Viability/Cytotoxicity Assay Kits for Animal Cells

Catalog No.: RA20074

Basic Information

Product name	Viability/Cytotoxicity Assay Kits for Animal Cells
Sizes	30T/150T/300T
Storage	-20℃, keep away from light
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em	Calcein：494/517 nm； EthD-I：528/617 nm（combine DNA）

Product Introduction

The Calcein AM/EthD-I Dual Fluorescence Assay Kit is a dual-labeled reagent for detecting viable and dead animal cells. The kit uses two probes to measure intracellular lysis enzyme activity and plasma membrane integrity respectively, providing a more accurate assessment of cell viability. Compared to the conventional Trypan blue method, this kit offers faster detection, enhanced safety, and higher sensitivity. It is compatible with fluorescence microscopy, flow cytometers, microplate readers, and other fluorescence detection systems for most eukaryotic mammalian cells, including certain tissue cultures with adherent cell nuclei. However, it is not suitable for fungal or yeast cells.

Product features

Good stability: the fluorescence staining has strong fluorescence brightness and good anti-quenching properties;

Easy to use: our company provides a variety of cell viability detection dyes for flexible and convenient selection;

Safe and sensitive: compared with the same acting trypan blue, it is faster, safer and more sensitive.

Product Components

Components	30 T	150 T	300 T
A. Calcein AM (4 mM in anhydrous DMSO)	10 μL	50 μL	100 μL
B. Ethidium homodimer-I (EthD-I) (2 mM in DMSO/H_2O 1:4(v/v))	30 μL	150 μL	300 μL

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Note: The number of times in this kit is based on the specification that 0.5 mL working fluid is used for one sample in the flow cytometer. Note that Calcein AM is easily hydrolyzed and should be stored in a sealed and dry place. Diluted working fluid should be prepared on the same day.

Perimental procedure

1.Fluorescence Microscopy Protocol

(1) Preparation of Working Solution Prepare a 2 μM Calcein AM and 4 μM EthD-I staining working solution: Allow Calcein AM and EthD-I stock solutions to recover at room temperature. Mix 20 μL 2 mM EthD-I with 5 μL 4 mM Calcein AM in 10 mL PBS or other serum-free buffer or medium, then vortex-blend. This working solution can be used directly for cell staining.

Note: The Calcein AM aqueous solution is prone to hydrolysis and should be used immediately. The concentration of Calcein AM and EthD-I should be adjusted according to the cell type used, with recommended ranges of 0.1-10 μM.

(2) Cell Preparation and Experiment Execution

1) For adherent cells: Wash 2-3 times with 1× PBS before staining. For suspension cells: Perform 5 min centrifugation at 250-1000×g at room temperature.

2) Collect cells for staining. Thoroughly wash cells 2-3 times with 1× PBS to remove residual esterase activity.

3) For adherent cells: Add sufficient Calcein AM/EthD-I staining working solution. For suspension cells: Add appropriate staining working solution to maintain cell density at 1-5×10^5/mL.

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

5) Observe the labeled cells under the fluorescence microscope.

2. Flow Cytometry Analysis

(1) Reagent Preparation: Allow reagents to recover to room temperature. Prepare staining working solutions with 2 μM Calcein AM and 4 μM EthD-I: Dissolve Calcein AM and EthD-I stock solutions at room temperature. Vortex mix 20 μL 2 mM EthD-I with 5 μL 4 mM Calcein AM in 10 mL PBS or other serum-free buffers or culture media.

(2) The working solution can be directly used for cell staining.

(3) Wash cells 2-3 times with 1× PBS.

(4) Resuspend cells in 0.5 mL staining working solution to achieve cell density of 1-5× 10^5/mL.

Note: It is recommended to prepare two additional cell samples, each containing only one dye (Calcein AM or EthD-I) for compensation adjustment during single-color flow cytometry. Additionally, prepare a buffer-containing sample (matching the buffer used in Calcein AM/EthD-

I detection solutions) as a negative control.

(5) Incubate at 4°C in the dark for 15-20 minutes.

(6) Detect cell viability using a flow cytometer within 1-2 hours. Calcein AM fluorescence emission peaks at 530 nm when excited by 488 nm laser, while EthD-I emits fluorescence at approximately 610 nm.

Note: When the cell circle is stained, pay attention to exclude cell fragments and adjust compensation with a single staining tube. Flow detection with a double staining tube should obtain two relatively independent cell groups: a group of live cells showing green fluorescence and a group of dead cells showing red fluorescence.

