

Viability/Cytotoxicity cell staining kit (Calcein AM, PI)

Flow cytometry

- 1. Thaw reagents and prepare staining working solutions by adding 5 μ L of component A (Calcein AM) and 30 μ L of component B (PI) to 10 mL of PBS, and vortex to mix.
- 2. Wash the cells 2-3 times with 1X PBS through centrifugation.
- 3. Resuspend cells in 0.5 mL of staining working solution. The cell density should be 1-5 X 10^5 cells/mL.

Note: We recommend preparing two tubes of additional samples, each tube with only one dye (Calcein AM and PI) for compensation adjustment of each dye.

- 4. Incubate for 15-20 min in the dark at room temperature.
- 5. Assay the sample by flow cytometry. Calcein AM's excitation/ emission maxima are 488/520 nm and PI excitation/emission maxima are 493/636 nm.

Note: Use single dye tubes to adjust and compensate, and the result should be two relatively independent cell populations: a live cell population with green fluorescence and a dead cell population with red fluorescence.

Fluorescence microscopy

1. Thaw reagents and prepare staining working solutions by adding 5 μ L of component A (Calcein AM) and 30 μ L of component B (PI) to 10 mL of PBS, and vortex to mix.

Note: For adherent cells, staining can be done directly. For suspension cells, centrifuge to collect cells for staining.

- 2. Wash the cells 2-3 times with 1X PBS.
- 3. Aspirate and discard PBS. For adherent cells, add Calcein AM/ PI staining solution to cover the sample. For suspended cells, add enough working solution to bring cell density to 1-5 X 10^5 cells/mL.
- 4. Incubate for 15-20 min at room temperature, protected from light.
- 5. Mount and observe the labeled cells under a fluorescence microscope.

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