, the following stains may be used to separate dead/dying cells from healthy cells. Dyes like PI/7-AAD and DAPI are not able to transit across intact cell membranes and are not fluorescent or have only weak fluorescence until intercalated between the DNA strands. This makes them excellent dead cell probes as they yield fluorescence once inside the cell.

For most flow cytometry experiments with these dyes, cells are stained with other fluorophores, using standard staining methods required for the primary assay, followed by the addition of the dead cell markers without a final wash step.

Note: All reagents below are available in working concentrations from various suppliers, but if you wish, they can be made from dry reagents at much less cost.

### PI (Propidium Iodide)

#### Preparation:

- Reagents: PI-Sigma-Aldrich P4170), PBS, Sodium Azide (Sigma-Aldrich S8032)
- Prepare a stock solution of PI at 1 mg/mL in PBS containing 0.01% Sodium Azide (Undiluted stock is stable for up to 6 months at 4°C).
- Aliquot into 2-4 mL portions and store at 4 degrees wrapped in foil.

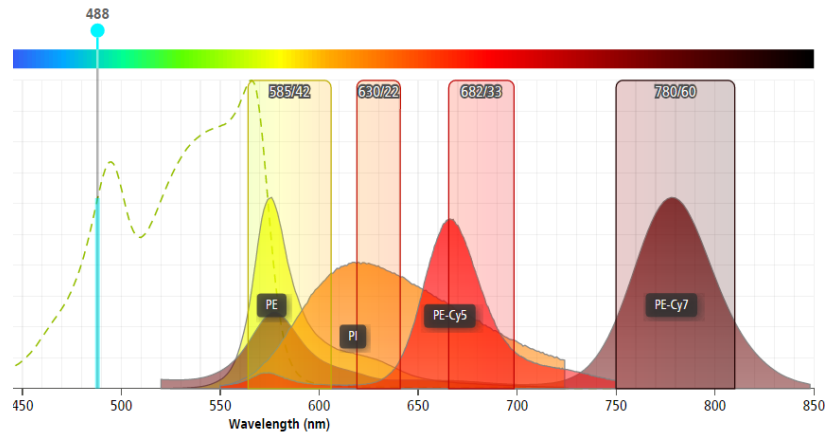
#### Procedure:

- Dilute an aliquot of the PI stock solution to 100 µg/mL
- Add PI washed and stained cells ready for flow cytometric analysis
- Add 1uL of PI stock solution per each 100 uL of staining buffer in your samples
- Incubate cells in the dark for 5-15 mins - Do not wash
- Acquire data on a flow Cytometer.

#### Spectral Properties

- Strong excitation at 488 nm (Blue laser), stronger excitation at 561 nm (yellow-green).
- Detected anywhere from 550 – 700 nm.
- Significant overlap between PE and PE-tandems to 700 nm
- Minimal compensation requirements required between FITC/GFP and PI if data acquired at longer wavelengths (~650-700 nm)

## Dead Cell Discrimination in Unfixed Samples



**Figure 1:** Spectral overlap of Propidium Iodide with PE and PE tandem fluorescent stains.

### 7-AAD (7-Aminoactinomycin-D):

#### Preparation:

- Reagents: 7-AAD (Sigma-Aldrich A9400), PBS, Sodium Azide (Sigma-Aldrich S8032), DMSO (D5897)
- Prepare a stock solution of 7-AAD at 1 mg/mL by dissolving 1.0 mg 7-AAD powder into 50  $\mu$ L of DMSO.
- Add 950  $\mu$ L of PBS containing 0.01% Sodium Azide
- Store in the fridge for 1 month in the dark.

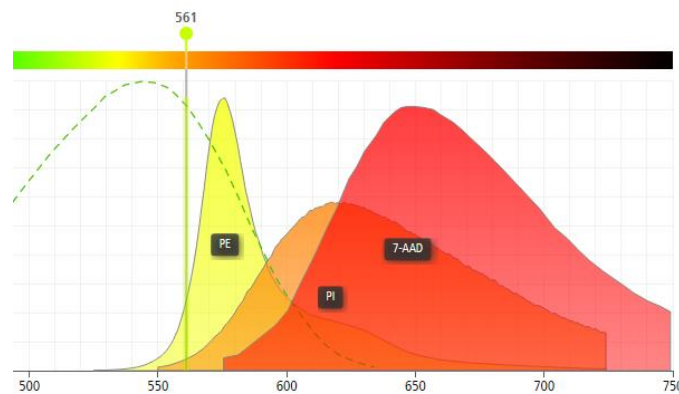
#### Procedure:

- Add 1  $\mu$ L of 7-AAD stock solution to approximately  $1 \times 10^6$  cells ready for analysis.
- Incubate cells in the dark for 15 mins at room temperature - do not wash
- Acquire data on a flow Cytometer.

#### Spectral Properties:

- 7-AAD is excited at 488 nm and emits at  $\sim 670$  nm flow cytometer or can be detected between 600 and 750 nm.
- 7-AAD is less bright than PI, but gives good resolution between live and dead cells.
- There is also less spillover with PE conjugates making it easier to compensate from these detectors.

