NutriCulture Live-Dead Cell (Calcein AM/PI) Staining Kit

500 tests, (96-well plate format)

Cat No: LDC500

Shipping : Ship with blue.
Storage : Store at -20°C with protecting from light. It is important to protect product from moisture to prevent hydrolysis of the *NutriCulture* Calcein AM during storage. If you aliquot the *NutriCulture* Calcein AM stock solution, store the vials in a desiccator, or inside an airtight secondary container containing desiccant.

General Information

NutriCulture Live-Dead Cell (Calcein AM/PI) Staining Kit can be used to distinguish dead cells and living cells in mammals with esterase activity. *NutriCulture* 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 be retained in the cell 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.

Protocol

General Considerations before use

1. Due to the different optimal staining conditions for various cell lines, it is recommended to conduct gradient concentration screening in the initial experiment. In principle, the lowest probe concentration should be used to obtain the best fluorescence results.

2. Due to the poor stability of *NutriCulture* Calcein AM in aqueous solutions, the staining solution can only be prepared before each experiment and used within one day.

3. *Attention:* PI has high carcinogenicity and mutagenicity, therefore gloves, safety goggles, and a face mask should be worn during operation. If it comes into contact with your skin, immediately rinse with plenty of tap water.

Assay Procedure for analysis with flow cytometry

1. Thaw the Calcein AM solution and PI solution at room temperature.

2. Preparation of staining solution: Add 5 µl *NutriCulture* Calcein AM and 15 µl *NutriCulture* PI to 5 mL 1x PBS (not provided) and mix thoroughly protected from light.

Note: *Use 0.5-20 µl NutriCulture Calcein AM to obtain the optimal working concentration. Use 3-25 µl NutriCulture PI to obtain the optimal working concentration. Then use these optimal concentrations to stain live/dead cells.*

3. For adherent cells, digest the cells with trypsin and collect them by centrifugation (1000 rpm, 3 minutes). For suspended cells, collect them by centrifugation (1000 rpm, 3 minutes).

4. Wash cells 2-3 times with 1x PBS to remove esterases from the culture medium.

5. Prepare cell suspension using 1x PBS, with cell density ranging from $5x10^5$ to $1x10^6$ cells/ml.

6. Transfer 200 µl of cell suspension to the new eppendorf tube. Then add 100 µl staining solution to cells. Mix well and incubate at 37°C for 15 minutes, protected from light.

Attention: *If necessary, extend the incubation time to 30 minutes.*

Note: *If the dye does not appear to remain in the living cells after staining, it may be because the living cells have discharged the dye due to cellular function, or insufficient reagents have been used.*

Note: *The staining temperature of 37°C can reduce the staining time. Staining at room temperature can reduce the side effect of fluorescent probe penetration into organelles*

7. 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°C refrigerator for detection within 2 hours.

Assay Procedure for analysis with fluorescence microscopy

1. Culture cells in 6-, 12-, or 24-well plates at a density of $5\text{-}10\times10^5$ cells/mL (cells can be cultured on pre-coated glass coverslips). Optimal conditions will be dependent on the cell type, it is recommended that cells be no more than 80% confluent by the time of visualization. The culture medium is recommended to be changed every other day until the cells reach the target confluency.

2. Culture and treat cells in duplicates or triplicates.

3. Thaw the Calcein AM solution and PI solution at room temperature.

4. Preparation of staining solution: Add 5 µl *NutriCulture* Calcein AM and 15 µl *NutriCulture* PI to 5 ml 1x PBS (not provided) and mix thoroughly protected from light.

Note: *Use 0.5-20 µl NutriCulture Calcein AM to obtain the optimal working concentration. Use 1-30 µl NutriCulture PI to obtain the optimal working concentration. Then use these optimal concentrations to stain live/dead cells. The standard 5 µl NutriCulture Calcein AM working solution is suitable for NIH3T3, PtK2, HeLa and MDCK cells.*

5. Wash cells 2-3 times with 1x PBS to remove esterases from the culture medium.

6. Add staining working solution in a ratio of 200 µL per well in a 24-well plate or 500 µL per well in a 6-well plate and incubate at 37°C 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.)

Note: *The staining temperature of 37°C can reduce the staining time. Staining at room temperature can reduce the side effect of fluorescent probe penetration into organelles*

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

Note: *If the dye does not appear to remain in the living cells after staining, it may be because the living cells have discharged the dye due to cellular function, or insufficient reagents have been used.*