Live-Dead Cell Staining Kit II (Calcein AM/EthD-III)

Introduction

Live-Dead Cell Staining Kit II (Calcein AM/EthD-III) is used for simultaneous fluorescence staining of live and dead mammalian cells. This kit contains two probes, Calcein AM and EthD-III. 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, EthD-III 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. Meanwhile, EthD-III in this kit has a higher quantum yield and affinity than the traditional probe PI for detecting dead cells. 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	K2248-500T
Calcein AM (4 mM)	50 μL
EthD-III (2.5 mM)	120 μL
PBS	2 x 120 mL

Store the kit at -20°C, stable for 1 year. The Calcein AM (4 mM) and EthD-III (2.5 mM) 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. The final concentrations of Calcein AM and EthD-III in the staining solution were 2 μM and 3 μM, respectively. Prepare fresh staining solution every time.

Reagents	Reactions number	
	100	1000
Calcein AM (4 mM)	5 μL	50 μL
EthD-III (2.5 mM)	12 μL	120 μL
PBS	10 mL	100 mL
Total	10 mL	100 mL

***Note:**

a) The concentration of Calcein AM and EthD-III in the staining solution can be adjusted depending on the specific experiment. The recommended final concentration range is 0.1-10 μM.

b) When the volume of the required staining solution is too small (e.g., 1 mL), to ensure the accuracy of the probe concentration, Calcein AM (4 mM) and EthD-III (2.5 mM) can be diluted sequentially, and then use the diluted probe to prepare the staining solution.

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, EthD-III is toxic to cells, so EthD-III 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; EthD-III, Ex/Em=530/620 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 or scrape 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 PBS as a negative control, and two additional tubes of the single stained sample with only one probe (Calcein AM or EthD-III) for compensation adjustment.

***Note:** The optimal time for incubation varies depending on the cell types. Otherwise, EthD-III is toxic to cells, so EthD-III 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; EthD-III, Ex/Em=530/620 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 it 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.
1. This kit is not suitable for bacteria. If required, the Live-Dead Bacterial Staining Kit (Cat. No. K2239) can be chosen.
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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