## Dead Cell Discrimination in Unfixed Samples



**Figure 2:** Spectral overlap of PE, Propidium iodide and 7-AAD with 561 nm laser excitation.

### DAPI (4', 6-Diamidino-2-Phenylindole, Dihydrochloride):

#### Preparation:

- Reagents: DAPI (Sigma – 10236276001)
- Preparation of stock solution: Dissolve in de-ionized water to a final concentration of 1 to 5 mg/ml.
  - Note: Do not use any buffers.
- Preparation of working solution: Dilute the stock solution with methanol to a final concentration of 1 µg/ml. The working solution is stable at 2 to 8 °C for about 6 months.
  - Storage conditions:
    - Stock solution (1 to 5 mg/ml) at -15 to -25 °C for 12 months.
    - Working solution (1µg/ml) at 2 to 8 °C for about 6 months.

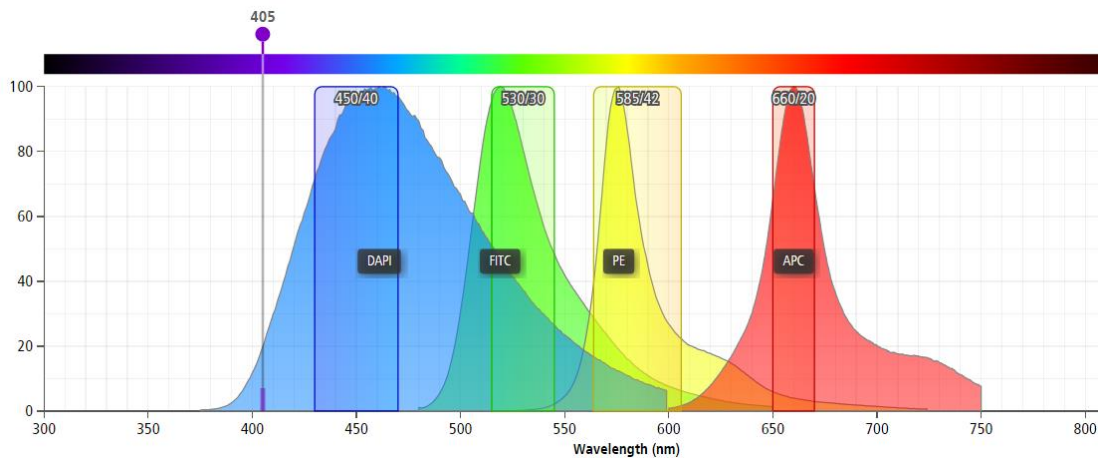
#### Procedure:

- Prepare samples for flow cytometry
- After the final wash step resuspend the cells in PBS with 1- 2% FBS and sodium azide containing 0.05-0.2 µ g/mL DAPI.
  - The optimal concentration of DAPI for viability analysis may vary by cell type. Please titrate the reagent for your cell type to ensure good resolution without oversaturation.
  - Do not add azide to the buffer if cells are being prepared for sorting.
- Incubate 5 minutes at room temperature. Do not wash.
- Acquire flow cytometry data.

## Dead Cell Discrimination in Unfixed Samples

### Spectral Properties:

- DAPI can be excited at 355, 375 or 405 nm with emission<sub>max</sub> at 460 nm (450/50 bandpass filter).
- DAPI cannot be used with fluorophores like Brilliant Violet 421, eFluor450, VioBlue Dye, V450 or Alexa Fluor® 405
- Use of the violet laser for DAPI excitation leaves commonly used detectors for the blue and red lasers free with little expectations for compensation between colours (depending on fluors chosen).



**Figure 3:** Compatibility of DAPI with commonly used flow cytometry fluorophores

### Other dyes available for unfixed live/dead discrimination include:

Reagent	Mechanism
NucGreen® Dead 488 Ready Probes® Reagent	DNA binding in porous cells.
<a href="#">SYTOX® DNA-binding dyes</a>	Binding of dsDNA/RNA in cells with poor membrane integrity
Cell Trace™ Calcein Esterase substrates for cell vitality	Cleavage of the AM esterase group in metabolically active cells yields fluorescence compared to dead cells where the AM group does not occur

### Useful Links:

ThermoFisher: [Checking Vital Signs: Don't Let Dead Cells Mislead You](#)

Biolegend: [Live Cell/Dead Cell Discrimination](#)

## Dead Cell Discrimination in Unfixed Samples

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:

Kuonen, F., et al. (2010), Fc block treatment, dead cells exclusion, and cell aggregates discrimination concur to prevent phenotypical artifacts in the analysis of subpopulations of tumor-infiltrating CD11b<sup>+</sup> myelomonocytic cells. *Cytometry*, 77A: 1082–1090.  
doi:10.1002/cyto.a.20969

Schmid I et. al, (2001), Simultaneous flow cytometric measurement of viability and lymphocyte subset proliferation, *Journal of Immunological Methods*, 247, Issue 1-2, pp. 175-186. [https://doi.org/10.1016/S0022-1759\(00\)00323-9](https://doi.org/10.1016/S0022-1759(00)00323-9)

Schmid, I., Krall, W. J., Uittenbogaart, C. H., Braun, J. and Giorgi, J. V. (1992), Dead cell discrimination with 7-amino-actinomycin D in combination with dual color immunofluorescence in single laser flow cytometry. *Cytometry*, 13: 204–208. [doi:10.1002/cyto.990130216](https://doi.org/10.1002/cyto.990130216)