MQ Calcein AMP/PI Double Stain Kit

Cat No. CAP-M-001-500
Size: 500 Tests

MOLEQULE-ON®

Component	Size
Calcein-AM Solution (2mM)	50 µL
PI Solution (1.5mM)	150 µL
10X Assay Buffer	50 ml

Storage

MQ Calcein AMP/PI Double Stain Kit should be stored dry at -20ºC.

Description

Calcein-AM is a cell staining reagent for fluorescent labeling of living cells, emitting green fluorescence (Ex=490nm, Em=515nm). The addition of acetomethoxymethyl ester (AM) group to traditional Calcein increases its hydrophobicity and enables it to easily penetrate the membrane of living cells. Once inside the cell, calcein-AM (which is not fluorescent on its own) is cleaved by intracellular esterases to form the nonpermeable polar molecule Calcein, which is trapped inside the cell and emits strong green fluorescence. Compared with other similar reagents such as BCECF-AM and CFDA, AM is the most suitable fluorescent probe for live cell staining due to its very low cytotoxicity and does not inhibit any cellular functions such as proliferation and lymphocyte chemotaxis.

Because dead cells lack esterase, Calcein-AM is used only for cell viability testing and short-term labeling of living cells. Therefore, Calcein-AM is often used in combination with dead cell fluorescent probes such as propidium iodide (PI) for simultaneous fluorescent double staining of live and dead cells. Propidium iodide (PI) can not pass through the cell membrane of living cells, but can only pass through the disordered region of the dead cell membrane to reach the nucleus and embed into the DNA double helix of the cell to produce red fluorescence (Ex=535 nm, Em=617 nm). Therefore, PI only stains dead cells. Since both Calcein and PI-DNA can be excited by 490 nm, both live and dead cells can be visualized by fluorescence microscopy. However, only dead cells could be observed with 545 nm excitation. The working principle of this kit is to double dye Calcein-AM and PI to double stain and label live and dead cells, so as to analyze the level of live and dead cells. According to our optimized experimental system, 200µl cell suspension can be stained for 500 times.

Procedure

1. Pretreatment of Working Solution

(1) Take out 10X Assay Buffer from the low-temperature refrigerator, take out an appropriate amount according to the single-use sterile conditions, and dilute it 10 times with deionized water to obtain 1X Assay Buffer.
(2) Since the stability of Calcein-AM is greatly affected by temperature, it is recommended that it be divided into small portions after receiving, sealed and stored at -20°C in a dark place, and it should not be repeatedly frozen and thawed. If the staining solution is diluted, it is recommended to use it up on the same day.

2. Cell Treatment

(1) Collect the suspended cells into a centrifuge tube, centrifuge at 450 xg for 5 minutes, and remove the supernatant. Wash the cells with 1X Assay Buffer, centrifuge at 450 xg for 5 minutes, twice, to remove residual esterase.
(2) After the adherent cells were digested with 0.25% trypsin-EDTA, the cells were collected and washed with 1X Assay Buffer and centrifuged at 450 xg for 5 min, twice, to remove trypsin and residual esterase.

3. Staining Steps

(1) Resuspend the cell pellet obtained by centrifugation in 1X assay buffer to count 1×10^5~10^6 cells/ml.
(2) Add 1-2ul of Calcein-AM (stock solution) to every 1ml of cells, mix well by pipetting, and incubate at 37°C in the dark for 20-25min.
(3) Take 3-5ul of the PI stock solution provided by the kit and add it to the above-stained cells. Stain for 5min at room temperature in the dark.
(4) Incubate the cells after fluorescence, centrifuge at 450 xg for 5 min to remove the staining solution.
Note: It is recommended to keep cells away from light during fluorescence staining.
(5) Wash the cells with 1X PBS at 450 xg for 5 min, centrifuge and resuspend the cells with 1X PBS, take 3-5ul and drop it on a clean glass slide, cover the slide with a clean cover glass, and examine it under a fluorescence microscope in time.
(6) Use a 490±10 nm excitation filter to detect both live cells (yellow-green fluorescence) and dead cells (red fluorescence) under a fluorescence microscope. In addition, use a 545nm emission filter to observe only dead cells. You can also use a suitable filter to directly detect under a fluorescence microplate reader.

Notes:

(1) For your safety and health, please wear lab coat and disposable gloves.
(2) Adherent cells can be directly removed from the culture medium without digestion and washed with 1X assay buffer for 2 min, twice. Stain according to the above ratio of cell volume and staining solution concentration. The staining time and working concentration of the staining solution can be adjusted according to the actual test.
(3) Calcein-AM acts on cells. Generally, when the number of cells is 1×10^5-10^6, the working concentration of Calcein-AM is basically 1-2 μM; the working concentration of PI is 5μM. However, due to the different optimal staining conditions of different cell lines, it is recommended to perform gradient experiments for the first experiment to determine the optimal concentrations of Calcein-AM and PI. The principle of gradient screening is to use the lowest probe concentration to obtain the best fluorescence results.
(4) Propidium iodide (PI) has certain carcinogenicity, so be sure to take precautions when operating. If it comes into contact with the skin, it needs to be washed immediately with tap water.

Note:

The following method can be used to optimize the best working concentrations of two fluorescent dyes for different cells.
a) Prepare dead cells by incubating cells with 0.1% saponin or 0.1-0.5% digoxigenin for 10 minutes, or incubating cells with 70% ethanol for 30 minutes.
b) Use 0.1-10 μM PI solution for dead cell staining to obtain the best working concentration that only stains the cell nucleus but not the cytoplasm.
c) Use 0.1-10 μM Calcein-AM for dead cell staining to obtain the best working concentration that does not stain the cytoplasm. Then use this concentration to stain live cells to observe whether live cells can be stained.