Cell Meter™ Cell Viability Assay Kit *NIR Fluorescence Optimized for Fluorescence Microplate Reader*

Catalog number: 22787
Unit size: 200 Tests

Component	Storage	Amount
Component A: CytoCalcein™ NIR	Freeze (<-15 °C), Minimize light exposure	2 vials, lyophilized
Component B: DMSO	Freeze (<-15 °C)	1 vial (100 µL)
Component C: Assay Buffer	Freeze (<-15 °C)	2 bottles (10 mL/bottle)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. 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 hydrolysis of the weakly fluorescent substrate 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. Using 100 uL of reagents per well in a 96-well format, this kit provides sufficient reagents to perform 200 assays. Using 25 uL of reagents per well in a 384-well format, this kit provides sufficient reagents to perform 800 assays.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds
2. Remove the medium
3. Add CytoCalcein™ NIR working solution (100 µL/well/96-well plate or 25 µL/well/384-well plate)
4. Incubate at room temperature or 37°C for 1 hr
5. Read fluorescence intensity (bottom read mode) at Ex/Em = 635/670 nm (Cutoff = 665 nm)

Important Thaw one of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	635 nm
Emission:	670 nm
Cutoff:	665 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. CytoCalcein™ NIR stock solution:
Add 20 µL of DMSO (Component B) into the vial of CytoCalcein™ NIR (Component A) and mix them well to make CytoCalcein™ NIR stock solution.

Note 20 µL of CytoCalcein™ NIR stock solution is enough for one plate. Protect from light. For storage, seal tubes tightly.

PREPARATION OF WORKING SOLUTION

Add 20 µL of CytoCalcein™ NIR stock solution into the bottle of Assay Buffer (10 mL, Component C) and mix well to make CytoCalcein™ NIR working solution.

Note This CytoCalcein™ NIR working solution is for one cell plate and stable for at least 2 hours at room temperature.

SAMPLE EXPERIMENTAL PROTOCOL

Cells Preparation:

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; for cytotoxicity assays, use more cells to start with.

Sample Protocol:

1. Treat cells with test compounds as desired.

   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.

2. Remove the medium from the cells.

   Note Medium must be removed before loading CytoCalcein™ NIR working solution.

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of CytoCalcein™ NIR working solution.

4. Incubate plate at room temperature or 37°C for 1 hour, protected from light.

   Note The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment. For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after incubation.

5. Monitor the fluorescence intensity with a fluorescence plate reader (bottom

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read mode) at Ex/Em = 635/670 nm (Cutoff = 665 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate CHO-K1 Cells samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

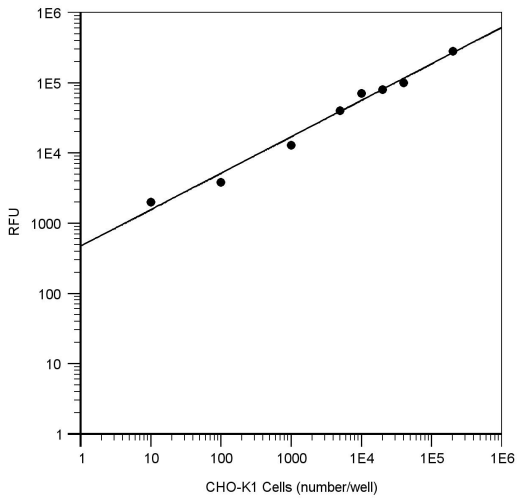

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Cell Viability Assay Kit. CHO-K1 cells at 0 to 50,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of CytoCalcein™ NIR dye-working solution for 1 hour at room temperature. The fluorescence intensity was measured at Ex/Em = 635/670 nm (Cutoff = 665 nm) with FlexStation™ microplate reader (Molecular Devices).

DISCLAIMER