Live-Dead Cell Staining Kit I (Calcein AM/PI)

Introduction

Live-Dead Cell Staining Kit I (Calcein AM/PI) is used for simultaneous fluorescence staining of live and dead mammalian cells. This kit contains two probes, Calcein AM and propidium iodide (PI). Calcein AM is a live-cell probe, which is highly lipophilic and cell permeable. Calcein AM itself is no-fluorescent, but it is hydrolyzed into green-fluorescent Calcein by esterase in the viable cells. On the other hand, PI is a dead cell probe that can only pass through damaged cell membranes and stain the nucleus red. The fluorescence intensity of these two probes can reflect cell viability and toxicity.

The kit is sensitive, quick and easy to use. However, Calcein AM does not penetrate the cell walls of bacteria or fungi, so this kit is not suited for bacteria or fungi.

Components and Storage

Components	K2247-100T	K2247-500T	K2247-2500T
Calcein AM (1000x)	10 μL	50 μL	250 μL
PI (1000x)	10 μL	50 μL	250 μL
Staining Buffer	10 mL	50 mL	250 mL

Store the kit at -20°C, stable for 1 year. The Calcein AM (1000x) and PI (1000x) should be stored away from light and moisture, avoiding repeated freeze/thaw cycles.

Protocol

1. **Preparation of staining solution:** For 96-well plates, refer to the following table to prepare the staining solution and mix it thoroughly. Prepare fresh staining solution every time. The concentration of Calcein AM and PI in the staining solution can be adjusted depending on the specific experiment.

Reagents	Reactions number		
	10	100	1000
Calcein AM (1000x)	1 μL	10 μL	100 μL
PI (1000x)	1 μL	10 μL	100 μL
Staining Buffer	1 mL	10 mL	100 mL
Total	1 mL	10 mL	100 mL

2. **Fluorescence microscope detection：**

   1) **Cell culture:** Seed cells in appropriate plates, petri dishes or flasks. Treat cells with the interested drug

according to the experimental design.

2) **Washing:** Remove the culture medium and wash 1-2 times with PBS.

***Note:** Serum or phenol red in the medium may cause an increase in extracellular fluorescence by hydrolyzing Calcein AM, so it is needed to wash cells with PBS prior to staining.

3) **Staining:** Incubate with staining solution for 20 min at room temperature in the dark. For 96-well plates, add 100 μL of staining solution per well. Other plate sizes can be used by scaling as necessary, but make sure that the staining solution can fully cover the cells.

***Note:** The optimal time for incubation varies depending on the cell types. Otherwise, PI is toxic to cells, so PI staining should not exceed 30 minutes to avoid false positives.

4) **Washing:** Remove the staining solution and wash 1-2 times with PBS.

5) **Detection:** After adding an appropriate amount of PBS or other suitable buffers, observe using a fluorescence microscope (Calcein AM, Ex/Em=494/517 nm; PI, Ex/Em=535/617 nm). Further fluorescent counterstains can be performed if necessary.

***Note:**

a) If a black 96-well plate is used, it can also be detected with a fluorescence microplate reader.

b) For suspension cells, after harvesting the cells, resuspend cells with the staining solution to a cell density of 1x 10^6 cells/mL. Incubate at room temperature for 20 min in the dark, and add a drop of the cell suspension to the glass slide. Then observe under the microscope after mounting.

3. Flow cytometry detection:

1) **Cell culture:** Seed cells in appropriate plates, petri dishes or flasks. Treat cells with the interested drug according to the experimental design.

2) **Cell harvest:** For adherent cells, trypsinize cells, neutralize with medium and centrifuge at 1000 rpm for 5 minutes. For suspension cells, centrifuge directly at 1000 rpm for 5 minutes.

3) **Washing:** Remove the culture medium and wash 1-2 times with PBS.

***Note:** Serum or phenol red in the medium may cause an increase in extracellular fluorescence by hydrolyzing Calcein AM, so it is needed to wash cells with PBS prior to staining.

4) **Staining:** Resuspend cells in the staining solution to give a cell density of 10^6 cells/mL. Incubate at room temperature in the dark for 20 min. In addition, prepare one tube of sample only containing Staining Buffer as a negative control, and two additional tubes of the single stained sample with only one probe (Calcein AM or PI) for compensation adjustment.

***Note:** The optimal time for incubation varies depending on the cell types. Otherwise, PI is toxic to cells, so PI staining should not exceed 30 minutes to avoid false positives.

5) **Washing:** Centrifuge at 1000 rpm for 5 minutes to remove the staining solution, and wash 1-2 times with PBS.

6) **Detection:** Resuspend cells in an appropriate amount of PBS or other suitable buffers. Then use the flow cytometer for detection (Calcein AM, Ex/Em=494/517 nm; PI, Ex/Em=535/617 nm).

***Note:**

a) If the detection cannot be carried out immediately, it is recommended to keep the sample on ice in the dark and perform the detection within 1 h.

b) When using flow cytometry, the concentration of the probes may be lower than fluorescence microscopy needed because flow cytometry is more sensitive, and the probes' concentration can be adjusted according to the specific experiment.

Note

1. Fluorescent probes are easy to quench and need to be protected from light when storing and using.
2. Calcein AM (1000x) may hydrolyze if exposed to moisture. So, it should be stored sealed, desiccated, and protected from light. And it is better to distribute into single-use aliquots.
3. Calcein AM is unstable in the aqueous solution, so please prepare a fresh staining solution every time.
4. Serum or phenol red in the medium may cause an increase in extracellular fluorescence by hydrolyzing Calcein AM, so it is needed to wash cells with PBS prior to staining.
5. This kit is not suitable for bacteria. If required, the Live-Dead Bacterial Staining Kit (Cat. No. K2239) can be chosen.
6. For your safety and health, please wear lab coats and gloves during the experiment.
7. For research use only. Not to be used in clinical diagnostic or clinical trials.

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