Live or Dead™ Cell Viability Assay Kit
Green/Red Dual Fluorescence

Catalog number: 22789, 22760
Unit size: 200 Tests, 1000 Tests

Component	Storage	Amount	
		Cat No. 22789	Cat No. 22760
Component A: CytoCalcein™ Green	Freeze (<-15 °C), Dessicated, Avoid Light	2 vials, lyophilized	10 vials, lyophilized
Component B: Propidium Iodide	Freeze (<-15 °C)	1 vial (10 mM, 40 µL)	1 vial (10 mM, 200 µL)
Component C: DMSO	Freeze (<-15 °C)	1 vial (100 µL)	1 vial (500 µL)
Component D: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)	1 bottle (100 mL)

OVERVIEW

This Live or Dead™cell viability uses two fluorogenic indicators: calcein AM for viable cells and a cell-impermeable DNA-binding dye for the cells with compromised membranes. Calcein AM is a hydrophobic compound that easily permeates intact live cells, and becomes strongly fluorescent upon hydrolysis by esterases. The hydrolysis of the non-fluorescent calcein AM by intracellular esterases generates the strongly fluorescent hydrophilic calcein that is well-retained in the cell cytoplasm. The esterase activity is proportional to the number of vial cells. The DNA-binding dye is quite polar and impermeable for viable cells that have intact membranes. It becomes fluorescent only upon binding to DNA of dead cells. Cells grown in black-walled plates can be stained and quantified in less than two hours. The assay is more robust and accurate than the other viability assays. It can be readily adapted for high-throughput assays in a wide variety of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. The kit provides all the essential components with an optimized assay protocol. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells. Using 100 ul of reagents per well in a 96-well format, this kit provides sufficient reagents to perform 100 assays. Using 25 ul of reagents per well in a 384-well format, this kit provides sufficient reagents to perform 400 assays.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds
2. Add the same volume of CytoCalcein™ Green/Propidium Iodide dye-working solution (100 µL/well/ 96-well plate or 25 µL/well/384-well plate)
3. Incubate at room temperature or 37°C for 1 hour
4. Monitor fluorescence at intensity (bottom read mode) Ex/Em = 490/525 nm (Cutoff = 515 nm, live) and 540/620 nm (Cutoff = 590 nm, dead), fluorescence microscope with FITC filter (live) and TRITC filter (dead), or flow cytometer with FL1 and FL2 channels

Important Thaw one of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm (live), 540 nm (dead)
Emission:	525 nm (live), 620 nm (dead)
Cutoff:	515 nm, 590 nm
Recommended plate:	Solid black
Instrument:	Fluorescence microscope
Excitation:	FITC filter (live), TRITC filter (dead)
Emission:	FITC filter (live), TRITC filter (dead)
Recommended plate:	Black wall/clear bottom
Instrument:	Flow cytometer
Excitation:	FL1 channel
Emission:	FL2 channel

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. CytoCalcein™ Green stock solution:
Add 20 µL of DMSO (Component C) into the vial of CytoCalcein™ Green (Component A) and mix well to make CytoCalcein™ Green stock solution. Protect from light.

Note 20 µL of CytoCalcein™ Green stock solution is enough for one plate. For storage, seal tubes tightly.

PREPARATION OF WORKING SOLUTION

Add the whole content (20 µL) of CytoCalcein™ Green stock solution and 20 µL Propidium Iodide (Component B) into 10 mL of Assay Buffer (Component C) and mix well to make CytoCalcein™ Green/Propidium Iodide dye-working solution. The CytoCalcein™ Green/Propidium Iodide dye-working solution is stable for at least 2 hours at room temperature.

