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Viability/Cytotoxicity cell staining kit (Calcein AM, EthD-1)

Flow cytometry

1. **Thaw reagents and prepare staining working solutions by adding 5 μL of component A (Calcein AM) and 20 μL of component B (EthD-I) to 10 mL of PBS, and vortex to mix.**
2. **Wash sample 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 EthD-I) for compensation adjustment of each dye.
4. **Incubate for 15-20 min at room temperature, protected from light.**
5. **Assay the sample by flow cytometry. Calcein AM's excitation/emission maxima are 488/520nm and EthD-I's excitation/emission maxima are 528/617nm.**
   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 μL of component A (Calcein AM) and 20 μL of component B (EthD-I) 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/EthD-I 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.**