Cell Meter™ Cell Viability Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22783 (2 plates)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

There are a variety of parameters that can be used to monitor cell viability. The proprietary red fluorescent dye used in the kit is a hydrophobic compound. It easily permeates intact live cells and gets enhanced fluorescence upon entering into live cells. The hydrolysis of the non-fluorescent substrate by intracellular esterases generates a strongly red fluorescent hydrophilic product that is well-retained in the cell cytoplasm. The red fluorophore generated by the non-fluorescent substrate used in the kit has the spectral properties of fluorescein at Ex/Em = ~570 nm/590 nm. When well excited at 540 nm, the fluoreophore emits intense red fluorescence at ~590 nm.

The kit provides all the essential components with an optimized cell-labeling protocol for fluorescence microplate assays. It can also be used with a fluorescence microscope equipped with a TRITC filter set. This Cell Meter™ Cell Viability Assay Kit provides an effective tool of labeling cells for fluorescence microplate and microscopic investigations of cellular functions. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The kit is suitable for proliferating and non-proliferating cells.

Kit Components

Components	**Amount**
Component A: CytoCalcein™ Red AM	2 vials, lyophilized
Component B: DMSO	1 vial (100 µL)
Component C: Assay Buffer	1 bottle (20 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells with test compounds → Remove growth medium → Add dye-loading solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at 37 °C for 30 min-1 hour → Monitor fluorescence intensity at Ex/Em = 540/590 nm

1. Prepare cells:

Plate 100 to 10, 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 for a 96-well plate, and 25 µL for a 384-well plate.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for proliferation or cytotoxicity induction. For proliferation assays, use fewer cells; 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™ Red stock solution: Add 20 µL of DMSO (Component B) into one vial of CytoCalcein™ Red (Component A), and mix well.

Note: 20 µL of CytoCalcein™ Red stock solution is enough for one plate. Unused CytoCalcein™ Red 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™ Red dye-loading solution for one cell plate: Add the whole content (20 µL) of CytoCalcein™ Red stock solution (from Step 2.2) into 10 mL of Assay Buffer (Component C), and mix well. The working solution is not stable, use it promptly.

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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 of 1-2.5 mM. Aliquot and store the unused probenecid stock solution at ≤ -20 °C.

3. Run the cell viability assay:

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 Remove growth medium.

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

3.4 Incubate the dye-loading plate at room temperature or 37 °C for 30 min-1 hour, protected from light.
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: For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after incubation.

3.5 Monitor the fluorescence intensity at Ex/Em = 540/590 nm (cutoff at 570 nm) with bottom read mode.

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.

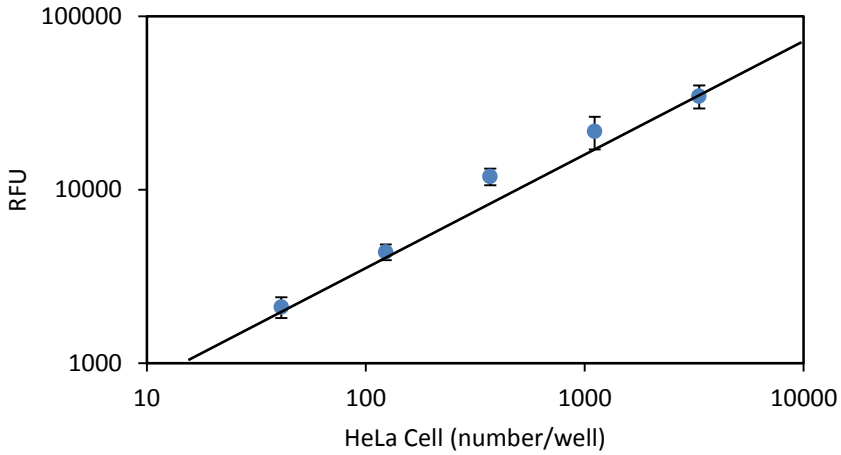

Figure 1. Hela cell number response was measured with Cell Meter™ Cell Viability Assay Kit. Hela cells at 0 to 3,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of CytoCalcein™ Red dye-loading solution for 30 min at 37 °C. The fluorescence intensity was measured at Ex/Em = 540/ 590 nm (cutoff at 570 nm) with bottom read mode using Flexstation (from Molecular devices).

References

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