Note If the cells such as CHO cells contain organic-anion transporters which cause the leakage of the fluorescent dye over time, a probenecid stock solution should be prepared and added to the loading buffer at a final in-well working concentration ranging from 1 to 2.5 mM. Unused probenecid stock solution can be stored at ≤ -20 °C. As the optimal staining conditions may vary depending on different cell types, it's recommended to determine the appropriate concentration of Component A and B individually.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Run the cell viability assay with plate reader or fluorescence microscope:

1. Treat cells with test compounds as desired.

   Note It is not necessary to wash cells before adding compound. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before adding compounds. Add 100 µL/well/96-well plate and 25 µL/well/384-well plate of 1X Hank's salt solution and 20 mM Hepes buffer (HHBS) or the buffer of your choice after aspiration. Alternatively, cells can be grown in serum-free media.

2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of CytoCalcein™ Green/Propidium Iodide dye-working solution.

3. Incubate the plate at room temperature or 37°C for 30 minutes to 1 hour, protected from light. (The incubation time could be from 15 minutes to overnight. We got the optimal results with the incubation time less than 4

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hours).

Note The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment. DO NOT wash the cells after loading. For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after incubation.

4. Monitor the fluorescence intensity with a fluorescence plate reader (bottom read mode) at Ex/Em = 490/525 nm (Cutoff = 515 nm, live) and Ex/Em = 540/620 nm (Cutoff = 590 nm, dead) or fluorescence microscope with FITC filter for live cells or TRITC filter for dead cells.

Run the cell viability assay with a flow cytometer:

1. Treat cells with test compounds for a desired period of time.
2. Centrifuge the cells to get 1 - 5 × 10^5 cells/tube.
3. Resuspend cells in 500 µL of CytoCalcein™ Green/Propidium Iodide dye-working solution.
4. Incubate at room temperature or 37°C for 10 to 30 minutes, protected from light.
5. **Optional:** Wash the cells with HHBS or buffer of your choice. Resuspend cells in 500 µL of HHBS to get 1 - 5 × 10^5 cells per tube.
6. Monitor the fluorescence intensity with flow cytometer at Ex/Em = 490/525 nm and Ex/Em = 490/620 nm (FL1 and FL2 channels).

EXAMPLE DATA ANALYSIS AND FIGURES

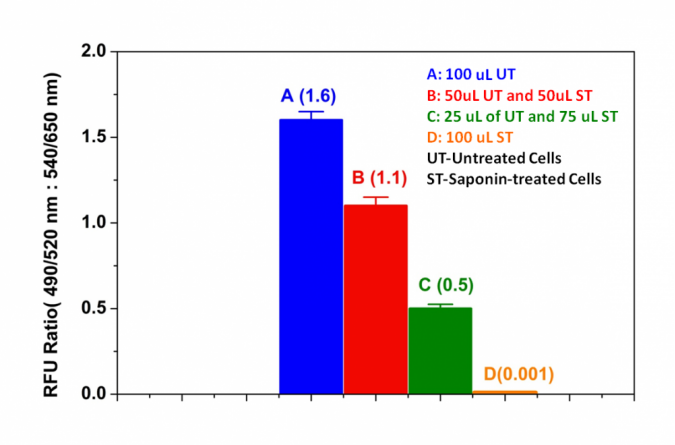

Figure 1. The Effect of Jurkat cells on Saponin induced cell death measured with Cell Meter™ Cell Viability Assay Kit. Jurkat cells at 2 x10^6 cells/mL were treated with or without 0.5% Saponin for 5 minutes. Cells were centrifuged and the supernatant were replaced with fresh medium. 100 uL of untreated cells (A), 50 uL each of untreated and treated cells (B), 25 uL of untreated and 75 uL treated cells (C), and 100 uL of 0.5% saponin treated cells (D) were plated in a 96-well black wall/clear bottom Poly-D-lysine plate. The cells were incubated with 100 µL/well of CytoCalcein™ Green/ Propidium Iodide dye-working solution for 1 hr at 37 °C. The fluorescence intensity was measured at Ex/Em = 490/525 nm and 540/650 nm with bottom read mode using NOVOstar instrument (BMG Labtech). The ratio of 490/525 nm to 540/650 nm fluorescence intensity on live and dead cells were showed as indicated (n=6).

DISCLAIMER