Cell Meter™ Cell Viability Assay Kit *Green/Red Dual Fluorescence*

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22789 (2 plates) 22789-B (10 plates)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

The Cell Meter™ assay kits are a set of tools for monitoring cell viability and cellular functions. This particular kit uses two non-fluorescent indicators: CytoCalcein™ Green for viable cells and a cell-impermeable DNA-binding dye for the cells with compromised membranes. CytoCalcein™ Green can easily permeate intact live cells and is hydrolyzed by intracellular esterase to generate the strongly fluorescent hydrophilic CytoCalcein™ Green which is well-retained in the cell cytoplasm. The esterase activity is proportional to the number of viable cells. The DNA-binding dye is quite polar and impermeable for viable cells that have intact membranes. It becomes fluorescent only upon binding to the DNA of dead cells. Cells grown in black-wall 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 a wide variety of fluorescence platforms such as microplate assays, fluorescence microscope, and flow cytometry. The kit provides all the essential components with an optimized assay protocol. It is suitable for both proliferating and non-proliferating cells (either suspension or adherent cells).

Kit Components

Component	**Amount**	
	#22789	**#22789-B**
	200 assays (96-well) 800 assays (384-well)	1,000 assays (96-well) 4,000 assays (384-well)
Component A: CytoCalcein™ Green	2 vials, lyophilized	10 vials, lyophilized
Component B: Propidium Iodide	1 vial (10 mM, 40 uL)	1 vial (10 mM, 200 uL)
Component C: DMSO	1 vial (100 µL)	1 vial (500 µL)
Component D: Assay Buffer	1 bottle (20 mL)	1 bottle (100 mL)

Caution: *Propidium Iodide is suspected to be highly carcinogenic, so careful handling is required.*

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells with test compounds → Add the same volume of dye-loading solution (100 µL/well/ 96-well plate or 25 µL/well/384-well plate) → Incubate at room temperature or 37 °C for 1 hour → Monitor fluorescence at intensity Ex/Em = 490/525 nm (live) and 540/620 nm (dead)

1. Prepare cells:

Plate 100 to 100, 000 cells/well in a tissue culture microplate with black wall and clear bottom. Add test compounds into the cells and incubate for a desired period of time (such as 24, 48 or 96 hours) in a 37 °C, 5% CO_2 incubator. For blank wells (medium without the cells), add the same amount of compound buffer. The suggested total volume is 100 µL/well/96-well plate and 25 µL/well/384-well plate.

Note: Each cell line should be evaluated on the individual basis to determine the optimal cell density for proliferation or cytotoxicity induction. For proliferation assays, use fewer cells, and for cytotoxicity assays, use more cells to start with.

2. Prepare dye-loading solution:

2.1 Thaw one of each kit component at room temperature before use.

2.2 Make CytoCalcein™ Green stock solution: Add 20 µL of DMSO (Component C) into the vial of CytoCalcein™ Green (Component A), and mix them well.

Note: 20 µL of CytoCalcein™ Green stock solution is enough for one plate. Unused CytoCalcein™ Green stock solution can be aliquoted and stored at ≤ -20 °C for one month if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles.

2.3 Make CytoCalcein™ Green/Propidium Iodide dye-loading solution for one cell plate: Add the whole content (20 µL) of CytoCalcein™ Green stock solution (from Step 2.2) and 20 µL Propidium Iodide (20 Component B) into 10 mL of Assay Buffer (Component C), and mix well. The working solution is stable for at least 2 hours at room temperature.

Note 1: 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

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working concentration ranging from 1 to 2.5 mM. Aliquot and store the unused probenecid stock solution at ≤ -20 °C.
Note 2: 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.

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

3.1 Treat cells with test compounds as desired (from Step 1).
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.

3.2 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of dye-loading solution (from Step 2.3).

3.3 Incubate the dye-loading 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 hours.)
Note 1: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
Note 2: DO NOT wash the cells after loading.
Note 3: For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after incubation.

3.4 Monitor the fluorescence intensity at Ex/Em = 490/525 nm (FITC filter) for live cells, and 540/620 nm (TRITC filter) for dead cells with a bottom read mode.

4. Run the cell viability assay with a flow cytometer:

4.1 Treat cells with test compounds for a desired period of time.

4.2 Centrifuge the cells to get 1-5 × 10^5 cells/tube.

4.3 Resuspend cells in 500 µL of CytoCalcein™ Green/ Propidium Iodide dye-loading solution (from Step 2.3).

4.4 Incubate at room temperature or 37 °C for 10 to 30 minutes, protected from light.
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.*

4.5 Monitor the fluorescence intensity at Ex/Em = 490/525 and 620 nm with a flow cytometer (using FL1 and FL2 channels).

Data Analysis

The fluorescence in blank wells with the growth medium is subtracted from the values for those wells with cells treated with the test compounds. The background fluorescence of the blank wells may vary depending on the sources of the microtiter plates or the growth media.

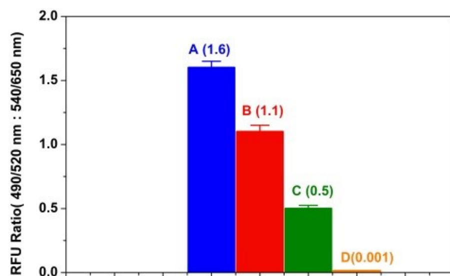

Figure 1. The Effect of Jurkat cells on Saponin induced cell death measured with Cell Meter™ Cell Viability Assay Kit**.** Jurkat cells at 2 X10^6 cells/mL were treated with or without 0.5% Saponin for 5 minutes, cells then centrifuged and the supernatant were replaced with fresh medium. 100 uL of untreated 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-loading 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).

References

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