

04511 Cellstain double staining kit

Application

Double Staining Kit is utilized for simultaneous fluorescence staining of viable and dead cells. This kit contains Calcein-AM and Propidium Iodide (PI) solutions, which stain viable and dead cells, respectively. Calcein-AM, acetoxymethyl ester of calcein, is highly lipophilic and cell membrane permeable. Though Calcein-AM itself is not a fluorescent molecule, the calcein generated from Calcein-AM by esterase in a viable cell emits strong green fluorescence (excitation: 490 nm, emission: 515 nm). Therefore, Calcein-AM only stains viable cells. On the other hand, PI, a nuclei staining dye, cannot pass through a viable cell membrane. It reaches the nucleus by passing through disordered areas of dead cell membrane, and intercalates with the DNA double helix of the cell to emit red fluorescence (excitation: 535 nm, emission: 617 nm). Since both calcein and PI-DNA can be excited with 490 nm, simultaneous monitoring of viable and dead cells is possible with a fluorescence microscope. With 545 nm excitation, only dead cells can be observed. Since optimal staining conditions differ from cell line to cell line, we recommend that a suitable concentration of PI and Calcein-AM be individually determined. Please note that PI is suspected to be highly carcinogenic; careful handling is required.

Content

Solution A: 4 vials

Solution B: 1 vial

Methods

1. Add 10 μ l Solution A and 5 μ l Solution B to 5 ml PBS to prepare assay solution.^{a)}
2. Prepare a cell suspension with a trypsin-EDTA treatment if cells are adhered to a culture plate.^{b)}
3. Centrifuge the cell suspension at 1,000 rpm for 3 min.
4. Wash the cell pellet with PBS several times to remove residual esterase activity.
5. Prepare a cell suspension with PBS in which the cell density is 1×10^5 to 1×10^6 cells/ml.
6. Mix 200 μ l of cell suspension and 100 μ l of assay solution and incubate the mixture at 37 °C for 15 min.
7. Detect fluorescence using a fluorescence microscope with 490 nm excitation for simultaneous monitoring of viable and dead cells. With 545 nm excitation, only dead cells can be observed.

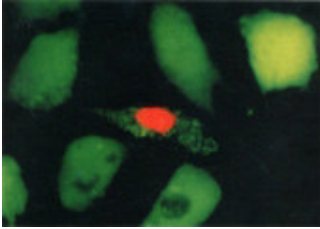
a) The concentration of each reagent should be optimized. Following steps may be necessary to determine the suitable concentration of each reagent:

1. Prepare dead cells by 10 min incubation in 0.1% saponin or 0.1-0.5% digitonin or by 30 min incubation in 70% ethanol.
2. Stain dead cells with 0.1-10 μ M PI solution to find a PI concentration that stain nucleus only, does not stain cytosol.
3. Stain dead cells with 0.1-10 μ M Calcein-AM solution to find a Calcein-AM concentration that does not stain cytosol. Then stain viable cells with that Calcein-AM solution to check whether the viable cell can be stained.

b) Or you may remove culture medium and wash cells with PBS several times. Add assay solution and incubate at 37 °C for 15 min.

Storage

Reagent solution is stable for 12 months at -20 °C with protection from light and moisture. Since the buffer solution of Calcein-AM is gradually hydrolyzed to generate fluorescent Calcein, the working solution is not storable. Close the bottle cap tightly after using a portion of Calcein AM solution to avoid moisture.



HeLa cell, incubated with assay solution for 15 min.

A) viable cell

B) dead cell

References

1. Kimura, K., et al., *Neurosci. Lett.*, **208**, 53 (1998).
2. Matsuse, S., et al., *J. Clin. Pathol.*, *in press*, , (1998).
3. Shimokawa, I., et al., *J. Geronto.*, **51a**, b49 (1998).
4. Yoshida, S., et al., *Clin. Nephrol.*, **49**, 273 (1998).
5. Tominaga, H., et al., *Anal. Commun.*, **36**, 47 (1999).