

## General Information

-Cellstain- Double Staining Kit consists of two staining compounds, Calcein-AM and Propidium Iodide (PI). This kit is utilized for a simultaneous detection of viable cells and dead cells in a cell culture dish by using a fluorescent microscope. Calcein-AM is cell membrane permeable and the generated Calcein by esterase activity in the viable cell emits strong green color fluorescence. Therefore, viable cells can be monitored as green fluorescent-colored cells under the microscope. On the other hand, PI can enter a dead cell through a damaged cell membrane and intercalate DNA of a dead cell. PI emits red color fluorescence in DNA double helix, so dead cells can be monitored as red fluorescent-colored cells.

## Kit Contents

- Solution A (1 mmol/l Calcein-AM/DMSO)..... 50 µl x 4
- Solution B (1.5 mmol/l PI/purified water)..... 300 µl x 1

## Storage Condition

Store at -20°C

## General Protocol

### Preparation of a staining solution\*

1. Before opening vials of Solution A and B, allow them to warm to room temperature.
2. 10 µl of Solution A and 15 µl of Solution B are mixed in 5 ml of D-PBS.

*\* This solution should be used up in one day because of the poor stability of Calcein-AM.*

### Preparation of cells

1. Remove cells from a culture dish by trypsin-EDTA.
2. Collect cells by centrifuge (1000 rpm, 3 minutes).
3. Discard the supernatant, and add PBS(-) to suspend cells (preferably  $10^5$  -  $10^6$  cells/ml).
4. Repeat step 2 and step 3 several times in order to eliminate esterase activity of the media.
5. Mix 100 µl of the staining solution with 200 µl of the cell suspension, and incubate the mixture for 15 minutes at 37°C.
6. Observe both living cells stained yellowish-green fluorescent and dead cells stained red fluorescent using an excitation filter at  $490 \pm 10$  nm under fluorescence microscope. In addition, use a 545 nm emission filter to observe only dead cells.

*As the optimal staining conditions differ from cell to cell, we recommend that suitable concentration of PI and Calcein-AM be determined individually.*

## Precaution

1. Since Calcein-AM/DMSO solution is moisture sensitive, please close the cap of Solution A tightly after using.
2. PI is suspected to be highly carcinogenic, so careful handling is required. When PI solution contacts skin, immediately wash off using water.

*If you need more information, please contact Dojindo technical service.*

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