Cell Meter™ Viability Assay Kit

Blue Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: #22785 (5 plates)	Keep in freezer and avoid 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 cell viability dye that gets enhanced fluorescence upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the weakly CytoCalcein™ Violet 460, AM by intracellular esterases generates 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 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. 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. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells.

Kit Key Features

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

Kit Components

Components	**Amount**
Component A: CytoCalcein™ Violet 460, AM	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 for 96-well plate or 25 µL for 384-well plate) → Incubate at room temperature or 37°C for 1 hr → Read Fluorescence at Ex/Em = 405/460 nm

1. Prepare cells:

1.1 Plate 100 to 10,000 cells per well in a tissue culture microplate with black wall and clear bottom. Add test compounds into the cells 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 corresponding amount of compound buffer. The total suggested 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, use fewer cells, and for cytotoxicity assays, use more cells to start with.

2. Prepare dye-loading solution (for 1 plate):

2.1 Thaw 1 vial each of all the components at room temperature before use.

2.2 Make CytoCalcein™ Violet 460, AM stock solution by adding 20 µL Component B (DMSO) into the vial of Component A (CytoCalcein™ Violet 460, AM), mixing them well.
Note: 20 µL of reconstituted CytoCalcein™ Violet 460, AM is enough for 1 plate, unused reconstituted CytoCalcein™ Violet 460 can be aliquoted and stored at ≤ -20°C for one month if the tubes are sealed tightly, avoiding light and repeated freeze-thaw cycles.

2.3 Make CytoCalcein Violet 460, AM dye-loading solution for one cell plate by adding whole content (20 µL) of DMSO reconstituted CytoCalcein Violet 460, AM stock solution (from step 2.2) into 10 mL of Component C (Assay Buffer), mixing them well. This working solution is stable for at least 2 hours at room temperature.
Note: If your cells (such as CHO cells) containing organic-anion transporters which promote the leakage of the fluorescent dye over time, a stock probenecid solution should be prepared and add to the loading buffer at a final in-well working concentration of 1-2.5 mM. Aliquot and store the unused stock probenecid 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 compound addition. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before adding compounds, provide residual volumes after the aspirate step. 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 hr (15 minutes to overnight, best results within 4 h), protecting 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: DO NOT wash the cells after loading.
Note 3: For non-adherent cells, it is recommended that centrifuge cell plates at 800 rpm for 2 minutes, brake off, after the incubation of the dye.

3.4 Monitor the fluorescence at Ex/Em = 405/460 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 can be varied depending on the sources of the microtiter plates or of the growth media.

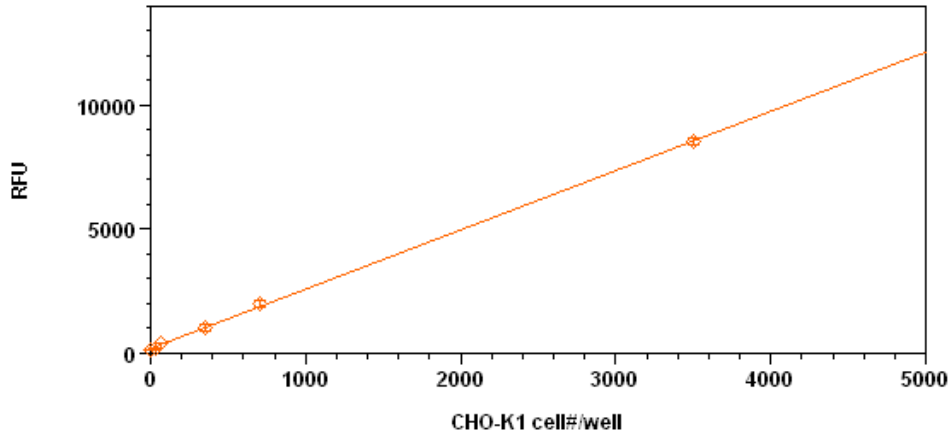

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Viability Test Kit. CHO-K1 cells at 0 to 5,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom Costar plate. The cells were incubated with 100 µL/well of CytoCalcein Violet 460, AM dye-loading solution for 1 hr at room temperature. The fluorescence intensity was measured at Ex/Em = 405/460 nm using NOVOstar instrument (from BMG Labtech). The fluorescence intensity was linear (R^2 = 1) to the cell number as indicated. The detection limit from the blank controls was 70 cells/well (n=6).

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.