# Product Information

## Live & Dead™ Viability/Cytotoxicity Assay Kit for Animal Cells (Calcein AM, PI)

Catalog Number: L6037S, L6037M, L6037L

Product Size: 30T, 150T, 300T

Contents:

| Component | L6037S (30T) | L6037M (150 T) | L6037L (300T) |
|---|---|---|---|
| A. Calcein AM (4 mM in DMSO) | 10 μL | 50 μL | 100 μL |
| B. PI, 1.5 mM in $H_2O$ | 50 μL | 250 μL | 500 μL |

Note: The number of times(T) of this kit is specified according to the usage of 0.5 mL working solution for one sample of flow cytometer. Calcein AM：Ex/Em = 494/517 nm，PI：Ex/Em（binding with DNA）=535/617 nm

## Storage

Store at -20℃ and protect from light. Calcein AM is easy to be hydrolyzed, which needs to be sealed and dried for storage. The diluent working solution needs to be prepared on the same day you used. Expiration date marked on the outer packing.

## Description

Live & Dead™ Viability/Cytotoxicity Assay Kit for Animal Cells (Calcein AM, PI) is a kind of double fluorescent staining kit for the detection of animal cell death. The two probes in the kit can respectively measure the esterase activity and the integrity of plasma membrane to reflect the activity of cells. The kit can be used in fluorescence microscope, flow cytometer, enzyme labeling instrument and other fluorescence detection systems.

Live & Dead™ Viability/Cytotoxicity Assay Kit for Animal Cells (Calcein AM, PI) can be applied to most eukaryotic mammalian cells, including some tissues of adherent nuclei, but not to fungi and yeast. Compared with trypan blue, detection results of this kit is faster, safer and more sensitive.

## Protocol

**Fluorescence microscopy**

1. Preparation of working solution

Prepare 2 μM Calcein AM and 4.5 μM PI staining solution: take out the original solution of Calcein AM and PI and restore it to room temperature. 30 μL 1.5 mM PI and 5 μL 4 mM Calcein AM were mixed with 10 mL PBS or other serum-free buffer or medium, and vortex mixed. The working solution can be directly used for cell staining.

Note: the aqueous solution of Calcein AM is easy to hydrolyze and should be used up the same day. The concentration selection of Calcein AM and PI varies according to the cell type used. The recommended concentration range is 0.1-10 μM.

2. Prepare cells and conduct experiments

(1) Adherent cells can be stained directly. For suspension cells, centrifugation was used to collect cell staining.

(2) Wash cells 2 or 3 times with 1 × PBS to remove the esterase activity.

(3) Absorb and discard PBS, and for adherent cells, add enough Calcein AM/PI staining solution. For suspension cells, add a proper amount of dye working solution to control the cell





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density at 1-5 × $10^5$/mL.

(4) Incubation at room temperature for 15-20 min (if concentration of working solution is high or the incubation temperature is high, the incubation time should be appropriately reduced).

(5) Observe labeled cells under fluorescence microscope.

**Flow cytometry**

1. Take out the reagent and restore to room temperature.

2. Prepare 2 μM Calcein AM and 4.5 μM PI staining solution: take out the original solution of Calcein AM and PI and restore it to room temperature. 30 μL 1.5 mM PI and 5 μL 4 mM Calcein AM were mixed with 10 mL PBS or other serum-free buffer or medium, and vortex mixed. The working solution can be directly used for cell staining.

3. Wash cells 2 or 3 times with 1 × PBS to remove the esterase activity.

4. The cells were suspended with 0.5 ml dye solution, and the cell density was controlled to be 1-5 × $10^5$/mL.

Note: it is recommended to prepare two additional tubes of sample, each of which is only added with one dye (Calcein AM or PI) for compensating adjustment.

5. Incubate at room temperature in dark for 15-20 min.

6. In 1-2 hours, detected cell activity by flow cytometry. Calcein AM can be excited by 488 nm laser and fluorescence emission spectrum is about 530 nm, and the emission spectrum of PI is about 617 nm.

Note: when the cell circle gate is used, the cell fragments should be excluded, and the single dye tube should be used to adjust compensation. The double dye tube should obtain two relatively independent cell groups: the living cell group showing green fluorescence and the dead cell group showing.

**Microplate Reader**

1. Culture appropriate number of adherent or suspension cells in 96 well plates.

Note: dead cells samples can be obtained by treating cells with 1% saponin or 0.1-0.5% digitalis saponin for 10 min.

2. Prepare 2 μM Calcein AM and 4.5 μM PI staining solution: take out the original solution of Calcein AM and PI to restore it to room temperature. 30 μL 1.5 mM PI and 5 μL 4 mM Calcein AM were mixed with 10 mL PBS or other serum-free buffer or medium, and vortex mixed. The working solution can be directly used for cell staining.

Note: 10 mL dye solution is enough for a 96 well plate, and the volume of dye solution can be adjusted according to the experimental needs. The concentration of Calcein AM and PI can be fumble between 0.1 to 10 μM.

3. Wash cells 2 or 3 times with 1 × PBS to remove the esterase activity.

4. Add 100 μL PBS into each well.

5. Add 100 μL dye working solution into each well, so that the total volume of each well was 200 μL, the final concentration of Calcein AM was 1 μM, and the final concentration of PI was 2.25 μM. Shake the plate gently so that the liquid covers cells evenly.

6. Incubate at room temperature in dark for 30-45 min.

7. Detection with Microplate Reader. When the program is set to fluorescein, it can detect Calcein AM; when the program is set to rhodamine or Texas Red, it can detect PI. According to the spectral characteristics, you can selecte the best emission and excitation wavelengths.

Note: by comparing with relative fluorescence values (RFU) measured in the sample group and the control group, you can get the change of the number of dead cells and living cells.

**Calculate the ratio of live cells to dead cells in a certain area**

The following method can calculate the ratio of dead cells to living cells. Samples required dead cell control group, living cell control group and sample group.1% saponin Or 0.1-0.5% digitalis saponin treatment for 10 min, dead cells can be obtained.

1. Prepare staining working solution and stain cells according


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to the above steps. In addition, respectively prepare 1 mL of 2 μM Calcein AM and 4 μM PI solution, follow the instructions below to stain the control group.


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