Calcein AM/PI Double Staining Kit

Cat. No: E-CK-A354 **Size: 100 Assays/500 Assays/2000 Assays**

Cat.	Products	100 Assays	500 Assays	2000 Assays	Storage
E-CK-A164	Calcein AM Solution (100 μM)	100 μL	500 μL	500 μL × 4	-20 ℃, shading light
E-CK-A165	PI Solution (750 μM)	100 μL	500 μL	500 μL × 4	-20 ℃, shading light
E-CK-A153	Calcein AM Assay Buffer	25 mL	55 mL × 2	110 mL × 4	-20 ℃
Manual			One Copy		

Storage

Store protected from light at -20 ℃ for 12 months. It is recommend that Calcein AM Solution (100 μM) be properly packaged and stored protected from light for the first time to prevent spontaneous ester hydrolysis in a damp environment.

Introduction

Elabscience® Calcein AM/PI Double Staining Kit can be used to distinguish dead cells and living cells in mammals with esterase activity. Calcein AM is the addition of acetyl methoxy methyl ester (AM) group to traditional Calcein, which increases hydrophobicity and can easily penetrate the living cell membrane and enter the cell. Calcein AM itself has no fluorescence. After entering the cell, it is hydrolyzed by endogenous esterase in the cell to produce Calcein, a polar molecule with strong negative charge and cannot pass through the cell membrane, while Calcein can emit strong green fluorescence (Ex/Em = 494nm/517nm). Due to the lack of esterase, dead cells cannot or rarely produce Calcein, so only living cells are stained with strong green fluorescence, and dead cells cannot be stained or stained very weakly. The selective membrane permeability of dead cells is lost, and Propidium Iodide (PI) can enter the cell to specifically bind to double-stranded DNA and produce strong red fluorescence(Ex/Em = 535nm/617nm) to label dead cells. Therefore, the combination of Calcein AM and PI can perform double fluorescence staining on living cells and dead cells at the same time, which can be used for the detection of cell activity and cytotoxicity.

Reagent Not Supplied

PBS buffer (pH7.2~7.4).

Experimental Procedure

1 Flow cytometry detection

1.1 Preparation of working solution

1.1.1 Reagent preparation: Take out the frozen Calcein AM/PI Double Staining Kit, after thawing at room temperature, vortex mixing each reagent.

1.1.2 Preparation of Calcein AM/PI staining working solution: After thawing at room temperature, the vortex-mixed Calcein AM Solution and PI solution were prepared into the staining working solution

at a ratio of 1~5×10^5 cells/200 μL. Prepare the staining working solution according the number of samples. Please refer to the table below.

Component	Volume of Calcein AM/PI staining working solution		
	1 mL	5 mL	10 mL
Calcein AM Solution (100 μM)	0.1 μL	0.5 μL	1 μL
PI Solution (750 μM)	1 μL	5 μL	10 μL
Calcein AM Assay Buffer	1 mL	5 mL	10 mL

Note: Calcein AM in the staining working solution is easily deliquescent, prepare the fresh solution before use.

Tips: It is suggested that in order to save reagents and ensure the accuracy of the experiment, Calcein AM Solution can be diluted gradiently, such as 100 times to 1 μM with Calcein AM Assay Buffer. Before staining, use Calcein AM Assay Buffer to dilute 1μM Calcein AM Solution 100 times to the dyeing concentration (0.01m), that is, 2 μL 1 μM Calcein AM Solution was added to 200 μL Calcein AM Assay Buffer.

1.1.3 For the preparation of negative control and the single dye pipe working solution applied to compensation adjustment, please refer to the table below.

Component	Calcein AM single staining working solution (1 mL)	PI single staining working solution (1 mL)	Negative control(1 mL)
Calcein AM Solution (100 μM)	0.1 μL	0 μL	0 μL
PI Solution (750 μM)	0 μL	1 μL	0 μL
Calcein AM Assay Buffer	1 mL	1 mL	1 mL

Note: Calcein AM in the staining working solution is easily deliquescent, prepare the fresh solution before use. Negative control and single-stained tube cells were selected positive drug group cells.

