Product Information

Catalog number: REK0010

Size: 150T/300T

Storage: Please store at 4℃ for 6 months, Avoid freeze / thaw cycles.

Product components:

Components	150 T	300 T
4 mM Calcein AM	50μl	100μl
1.5 mM Propidium Iodide	250μl	500μl

Note: The number of tests is based on the use of 0.5 mL working solution per sample for flow cytometry.

Spectral Information:

- Calcein AM: Ex/Em = 494/517 nm, Propidium Iodide (PI): Ex/Em = 535/617 nm (when bound to DNA)

Product Description

The Animal Cell Viability/Toxicity Detection Kit (Calcein AM, PI) is a dual-fluorescence staining kit for detecting the viability of animal cells. The kit contains two probes that can reflect cell viability by measuring intracellular esterase activity and plasma membrane integrity. This kit is suitable for fluorescence microscopy, flow cytometry, microplate readers, and other fluorescence detection systems. It can be applied to most eukaryotic mammalian cells, including adherent cells and certain tissues, but is not suitable for fungi and yeast. Compared to Trypan Blue, this kit offers faster, safer, and more sensitive detection.

Storage Conditions

Store at -20°C, protected from light. Note that Calcein AM is prone to hydrolysis and should be stored sealed and dry. Diluted working solutions should be prepared on the day of use. The expiration date is indicated on the outer packaging.

Instructions for Use

Fluorescence Microscopy Detection

1. Preparation of Working Solution:

Prepare a staining working solution of 2 μM Calcein AM and 4.5 μM PI: Take the Calcein AM and PI stock solutions out and allow them to reach room temperature. Mix 30 μL of 1.5 mM PI and 5 μL of 4 mM Calcein AM

with 10 mL of PBS or other serum-free buffer or culture medium, and vortex to mix thoroughly. This working solution can be used directly for cell staining.

Note: The aqueous solution of Calcein AM is prone to hydrolysis and should be used on the same day. The concentrations of Calcein AM and PI may vary depending on the cell type used, with a recommended range of 0.1-10 μM.

2. Cell Preparation and Experiment:

(1)For adherent cells, wash 2-3 times with 1×PBS before staining. For suspension cells, centrifuge at 250-1000×g for 5 min at room temperature to collect the cells for staining.

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

(3)For adherent cells, add an appropriate amount of Calcein AM/PI staining working solution. For suspension cells, add an appropriate amount of staining working solution to achieve a cell density of 1-5×10^5/mL.

(4)Incubate in the dark at room temperature for 15-20 min (reduce incubation time if the working solution concentration or incubation temperature is higher).

(5)Observe the stained cells under a fluorescence microscope.

Flow Cytometry Detection

1. Preparation of Reagents:

Take out the reagents and allow them to reach room temperature.

2. Preparation of Working Solution:

Prepare a staining working solution of 2 μM Calcein AM and 4.5 μM PI as described above.

3. Cell Preparation:

- Wash the cells thoroughly 2-3 times with 1×PBS.
- Suspend the cells in 0.5 mL of staining working solution to achieve a cell density of 1-5×10^5/mL.

Note: Prepare two additional tubes of cells, each stained with only one dye (Calcein AM and PI) for single-stain compensation adjustment in flow cytometry; also prepare a tube of cells with only buffer (consistent with the buffer used for staining) as a negative control for flow cytometry.

4. Incubation:

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

5. Detection:

Detect cell viability by flow cytometry within 1-2 h. Calcein AM can be excited by a 488 nm laser, with fluorescence emission detected around 530 nm, while PI emission is detected around 617 nm.

Note: When gating cells, exclude cell debris. Use single-stain tubes for compensation adjustment. Flow cytometry of double-stained cells should yield two distinct cell populations: live cells showing green fluorescence and dead cells showing red fluorescence.

Microplate Reader Detection

1. Cell Culture:

Culture an appropriate number of adherent or suspension cells in a 96-well black microplate.

Note: Treatment of cells with 1% saponin or 0.1-0.5% digitonin for 10 min can produce dead cells.

