Cell Viability Assay Kit, Green/Red Fluorescence

For detection and distinction of viable and non-viable cells, using two fluorogenic dyes

Catalog #100-1545 1 Kit 200 Tests

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Product Description

Cell Viability Assay Kit, Green/Red Fluorescence provides a convenient and robust method to determine cell viability through the use of two fluorogenic dyes, Calcein AM and Propidium Iodide, that permit the simultaneous detection and distinction of viable and non-viable cells. As a fluorogenic dye, Calcein AM is initially non-fluorescent. After its passive entry into cells, intracellular esterases, which are present only in viable cells, hydrolyze Calcein AM into Calcein, a bright green fluorescent molecule (Bratosin et al.). The intensity of the green fluorescence is proportional to the amount of esterase activity and thus can correlate to the number of viable cells. Propidium Iodide is the second fluorogenic dye; however, unlike Calcein AM, it can only cross the compromised membranes of dead cells. Once inside dead cells, Propidium Iodide fluoresces red upon its binding to DNA. The dyes in this kit are well suited for use with a fluorescence microscope or fluorescence microplate plate reader that is capable of detecting in FITC (for Calcein) and TRITC (for Propidium Iodide) channels. This assay can detect and quantify cell proliferation in adherent or suspension cultures or incorporate it as a useful readout of in vitro cytotoxicity assays.

NOTE: This kit includes sufficient reagents for staining 200 tests (wells) in a 96-well plate or 800 tests (wells) in a 384-well plate.

Excitation Wavelength: 490 nm for live cells; 540 nm for dead cells
Emission Wavelength: 525 nm for live cells; 620 nm for dead cells

Product Information

The following components are sold as a complete kit (Catalog #100-1545) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
Calcein AM	300-1013	2 vials	Store at -20°C. Protect from light.	Product stable until expiry date (EXP) on box label.
Propidium Iodide†	300-1014	40 µL	Store at -20°C. Protect from light.	Product stable until expiry date (EXP) on box label.
DMSO*	300-1015	100 µL	Store at -20°C.	Product stable until expiry date (EXP) on box label.
Assay Buffer	300-1016	20 mL	Store at -20°C.	Product stable until expiry date (EXP) on box label.

†Please refer to the Safety Data Sheet (SDS) for hazard information.

*Please refer to the Safety Data Sheet (SDS) for hazard information. DMSO is a strong solvent and skin penetrant and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.

Directions for Use

The following protocol is for staining cells in a 96-well or 384-well plate. If using other cultureware, adjust volumes accordingly. Using a black-wall/clear-bottom plate is recommeneded.

NOTE: Thaw reagents at room temperature (15 - 25°C). If not used immediately, aliquot and store stock solutions at -20°C. Avoid repeated freeze-thaw cycles.

A. PREPARATION OF CALCEIN AM STOCK SOLUTION

To prepare Calcein AM stock solution, add 20 µL of dimethyl sulfoxide (DMSO) to a vial of lyophilized Calcein AM. Mix thoroughly; protect from light.

NOTE: If not used immediately, aliquot and store the stock solution at -20°C. Avoid repeated freeze-thaw cycles.

B. PREPARATION OF CALCEIN AM AND PROPIDIUM IODIDE WORKING SOLUTION

To make Calcein AM and Propidium Iodide working solution of 500X, add 20 µL of Calcein AM stock solution and 20 µL of Propidium Iodide to 10 mL of Assay Buffer. This volume of the working solution is sufficient to stain 100 tests (wells) in a 96-well plate or 400 tests (wells) in a 384-well plate.

NOTE: Calcein AM and Propidium Iodide working solution is stable for at least 2 hours at room temperature; however, it is recommended to be prepared fresh before each use. Use the working solution immediately; do not store. Protect from light.

C. STAINING CELLS AND FLUORESCENCE DETECTION

1. Culture the cells in appropriate culture medium in a black-wall/clear-bottom (flat) 96- or 384-well plate. For cell proliferation or cytotoxic assays, treat cells with test compounds. Incubate cells at 37°C and 5% CO_2 for an appropriate length of time.
   - 96-well plate: Suggested working culture volume of 100 µL per well
   - 384-well plate: Suggested working culture volume of 25 µL per well

   NOTE: The optimal cell density and incubation time should be determined for different cell types.
2. Add an equal volume of Calcein AM and Propidium Iodide working solution to the cells as follows:
   - 96-well plate: 100 µL per well
   - 384-well plate: 25 µL per well

   Ensure bubbles are not introduced into each well, as they may interfere with absorbance readings.
3. Incubate at room temperature or 37°C for 15 - 60 minutes; protect from light. Do not wash cells after staining with Calcein AM and Propidium Iodide working solution.

   NOTE: The optimal incubation time should be determined for different cell lines.
4. Observe stained cells using a fluorescence microscope or microplate reader equipped with a FITC filter for live cell detection and a TRITC filter for dead cell detection.

References

Bratosin D et al. (2005) Novel fluorescence assay using calcein-AM for the determination of human erythrocyte viability and aging. Cytometry A66(1): 78–84.

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