Cell Meter™ Cell Viability Assay Kit *Blue Fluorescence with 405 nm Excitation*

Catalog number: 22784
Unit size: 500 Tests

Component	Storage	Amount
Component A: CytoCalcein™ Violet 450, AM	Freeze (<-15 °C), Minimize light exposure	5 vials, lyophilized
Component B: DMSO	Freeze (<-15 °C)	1 vial (200 µL)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)

OVERVIEW

There are a variety of parameters that can be used to monitor cell viability. The proprietary violet laser -excitable fluorescent dye used in the kit is a hydrophobic compound that easily permeates intact live cells and gets enhanced fluorescence upon entering into live cells. The hydrolysis of the non-fluorescent substrate by intracellular esterases generates a strongly blue fluorescent product that is well-retained in the cell cytoplasm. The blue fluorophore generated by the esterase hydrolysis of the non-fluorescent substrate has the spectral properties of fluorescein. When excited at 405 nm, the fluoreophore emits intense blue fluorescence at ~450 nm. The kit provides all the essential components with an optimized cell-labeling protocol for fluorescence microplate assays. This Cell Meter™ Cell Viability Assay Kit provides an effective tool of labeling cells for fluorescence flow cytometry, microplate and microscopic 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.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds
2. Add the same volume of CytoCalcein™ Violet 450, AM working solution (100 µL/well/96-well plate or 25 µL/well/384-well plate)
3. Incubate at room temperature or 37°C for 1 hour
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 405/460 nm (Cutoff = 435 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	405 nm
Emission:	460 nm
Cutoff:	435 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™ 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 to make CytoCalcein™ Violet 450, AM stock solution.

Note 20 µL of CytoCalcein™ Violet 450, AM stock solution is enough for one plate. Protect from light. For storage, seal tubes tightly.

PREPARATION OF WORKING SOLUTION

Add the whole content (20 µL) of CytoCalcein™ Violet 450, AM stock solution into 10 mL of Assay Buffer (Component C), and mix well to make CytoCalcein™ Violet 450, AM working solution. This CytoCalcein™ Violet 450, AM 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.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells with test compounds as desired.

   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.

2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of CytoCalcein™ Violet 450, AM working solution.

3. Incubate the 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 The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

   Note DO NOT wash the cells after loading.

   Note For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after incubation.

4. Monitor the fluorescence intensity with a fluorescence plate reader (bottom read mode) at Ex/Em = 405/460 nm (Cutoff = 435 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:

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

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

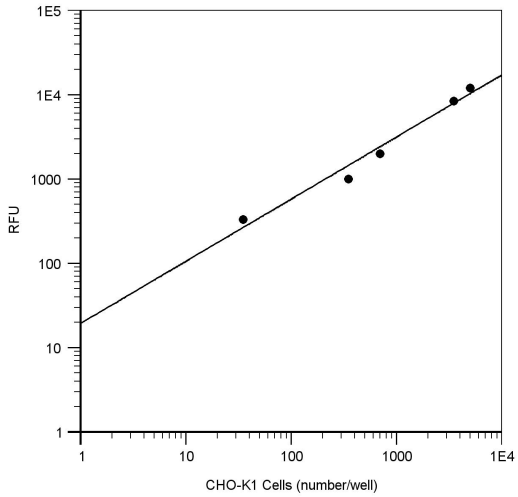

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Cell Viability Assay Kit. CHO-K1 cells at 0 to 5,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of CytoCalcein™ Violet 450, AM dye-working solution 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.