

Product Information

Live & Dead™ Viability/Cytotoxicity Assay Kit for Animal Cells (Calcein AM, EthD-1)

Catalog Number: L6023S, L6023M, L6023L

Product Size: 30T, 150T, 300T

Contents:

Component	L6023S(30T)	L6023M(150T)	L6023L(300T)
A. Calcein AM (4 mM in anhydrous DMSO)	10 µL	50 µL	100 µL
B. EthD-I, 2 mM in DMSO/H ₂ O=1:4(v/v)	30 µL	150 µL	300 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.

Parameters

Calcein AM: Ex/Em: 494/517 nm

EthD-I: Ex/Em: 528/617 nm (with DNA)

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.

Storage

Store at -20°C 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.

Protocol

Fluorescence microscopy

1. Preparation of working solution

Prepare 2 µM Calcein AM and 4 µM EthD-I staining solution: take out the original solution of Calcein AM and EthD-I and restore it to room temperature. 20 µL 2 mM EthD-I 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 EthD-I 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.

Description

Live & Dead™ Viability/Cytotoxicity Assay Kit for Animal Cells (Calcein AM, EthD-1) 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, EthD-1) can be applied to most eukaryotic



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

(3) Absorb and discard PBS, and for adherent cells, add enough Calcein AM/EthD-I staining solution. For suspension cells, add a proper amount of dye working solution to control the cell density at $1-5 \times 10^5/\text{mL}$.

(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 $2 \mu\text{M}$ Calcein AM and $4 \mu\text{M}$ EthD-I staining solution: take out the original solution of Calcein AM and EthD-I and restore it to room temperature. $20 \mu\text{L}$ 2 mM EthD-I and $5 \mu\text{L}$ 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 $1 \times$ 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 $1-5 \times 10^5/\text{mL}$.

Note: it is recommended to prepare two additional tubes of sample, each of which is only added with one dye (Calcein AM or EthD-I) 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 EthD-I is about 610 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 red fluorescence.

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 $2 \mu\text{M}$ Calcein AM and $4 \mu\text{M}$ EthD-I staining solution: take out the original solution of Calcein AM and EthD-I to restore it to room temperature. $20 \mu\text{L}$ 2 mM EthD-I and $5 \mu\text{L}$ 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 EthD-I can be fumble between 0.1 to $10 \mu\text{M}$.

3. Wash cells 2 or 3 times with $1 \times$ PBS to remove the esterase activity.
 4. Add $100 \mu\text{L}$ PBS into each well.
 5. Add $100 \mu\text{L}$ dye working solution into each well, so that the total volume of each well was $200 \mu\text{L}$, the final concentration of Calcein AM was $1 \mu\text{M}$, and the final concentration of EthD-I was $2 \mu\text{M}$. 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 labeler is set to fluorescein, it can detect Calcein AM; when the labeler is set to rhodamine or Texas Red, it can detect EthD-I. According to the spectral characteristics, you can select 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.