1.2 Staining procedure

1.2.1 Collect the cells, centrifuge at 300×g for 5 min, discard the supernatant. Add 1 mL of PBS to resuspend the cells, centrifuge at 300×g for 5 min, discard the supernatant. Wash repeatedly 1 time, discard the supernatant.

1.2.2 Add 200 μL of Calcein AM/PI staining working solution to resuspend 1~5×10^5 cells in each group and incubate for 5~15 min at room temperature in the dark.

1.2.3 After incubation, flow cytometry can be performed directly. If it cannot be detected in time, it is recommended to avoid light and place in a 4℃ refrigerator for detection within 2 hours.

Note: Calcein can be detected in FITC channel while PI can be detected in PE or Percp/Cy5.5 channel.

2 Fluorescence microscope detection

2.1 Preparation of working solution

2.1.1 Reagent preparation: Take out the frozen Calcein AM/PI Double Staining Kit, after thawing at room temperature, vortex mixing each reagent.

2.1.2 Preparation of Calcein AM/PI staining working solution: After thawing at room temperature, the

vortex-mixed Calcein AM Solution and PI solution were prepared into the staining working solution according to 100 μL per well of 96-well plate or 200 μL per well in a 24-well plate. Prepare the staining working solution according the number of samples. Please refer to the table below.

Component	Volume of Calcein AM/PI staining working solution		
	1 mL	5 mL	10 mL
Calcein AM Solution (10 μM)	10 μL	50 μL	100 μL
PI Solution (750 μM)	10 μL	50 μL	100 μL
Calcein AM Assay Buffer	1 mL	5 mL	10 mL

Note: Calcein AM in the staining working solution is easily deliquescent, prepare the fresh solution before use.

Calcein AM Assay Buffer is conducive to the loading of fluorescent probes and the maintenance of fluorescent signals. If adherent cells are easy to fall off and sensitive, it is recommended to use basic medium to prepare the above staining working solution.

2.2 Staining process

2.2.1 Carefully remove the culture medium of adherent cells, add an appropriate amount of PBS to each well to wash cells, repeat wash the slides and remove PBS.

2.2.2 Add Calcein AM/PI staining working solution in a ratio of 100 μL per well in a 96-well plate or 200 μL per well in a 24-well plate and incubate at 37 ℃ for 10~30 min. (The staining time should be extended to 30-60 min for the preparation of staining working solution with basic medium, and PI solution should be added 10 min before the end of the reaction.)

2.2.3 Observe under fluorescence microscope after incubation (Calcein is green fluorescent, Ex/Em=494nm/517nm; PI is red fluorescent, Ex/Em=535nm/617nm).

Note: For suspended cells, after collecting cell precipitation, add Calcein AM/PI staining working solution at a ratio of 1~5×10^5 cells/200 μL and incubate at room temperature for 15~20 min. Add the cell suspension to the glass slide, cover the cover glass gently, and then observe under the microscope.

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Please store the product at the appropriate temperature to avoid failure.
4. Wash cells with serum-free medium (serum may contain esterase) or PBS before staining. The Buffer should not contain primary or secondary amines, as fatty histamine can lyse AM esters and hinder loading.
5. The staining temperature of 37 ℃ can reduce the staining time. Staining at room temperature can reduce the slide effect of fluorescent probe penetration into organelles.
6. Mn^{2+} has fluorescence quenching effect, so pay attention not to contain metal ions such as Mn^{2+} in the washing buffer.
7. Leakage: AM ester can be excluded by P glycoprotein multi-drug carrier.
8. It is suitable for any animal cells containing esterase activity. Calcein AM is not suitable for plants and bacteria because it cannot enter the cell wall.

9. Cells can be treated with 5%~20% DMSO for 2~4 hours or 70% alcohol for 30 min to obtain positive quality control samples.
10. Excessive acceleration and deceleration of centrifuge may cause cell loss. It is suggested to adjust the acceleration no more than 3 and deceleration no more than 2, that is, Acc≦3, Dec≦2.
11. When using fluorescence microscope for in situ detection of cells with weak adhesion ability, cell culture vessels can be subjected to anti-detachment treatment before cell inoculation and staining. The PI staining time should be less than 30 min, otherwise it may lead to false positive of PI. If you want to extend the staining time of Calcein AM, PI can be added within 10-30 min before the end of Calcein AM staining, and observe and take photos within 1-2 hours.

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