1. Introduction

Our product Live-Dead Cell Staining Kit is used for simultaneous fluorescence staining of viable and dead cells. This kit contains two dyes - Calcein-AM and Propidium Iodide (PI), which stain viable and dead cells, respectively.

Calcein-AM, acetoxymethyl ester of calcein, is highly lipophilic and cell membrane permeable. Though Calcein-AM itself isn't a fluorescent molecule, it will turn to Calcein after esterase's digestion in a viable cell, which could emit strong green fluorescence (excitation: 490 nm, emission: 515nm). Therefore, Calcein-AM only stains viable cells.

In contrast, PI, a nuclei staining dye, can't pass through a viable cell membrane. It reaches the nucleus by passing through disorderedareas of dead cell membrane, and intercalates with the DNA double helix of the cell to emit red fluorescence (excitation: 535 nm, emmision: 617 nm).

Since both calcein and PI-DNA can be excited with 490 nm, simultaneous monitoring of viable and dead cells is possible with a fluorescence microscope or flow cytometer. With 545 nm excitation, only dead cells can emit red fluorescence. Since optimal staining conditions constantly changes because of different cell lines, we recommend that a suitable concentration of PI and Calcein-AM should be individually determined.

Please note that PI is suspected to be highly carcinogenic; careful handling is required.

2. Guidelines

2.1. Bring Calcein-AM solution and PI solution to room temperature.

Add 5 µL of Calcein-AM solution (2 mM) and 15 µL of PI solution (1.5 mM) to 5 mL PBS, mix well. In this case, the working concentration of Calcein-AM is 2 µM, and the PI is 4.5 µM.

Because the optimal staining conditions for various cell lines are different, gradient screening is recommended for the initial experiments. The principle is to use the lowest probe concentration to get the best fluorescence results.

Due to the poor stability of Calcein-AM in water, prepare the staining solution only prior to each experiment and use it in one day.

Note: PI is highly carcinogenic and mutagenic, so wear gloves, safety goggles, and mask when handling. If it comes in contact with your skin, immediately wash with a copious amount of running water.

2.2. For adherent cells, digest the cells with trypsin, then collect the cells by centrifugation (1000 rpm, 3 min).

For suspended cells, collect the cells by centrifugation (1000 rpm, 3 min).

2.3. Wash cells with PBS 2-3 times to remove the esterase in the media.

2.4. Prepare a cell suspension with PBS in which the cell density is $1x10^5$ to $1x10^6$ cells/ml.

2.5. Remove 200 µl of the cell suspension to a microtube. Then add 100 µl of Staining solution to the cell. Mix and incubate the mixture at 37 ℃ for 15 min, protect from light.

Note: If needed, extend the incubation time to 30 min.

2.6. If fluorescence microscope is used, place 10 µl of the cell and staining solution on a glass slide and cover with a cover glass. Detect fluorescence with 490 nm excitation for simultaneous monitoring of viable and dead cells. With 545 nm excitation, only dead cells can be observed.

You can also take flow cytometer or other machines.

3. **Pre-Experiment**

If optimal concentration of each reagent should be determined, follow below steps:

3.1. Prepare dead cells by 10 min incubation in 0.1% saponin or 0.1-0.5% digitonin or by 30 min incubation in 70% ethanol.

3.2. Stain dead cells with 0.1-10 μM PI solution to find a PI concentration that stain nucleus only, does not stain cytosol.

3.3. Stain dead cells with 0.1-10 μM Calcein-AM solution to find a Calcein-AM concentration that does not stain cytosol. Then stain viable cells with that Calcein-AM solution to check whether the viable cell can be stained.

4. Note

4.1. When disposing of remaining dye solution, follow handling guidelines and regulations and entrust disposal to an industrial waste disposal company.

4.2. In previous reports, CFSE has been reported in a paper that the fluorescent dye was retained within cells for up to 8 weeks. And the fluorescence of Calcein-AM and BCECF-AM have been observed in cells for up to three days.

4.3. If the dye seems not to stay inside of the viable cell after staining, it may because viable cell expels the dye due to the cell function or not enough reagent was used for the cells.