3.Enzyme Immunoassay

(1) Cultivate appropriate amounts of adherent or suspension cells in 96-well black enzyme immunoassay plates.

Note: Cells treated with 1% saponins or 0.1-0.5% digitalis saponins for 10 minutes will be dead.

(2)Prepare staining working solutions with 2 μM Calcein AM and 4 μM EthD-I: Extract stock solutions of Calcein AM and EthD-I and allow them to recover at room temperature. Mix 20 μL 2 mM EthD-I with 5 μL 4 mM Calcein AM in 10 mL PBS or other serum-free buffers or culture media, then vortex mix thoroughly.

Note:

1)A 10 mL staining solution is sufficient for one 96-well plate. Adjust the volume as needed. The concentrations of Calcein AM and EthD-I can be adjusted between 0.1-10 μM.

2) Calcein AM aqueous solution is prone to hydrolysis and should be used immediately. EthD-I working solution can be stored at-20℃°C for at least one year.

(3) Wash cells thoroughly with 1× PBS to remove residual esterase activity. For adherent cells, add 100 μL PBS per well. For suspension cells, resuspend cells in 100 μL PBS, centrifuge to discard supernatant, and repeat the above steps.

(4) Add 100 μL PBS per well.

(5) Add 100 μL staining working solution per well to achieve a total volume of 200 μL per well, with final concentrations of Calcein AM at 1 μM and EthD-I at 2 μM. Gently shake the culture plate to ensure uniform liquid coverage over cells.

(6) Incubate at room temperature in the dark for 30-45 minutes.

Note: Optimal incubation times may vary among cell types. Use 30 minutes as the initial incubation time, then adjust based on staining effectiveness to optimize results.

(7) Perform detection using a microplate reader. When set for Fluorescein, detect Calcein AM; when set for Rhodamine or TexasRed, detect EthD-I. Select optimal emission and excitation wavelengths

according to spectral characteristics.

Note: Relative fluorescence values (RFU) measured from sample versus control groups indicate changes in dead versus live cell counts. An alternative data analysis method is also provided below.

4.Calculating the Ratio of Live to Dead Cells in a Sample

The following method can determine the ratio of dead to live cells. Required samples include a control group for dead cells, a control group for live cells, and the sample group to be tested. Cells are treated with 1% saponins or 0.1-0.5% digitalis saponins for 10 minutes to induce cell death.

(1)Prepare staining working solution and stain cells according to the above steps. Additionally, prepare 1 mL of 2 μM Calcein AM and 4 μM EthD-I solutions separately, and stain the control groups as follows: For subsequent cell groups or cell-free controls, ensure complete consistency in cell quantity, working solution concentration, incubation time, and incubation temperature.

(2) Measurement of sample and control groups:

A. Measure the 645 nm value for both Calcein AM and EthD-I, denoted as F(645) sam.

B. Measure the 530 nm value for both Calcein AM and EthD-I, denoted as F(530) sam.

C. For dead cells, measure the 645 nm value for the single-stained EthD-I control group, denoted as F(645) max.

D. For dead cells, measure the 645 nm value for the single-stained Calcein AM control group, denoted as F(645) min.

E. Live Cell EthD-I Single-Color Staining Control: The measurement at 530 nm is recorded as EthD-I = F(530) min.

F. Live Cell Calcein AM Single-Color Staining Control: The measurement at 530 nm is recorded as Calcein AM = F(530) max.

G. Blank Control Well (with or without dye): The detection value at 530 nm is recorded as F(530) 0.

H. Blank Control Well (with or without dye): The detection value at 645 nm is recorded as F(645) 0.

The ratio of dead to live cells is calculated based on the measured data:

% Live Cells = (B-E) ÷ (F-E)

% Dead Cells = (A-D) ÷ (C-D)

(3). Determining the Ratio of Live to Dead Cells in a Specific Region:

By establishing fluorescence spectrum standard curves at 530 nm and 645 nm, the quantities of dead and live cells can be determined. The fluorescence intensity of each dye shows a linear relationship with the number of dead or live cells in the sample.

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Notes

1. Before use, centrifuge the product to the tube bottom immediately before proceeding with subsequent experiments.
2. Phenol red or serum may interfere with the detection of this kit.
3. Fluorescent dyes are prone to quenching. During operation, take precautions against light exposure to minimize fluorescence quenching.
4. For your safety and health, wear lab coats and disposable gloves during handling.
5. This product is for scientific research purposes only and must not be used for clinical diagnosis or treatment.

This product is for research use only!