2. Preparation of Working Solution:

Prepare a staining working solution of 2 μM Calcein AM and 4.5 μM PI as described above.

Note: (1) 10 mL of staining solution is sufficient for one 96-well plate; adjust the volume of staining solution according to experimental needs. The concentrations of Calcein AM and PI can be optimized within the range of 0.1-10 μM. (2) The aqueous solution of Calcein AM is prone to hydrolysis and should be used on the same day.

3. Cell Washing:

Wash the cells thoroughly with 1×PBS to remove residual esterase activity. For adherent cells, add 100 μL of PBS per well. For suspension cells, resuspend the cells in 100 μL of PBS, centrifuge to remove the supernatant, and repeat the operation.

4. Addition of Staining Solution:

Add 100 μL of staining working solution per well, making the total volume per well 200 μL, with a final concentration of 1 μM for Calcein AM and 2.25 μM for PI. Gently shake the microplate to ensure even coverage of the cells.

5. Incubation:

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

Note: The optimal incubation time may vary for different cell types. Start with an initial incubation time of 30 min and adjust as needed based on staining results for optimal performance.

6. Detection:

Measure fluorescence using a microplate reader. When set to fluorescein, the reader can detect Calcein AM; when set to rhodamine or Texas Red, it can detect PI. Select the appropriate excitation and emission wavelengths based on the spectral characteristics.

Note: Compare the Relative Fluorescence Values (RFU) of the sample and control groups to determine changes in the number of dead and live cells. An alternative data analysis method is provided below.

Calculation of Live and Dead Cell Ratios

The following method can be used to calculate the ratio of dead to live cells. Prepare dead cell control, live cell control, and test sample groups. Dead cells can be obtained by treating cells with 1% saponin or 0.1-0.5% digitonin for 10 min.

1. Preparation of Staining Solution and Cell Staining:

Prepare the staining working solution and stain the cells as described above. Additionally, prepare 1 mL of 2 μM Calcein AM and 4 μM PI solutions for staining the control groups. Ensure that the cell number, detection working solution concentration, incubation time, and temperature are consistent across all groups.

2. Measurement of Samples and Controls:

- Measure the fluorescence intensity of the sample group at 517 nm, denoted as F(517)sam.
- Measure the fluorescence intensity of the sample group at 617 nm, denoted as F(617)sam.
- Measure the fluorescence intensity of the dead cell PI single-stain control group at 617 nm, denoted as F(617)max.
- Measure the fluorescence intensity of the dead cell Calcein AM single-stain control group at 617 nm, denoted as F(617)min.
- Measure the fluorescence intensity of the live cell PI single-stain control group at 517 nm, denoted as F(517)min.
- Measure the fluorescence intensity of the live cell Calcein AM single-stain control group at 517 nm, denoted as F(517)max.
- Measure the fluorescence intensity of the blank control well (with or without dye) at 517 nm, denoted as F(517)0.
- Measure the fluorescence intensity of the blank control well (with or without dye) at 617 nm, denoted as F(617)0.

3. Calculation of Ratios:
- % Live Cells = (F(517)sam - F(517)min) ÷ (F(517)max - F(517)min)
- % Dead Cells = (F(617)sam - F(617)min) ÷ (F(617)max - F(617)min)

Note: Subtract the corresponding F(517)0 and F(617)0 values from all F(517) and F(617) measurements.

Determination of Live and Dead Cell Ratios

The ratio of dead to live cells can be determined by creating fluorescence intensity standard curves at 517 nm and 617 nm. The fluorescence intensity of each dye is linearly related to the number of dead or live cells in the sample.

Precautions

1. Before use, briefly centrifuge the reagents to the bottom of the tube before proceeding with the experiment.
2. Phenol red or serum may interfere with the detection of this kit.
3. Fluorescent dyes are prone to quenching. Minimize exposure to light during the experiment to slow down fluorescence quenching.
4. For your safety and health, wear a lab coat and disposable gloves when handling the reagents.