Cell Meter™ Cell Viability Assay Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22785 (5 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 to monitor cell viability. This kit uses our proprietary cell viability dye whose fluorescence is strongly enhanced upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The weakly fluorescent CytoCalcein™ Violet 450, AM 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 wall/clear bottom plates can be stained and quantified in less than two hours. The assay is more robust than tetrazolium salt or Alarmar Blue® based ones. It can be readily adapted for many different types of fluorescence platforms such as microplate assays, fluorescence microscope, and flow cytometry. The kit is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. It provides all the essential components with an optimized cell-labeling protocol and can be used for both suspension and adherent 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 hour.
Versatile Applications:	Compatible with many cell lines and targets.

Kit Components

Components	**Amount**
Component A: CytoCalcein™ Violet 450, 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/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 = 405/460 nm

1. Prepare cells:

Plate 100 to 10,000 cells/well in a tissue culture microplate with black wall and clear bottom, and add test compounds into the cells. 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.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

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™ Violet 450, AM stock solution: Add 20 µL of DMSO (Component B) into the vial of CytoCalcein™ Violet 450, AM (Component A), and mix well.
Note: 20 µL of CytoCalcein™ Violet 450, AM stock solution is enough for one plate. Unused CytoCalcein™ Violet 450 stock solution could 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 Violet 450, AM dye-loading solution for one cell plate: Add the whole content (20 µL) of CytoCalcein Violet 450, AM stock solution (from Step 2.2) into 10 mL of Assay Buffer (Component C), and mix them well. This 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 promote 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 ranging from 1 to 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 compounds. 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 a 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 = 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 may vary depending on the sources of the microtiter plates or the growth media.

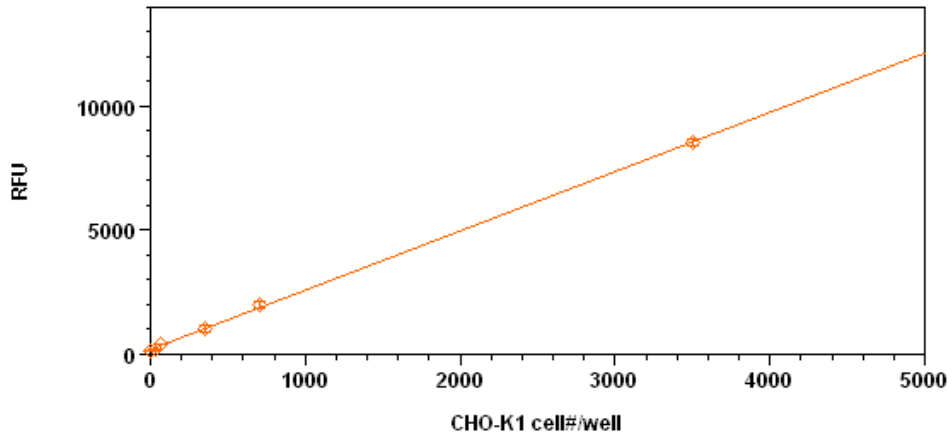

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Viability Test Kit. CHO-K1 cells at 0 to 5,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of CytoCalcein Violet 450, AM dye-loading solution for 1 hour at room temperature. The fluorescence intensity was measured at Ex/Em = 405/460 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 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.