AAT Bioquest®

PRODUCT INFORMATION SHEET

Cell Meter™ Cell Viability Assay Kit *Green Fluorescence*

Catalog number: 22786
Unit size: 500 Tests

Component	Storage	Amount
Component A: CytoCalcein™ Green	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

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 the non-fluorescent calcein AM that becomes strongly fluorescent upon entering into live cells. Calcein AM is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the non-fluorescent calcein AM by intracellular esterases generates the strongly fluorescent hydrophilic calcein 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 calcein generated from the esterase-catalyzed hydrolysis of calcein AM. 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 500 assays. Using 25 uL of reagents per well in a 384-well format, this kit provides sufficient reagents to perform 2000 assays.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds
2. Add the same volume of working solution as the cell medium
3. Incubate at room temperature or 37°C for 1 hour
4. Monitor fluorescence with fluorescence microplate reader at Ex/Em= 490/525 nm (Cutoff=515 nm)

Important Thaw one of each kit component at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 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™ Green stock solution:
Add 20 µL of DMSO (Component B) into the vial of CytoCalcein™ Green (Component A) and mix well. Protect from light.

Note 20 µL of CytoCalcein™ Green stock solution is enough for one plate.

PREPARATION OF WORKING SOLUTION

Add the whole content (20 µL) of CytoCalcein™ Green stock solution into 10 mL of Assay Buffer (Component C) and mix well.

Note The 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 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.

SAMPLE EXPERIMENTAL PROTOCOL

Cells Preparation:

Plate 100 to 100, 000 cells/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 for a 96-well plate, and 25 µL for a 384-well plate.

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.

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) or 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. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of dye-loading solution.

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 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 at Ex/Em = 490/525 nm with cutof=515 nm.

©2008 AAT Bioquest, Inc. Last revised November 2019. For more information and tools, please visit https://www.aatbio.com

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

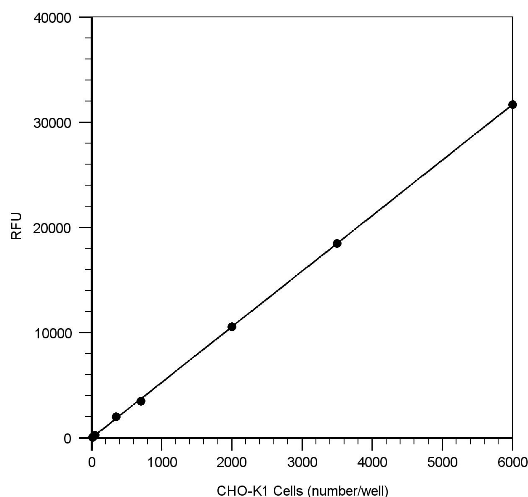

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Cell Viability Assay Kit. CHO-K1 cells at 0 to 5,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of dye-loading solution for 1 hour at 37°C. The fluorescence intensity was measured at Ex/Em = 490/ 525 nm with NOVOstar instrument (from BMG Labtech). The fluorescence intensity was linear (R□□ square = 1) to the cell number as indicated.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com
©2008 AAT Bioquest, Inc. Last revised November 2019. For more information and tools, please visit https://www.aatbio.com