Protocol for Staining Dead Cells with Viability Dyes

The Viability Dyes can be used to exclude non-viable cells in flow cytometry assays. They irreversibly bind to cell surface and intracellular amines. The dye can covalently bind to a higher concentration of amines in cells that have lost plasma membrane integrity, such as those in the late stages of apoptosis or dead cells, and generate a significantly more intense fluorescence signal. Dead dells stained with the viability dye will maintain their staining intensity after washing, fixation, permeabilization, or additional intracellular staining.

1. Allow vial of Viability Dye to equilibrate to room temperature and quickly spin before use.
2. Wash cells twice in PBS solution free of azide, serum or protein.
3. Resuspend cells in azide,serum, and protein free PBS at a concentration of 1-10x10^6/mL.
4. Add 1 µL of Fixable Viability Dye per 1 mL of cells and vortex immediately.
5. Incubate the cells for 30 minutes protected from light at 2-8 °C.
6. Wash the cells with flow staining buffer or equivalent.
7. Cells are ready to be stain, fixed, and permeabilized as desired.