Cell Meter™ Cell Viability Assay Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22786 (5 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 green 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 green fluorescent hydrophilic product that is well-retained in the cell cytoplasm. The green fluorophore generated by the non-fluorescent substrate used in the kit has the spectral properties of fluorescein at Ex/Em = ~490 nm/520 nm. When well excited with the Argon Laser at 488 nm, the fluoreophore emits intense green fluorescence at ~520 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 FITC 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 Key Features

Robust:	Higher maximum signal with lower variation across the plate.
Convenient:	Formulated to have minimal hands-on time.
Rapid Dye Loading:	Dye loading at RT for 30 min to 1 hr.
Versatile Applications:	Compatible with many cell lines and targets.

Kit Components

Components	**Amount**
Component A: CytoCalcein™ Green	5 vials, lyophilized
Component B: DMSO	1 vial (200 µL)
Component C: Assay Buffer	1 bottle (50 mL)

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 intensity at Ex/Em = 490/525 nm

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 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™ Green stock solution: Add 20 µL of DMSO (Component B) into the vial of CytoCalcein™ Green (Component A), and mix 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 dye-loading solution for one cell plate: Add the whole content (20 µL) of CytoCalcein™ Green stock solution (from Step 2.2) 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: 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 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 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.

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.

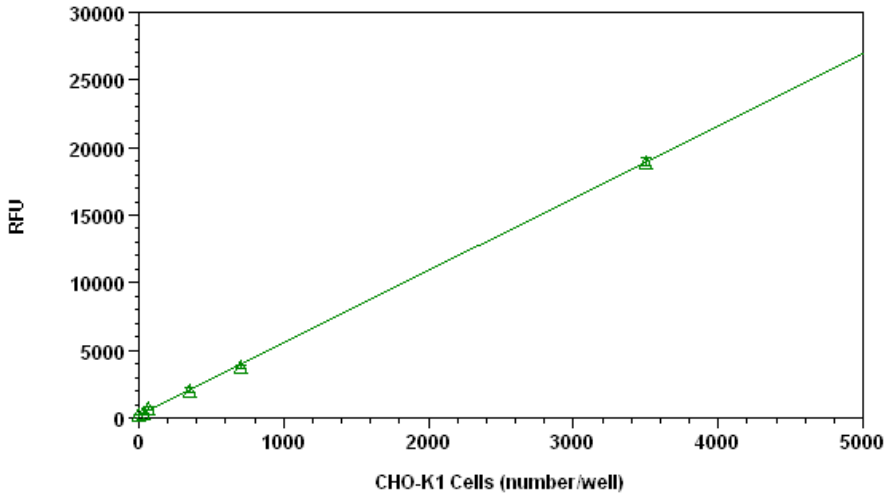

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Cell Viability Assay Kit. CHO-K1 cells at 0 to 5,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of CytoCalcein™ Green dye-loading solution for 1 hour at 37 ^{o}C. The fluorescence intensity was measured at Ex/Em = 490/ 525 nm with NOVOstar instrument (from BMG Labtech). The fluorescence intensity was linear (R^2 = 1) to the cell number as indicated. The detection limit was 30 cells/well (n=6).

References

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