

Cell Meter™ Cell 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. The proprietary far red fluorescent dye used in the kit is a hydrophobic compound that can easily permeate intact live cells and gets enhanced fluorescence upon entering into live cells. The hydrolysis of the weakly 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 weakly fluorescent substrate has the spectral properties similar to those of Cy5® or Alexa Fluor® 647 at Ex/Em = ~640/660 nm (Cy5® or Alexa Fluor® 647 are the trademarks of GE Healthcare and Invitrogen respectively). When well excited with the He-Ne Laser at 633 nm, the red fluorophore emits intense red fluorescence at ~660 nm.

This Cell Meter™ Cell Viability Assay Kit is an effective tool of labeling cells for the 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, and can be used for both suspension and adherent cells. It comes with reagents sufficient to perform 200 assays in a 96-well plate or 800 assays in a 384-well plate. The kit provides all the essential components with an optimized protocol for microplate reader analysis of cell viability.

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% CO₂ 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; 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™ NIR stock solution:** Add 20 µL of DMSO (Component B) into the vial of CytoCalcein™ NIR (Component A), 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 Assay Buffer (10 mL, Component C), 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 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 = 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.

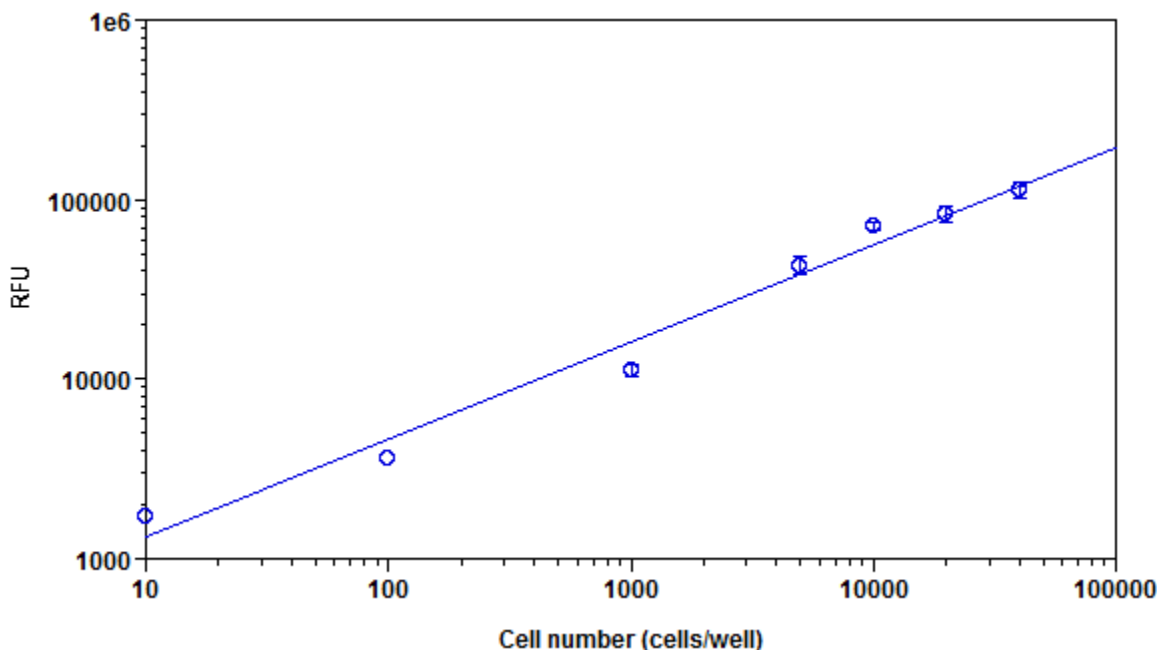


Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Viability Test Kit. CHO-K1 cells at 0 to 50,000 cells/well/100 μ L were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 μ L/well of CytoCalcein™ NIR dye-loading solution for 1 hour 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.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.