# Dead Cell Exclusion

Because dead cells tend to bind nonspecifically to many reagents, they often give rise to false positive results in flow cytometry. Identifying and removing data points representing dead cells is a critical step in ensuring accurate data.

Loss of membrane integrity is a definitive indicator of cell death in flow cytometric assays. Cells that exclude a dead cell dye are considered viable, whereas cells with a compromised membrane allow the dye inside into cell to stain an internal component, thus identifying the cell as dead. Nucleic acid intercalating dyes are impermeant dyes that enter a dead cell and bind to the DNA, where they undergo a significant fluorescence enhancement. In contrast fixable dyes are based on the reaction of a fluorescent reactive dye with cellular proteins (amine-reactive dyes). In live cells, only surface proteins bind to the reactive dye, resulting in dim fluorescence. The reactive dye can enter dead cells and label cytoplasmatic proteins, resulting in at least a 50-fold increase in fluorescence.

***Note**: Impermeant DNA intercalating dyes are not compatible with fixation or intracellular staining protocols.*

## Propidium Iodide (PI)

- ✓ Penetrates cells with compromised plasma membranes, excluded from viable cell
- ✓ DNA intercalating, impermeant
- ✓ Ex/Em: 535/617 nm

## 7-Aminoactinomycin D (7-AAD)

- ✓ Penetrates cells with compromised plasma membranes, excluded from viable cell
- ✓ DNA intercalating, semi-permeant
- ✓ Ex/Em: 546/647 nm

***Advantage:*** Large Stoke's shift makes 7-AAD compatible with most blue and green fluorophores

## DAPI

- ✓ Cell permeable; can also enter living cells, but a high concentration is required
- ✓ Ex/Em: 358/461 nm (can be excited by 355, 375 and 405 nm laser)

***Advantage:*** Availability due to frequent usage in microscopy

## Monomeric Cyanine Nucleic Acid Stains (Thermo Fisher Scientific)

- ✓ Penetrates cells with compromised plasma membranes, excluded from viable cell
- ✓ DNA intercalating

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|---|---|
| **TO-PRO®-1** | Ex/Em: 515/531 nm |
| **TP- PRO®-3** | Ex/Em: 642/661 nm |

***Advantage:*** Low background and bright fluorescence.

## Sytox® Dead Cell Stains (Thermo Fisher Scientific)

- ✓ Penetrates cells with compromised plasma membranes, excluded from viable cell
- ✓ DNA intercalating

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|---|---|
| **SYTOX® Blue** | Ex/Em: 444 /480 nm (DAPI) |
| **SYTOX® Green** | Ex/Em: 488 /525 nm (FITC) |
| **SYTOX® Red** | Ex/Em: 640 /660 nm (APC) |

***Advantage:*** Cell applicable without additional washing steps because they are non-fluorescent in aqueous media.

## LIVE/DEAD® Fixable Dead Cell Stains (Thermo Fisher Scientific)

- ✓ Fixable, amine-reactive dye (application prior to fixation)
- ✓ Penetrates cells with compromised plasma membranes and covalently react with primary amines of cell proteins

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|---|---|
| **Violet Dead Cell Stain** | Ex/Em: 488 or 561/615 nm |
| **Green Dead Cell Stain** | Ex/Em: 405/440 nm |
| **Red Dead Cell Stain** | Ex/Em: 640 /660 nm |

## Zombie Dyes (Biolegend)

- ✓ Fixable, amine-reactive dye
- ✓ Penetrates cells with compromised plasma membranes and covalently react with primary amines of cell proteins

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| **UV ™** | Ex/Em: UV/DAPI |
| **Violet™** | Ex/Em: Violet/ BV421 |
| **Green™** | Ex/Em: Blue/FTIC |
| **Red™** | Ex/Em: Red/Texas-Red |
| **NIR™** | Ex/Em: Red/APCCy-7 |