Cell Meter™ Viability Assay Kit

NIR Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 22787 (2 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. There are a variety of parameters that can be used for monitoring cell viability. This kit uses a proprietary dye that gets enhanced fluorescence upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The weakly fluorescent substrate is hydrolyzed by intracellular esterase to generate a strongly fluorescent hydrophilic product that is well-retained in the cell cytoplasm. The esterase activity is proportional to the number of viable cells, and thus directly related to the fluorescence intensity of the product generated from the esterase-catalyzed hydrolysis of the fluorogenic substrate. Cells grown in black-walled plates can be stained and quantified in less than two hours. The assay is more robust than the tetrazolium salt or Alarmar Blue™-based ones. The kit can be readily adapted for many different types of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The kit provides all the essential components with an optimized cell-labeling protocol and can be used for both proliferating and non-proliferating cells (either suspension or adherent cells). It contains sufficient reagents to perform either 200 assays ((100 uL/well for a 96-well format) or 800 assays (25 uL/well for a 384-well format).

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™ NIR	2 vials, lyophilized
Component B: DMSO	1 vial (100 µL)
Component C: Assay Buffer	2 bottles (10 mL/bottle)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells with test compounds →Remove the medium→Add 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 hr→ Read fluorescence intensity at Ex/Em = 635/670 nm

1. Prepare cells:

Plate 100 to 100,000×10 cells per 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 (for 1 plate):

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

2.2 Make CytoCalcein™ NIR stock solution: Add 20 µL of Component B (DMSO) into the vial of Component A (CytoCalcein™ NIR), and mix them well.
Note: 20 µL of CytoCalcein™ NIR stock solution is enough for one plate. Unused CytoCalcein™ NIR 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™ NIR dye-loading solution for one cell plate: Add 20 µL of CytoCalcein™ NIR stock solution (from Step 2.2) into the bottle of Component C (Assay Buffer, 10 mL), and mix them well. The dye-loading solution is stable for at least 2 hours at room temperature.

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 the medium from the cells.
Note: Medium must be removed before dye loading.

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 1 hr, 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.
Note2: 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 = 635/670 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.

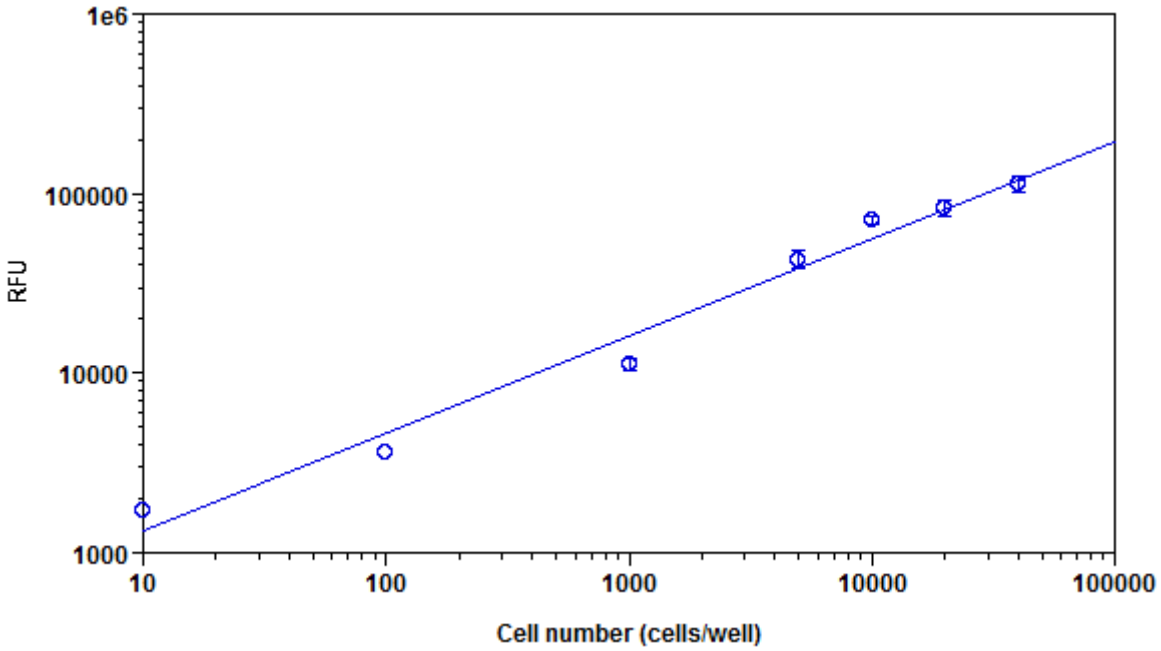

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Viability Test Kit. CHO-K1 cells at 0 to 50,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom Costar plate. The cells were incubated with 100 µL/well of CytoCalcein™ NIR dye-loading solution for 1 hr at room temperature. The fluorescence intensity was measured at Ex/Em = 635/670 nm with FlexStation (from Molecular devices).

References

1. Zibek S, Stett A, Koltay P, Hu M, Zengerle R, Nisch W, Stelzle M. (2006) Localized functional chemical stimulation of TE 671 cells cultured on nanoporous membrane by calcein and acetylcholine. Biophys J.
2. Klesius PH, Evans JJ, Shoemaker CA, Pasnik DJ. (2006) A vaccination and challenge model using calcein marked fish. Fish Shellfish Immunol, 20, 20.
3. Bratosin D, Mitrofan L, Palii C, Estaquier J, Montreuil J. (2005) Novel fluorescence assay using calcein-AM for the determination of human erythrocyte viability and aging. Cytometry A, 66, 78.
4. Schoonen WG, Westerink WM, de Roos JA, Debiton E. (2005) Cytotoxic effects of 100 reference compounds on Hep G2 and HeLa cells and of 60 compounds on ECC-1 and CHO cells. I mechanistic assays on ROS, glutathione depletion and calcein uptake. Toxicol In Vitro, 19, 505.