# Product Data Sheet

Product Name: Live-Dead Cell Staining Kit
Cat. No.: GK10030

## Components

| Components | 100 Tests | 500 Tests | 1000 Tests |
|---|---|---|---|
| Calcein-AM Solution (2 mM) | 10 μL | 50 μL | 50 μL × 2 |
| PI Solution (1.5 mM) | 30 μL | 150 μL | 150 μL × 2 |
| Storage | -20°C, protect from light | | |

## Protocol

Operation Guide

1. Thaw the Calcein AM solution and PI solution at room temperature.

Add 5 μ L Calcein AM solution (2 mM) and 15 μ Add L PI solution (1.5 mM) to 5 mL PBS and mix thoroughly. In this case, the working concentration of Calcein AM is 2 μ M. PI is 4.5 μ M.

Due to the different optimal staining conditions for various cell lines, it is recommended to conduct gradient concentration screening in the initial experiment. In principle, the lowest probe concentration should be used to obtain the best fluorescence results.

Due to the poor stability of Calcein AM in aqueous solutions, the staining solution can only be prepared before each experiment and used within one day.

Attention: PI has high carcinogenicity and mutagenicity, therefore gloves, safety goggles, and a face mask should be worn during operation. If it comes into contact with your skin, immediately rinse with plenty of tap water.

For adherent cells, digest the cells with trypsin and collect them by centrifugation (1000 rpm, 3 minutes).

For suspended cells, collect them by centrifugation (1000 rpm, 3 minutes).

3. Wash cells 2-3 times with PBS to remove esterases from the culture medium.

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4. Prepare cell suspension using PBS, with cell density ranging from 1x105 to 1x106 cells/ml.

5. Add 200 μ Transfer the cell suspension to the EP tube. Then add 100 μ Add staining solution to cells. Mix well and incubate at 37 °C for 15 minutes, away from light. Attention: If necessary, extend the incubation time to 30 minutes.

If using a fluorescence microscope, 10 μ Place the cells and staining solution on a glass slide and cover with a cover glass. By using an excitation wavelength of 490 nm to detect fluorescence, both live and dead cells can be monitored simultaneously. Under excitation at 545 nm, only dead cells can be observed. You can also use a flow cytometer or other machines.

Pre experiment

If you need to determine the optimal concentration for each reagent, please follow the following steps:

1. Incubate cells with 0.1% saponin or 0.1-0.5% digitalis saponin for 10 minutes to prepare dead cells. Alternatively, incubate in 70% ethanol for 30 minutes.

2. Use 0.1-10 μ M PI solution is used to stain dead cells to obtain the optimal working concentration for staining only the nucleus without staining the cytoplasm. Using 0.1-10 μ Stain dead cells with M Calcein AM solution to obtain the optimal working concentration without staining the cytoplasm. Then use this concentration to stain live cells and observe if they can be stained.

take care

When handling the remaining dye solution, please follow the guidelines and regulations for handling toxic solutions, and entrust the treatment to an industrial waste treatment company.

In previous experiments, CFSE was reported in a paper that fluorescent dyes were retained in cells for up to 8 weeks. The fluorescence of Calcein AM and BCECF-AM was observed to remain for up to three days.

3. If the dye does not appear to remain in the living cells after staining, it may be because the living cells have discharged the dye due to cellular function, or insufficient reagents have been used.

## Background

The Live-Dead Cell Staining Kit is used for simultaneous fluorescent staining of live and dead cells.

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This kit contains two dyes – Calcein-AM and Propidium Iodide (PI), which stain live and dead cells, respectively. Calcein-AM is the acetoxymethyl ester of Calcein, which is highly lipophilic and can penetrate cell membranes. Although Calcein-AM itself is not a fluorescent molecule, after being digested by esterases in live cells, it is converted into Calcein, emitting strong green fluorescence (maximum excitation wavelength: 490 nm, maximum emission wavelength: 515 nm). Therefore, Calcein-AM only stains live cells.

Conversely, PI (a nuclear staining dye) cannot penetrate the membranes of live cells. It can pass through the disordered regions of dead cell membranes to reach the nucleus and intercalate into the DNA double helix, emitting red fluorescence (maximum excitation wavelength: 535 nm, maximum emission wavelength: 617 nm).

Since both calcein and PI-DNA can be excited at 490 nm, live and dead cells can be monitored simultaneously using a fluorescence microscope or flow cytometer. Under 545 nm excitation, only dead cells will emit red fluorescence. Because optimal staining conditions vary depending on the cell line, we recommend determining the appropriate concentrations of PI and Calcein-AM separately.

Please note that PI is suspected of being highly carcinogenic; handle with caution.

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