Calcein AM Cell Viability Assay Kit

Introduction

The Calcein AM Cell Viability Assay Kit is a kit that uses the fluorescent probe calcein AM to detect cell viability. Calcein AM is a cell-permeable derivative of calcein, which makes it easier to enter cells. Once inside the cells, no-fluorescent calcein AM is hydrolyzed into green-fluorescent calcein by esterases. Calcein is highly negatively charged and is remained in the cells. Since dead cells lack esterase enzymes, calcein AM can only stain live cells, and its fluorescence signal is proportional to the number of living cells.

Calcein AM has low toxicity to cells, so it is currently one of the most ideal probes for live-cell staining. In addition, calcein AM is more sensitive than common reagents (e.g., MTT, CCK-8) for detecting cell viability/proliferation, as this kit can detect as few as 50 cells per well.

Components and Storage

Components	K2231-100 T	K2231-500 T	K2231-2500 T
Calcein AM（1000X）	12 μL	55 μL	260 μL
Dilution buffer	12 mL	55 mL	260 mL

This kit should be stored at -20°C, stable for 1 year. Calcein AM（1000X）should be stored at -20°C away from light and moisture, and avoid repeated freeze/thaw cycles.

Protocol

1. **Preparation of Calcein AM working solution:** Dilute appropriate Calcein AM (1000X) in dilution buffer at a ratio of 1:1000 (1 μL Calcein AM (1000X) + 1 mL dilution buffer), mix well to obtain Calcein AM working solution. The working solution is unstable, any unused working solution should be discarded after use.

***Note:** Calcein AM (1000X) is susceptible to hydrolysis. So, it is recommended to be prepared in single-use aliquots. Before use, allow the Calcein AM (1000X) to warm to room temperature.

2. **Fluorescence microplate reader or fluorescence microscopy detection:**

Here, we take adherent cells as an example. For suspension cells, harvest cells and resuspend them in Calcein AM working solution, then perform similarly to the adherent cells.

1) **Cell seeding:** Cells are seeded in 96-well plates or other plates, and cells can be treated as designed. For 96-well plates, it is recommended to plate 100-10000 cells/well, and 2000-5000 cells/well is optimal.

*Note: If use a fluorescence microplate reader for detection, please seed cells in black culture plates.

2) **Wash:** Remove the cell culture medium and wash cells in PBS one time.

*Note: Serum and phenol red can increase background fluorescence. This step is to reduce the background caused by residual serum and phenol red.

3) **Staining:** Add appropriate Calcein AM working solution to cover cells. Incubate at 37°C away from light for 30 min. For 96-well plates, 100 μL per well working solution is needed. For 6-well plates, 1 mL per well working solution is needed.

*Note: The optimal time for incubation varies depending on the type of cells.

4) **Detection:** After incubation, wash the cells with PBS 1-2 times. The fluorescence signal can be detected by a fluorescent microplate reader (Ex/Em: 494/517 nm). Or use a fluorescence microscope for observation. Other counterstains can be continued if needed.

3. **Flow cytometry detection:**

1) **Prepare cells:** For adherent cells, use trypsin to digest cells and then resuspend cells in culture medium. Washed cells in PBS one time. For suspension cells, centrifuge at 800 rpm for 5 min and washed with PBS one time.

2) **Staining:** Add Calcein AM working solution to resuspend the cells pellet and adjust the cell density to 10^6 cells/mL. Incubate at 37°C away from light for 30 min. Samples only containing dilution buffer need to be prepared as negative controls for flow cytometry.

*Note: The optimal time for incubation varies depending on the type of cells.

3) **Detection:** After incubation, wash cells in PBS 1-2 times. Then resuspend cells with appropriate dilution buffer for flow cytometry. Other counterstaining can also be performed if needed.

*Note: After staining, the samples need to be placed on ice and detected within 1 h as much as possible.

Note

1. Fluorescent probes are easy to quench, please protect them from light during storage and use.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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