Cell Meter™ Cell Viability Assay Kit *Red Fluorescence*

Catalog number: 22783
Unit size: 200 Tests

Component	Storage	Amount
Component A: CytoCalcein™ Red AM	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)	1 bottle (20 mL)

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 non-fluorescent dye that gets enhanced red fluorescence upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the non-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. The fluorogenic indicator has spectral properties compatible with Cy3/TRITC filter set.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds
2. Remove the medium
3. Add CytoCalcein™ Red AM working solution (100 µL/well/96-well plate or 25 µL/well/384-well plate)
4. Incubate at room temperature or 37°C for 30 minutes - 1 hr
5. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 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™ Red AM stock solution:
Add 20 µL of DMSO (Component B) into the vial of CytoCalcein™ Red AM (Component A) and mix well to make CytoCalcein™ Red AM stock solution.

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

Note Unused CytoCalcein™ Red stock solution can be aliquoted and stored at < -20 °C for one month if the tubes are sealed tightly. Avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

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

Note This CytoCalcein™ Red AM working solution is not stable, use it promptly.

Note If the cells, such as CHO cells, contain organic-anion transporters which cause 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 of 1-2.5 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 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 growth medium.

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

4. Incubate plate at room temperature or 37°C for 30 minutes to 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 read mode) at Ex/Em = 540/590 nm (Cutoff = 570 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

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curve and equation. This equation can be used to calculate HeLa Cell 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

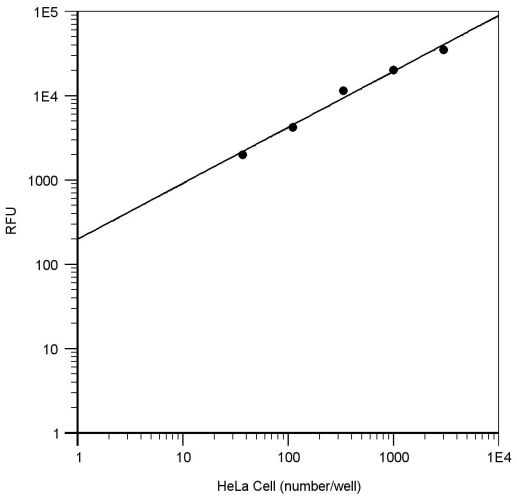

Figure 1. HeLa cell number response was measured with Cell Meter™ Cell Viability Assay Kit. HeLa cells at 0 to 3,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of CytoCalcein™ Red dye-working solution for 30 minutes at 37°C. The fluorescence intensity was measured at Ex/Em = 540/590 nm (Cutoff = 570 nm) with bottom read mode using Flexstation (from Molecular devices).

DISCLAIMER