Cell Meter™ Colorimetric Cell Cytotoxicity Assay Kit

Catalog number: 22779, 22780
Unit size: 5000 Tests, 1000 Tests

Component	Storage	Amount (Cat No. 22779)	Amount (Cat No. 22780)
Component A: Assay Solution	Freeze (< -15 °C), Minimize light exposure	5 bottles (20 mL)	1 bottle (20 mL)

OVERVIEW

Monitoring cell cytotoxicity is one of the most essential tasks for studying cellular functions. Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. There are a variety of parameters that can be used. This kit uses a proprietary water-soluble and cell-permeable dye that changes its absorption spectra upon cellular reduction. The absorption ratio change is directly proportional to the number of living cells. The characteristics of its high sensitivity, non-radioactivity and no-wash method make the kit suitable for high throughput screening of cell proliferation or cytotoxicity against a variety of compounds. This kit does not require pre-mixing of components and has higher sensitivity compared to the traditional tetrazolium-based colorimetric assays (such as WST-8, MTT and XTT). The kit provides the reagents sufficient to run 1000 assays (regular size) or 5000 assays (bulk package). All the kit components are quite stable with minimal cytotoxicity, thus a longer incubation time (such as 24 to 48 hours) is possible if required. Our Cell Meter™ Colorimetric Cell Cytoxicity Assay Kit is robust and convenient to use. It can be readily adapted for a wide variety of instrument platforms. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds (100 µL/well/96-well plate or 50 µL/well/384-well plate)
2. Add 1/5 volume of Assay Solution (Component A)
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 1 - 24 hours
4. Monitor absorbance ratio at A_{570nm}/A_{605nm}

Important Note

Thaw the component at room temperature before starting the experiment.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	570/605 nm
Recommended plate	Black wall/clear bottom

SAMPLE EXPERIMENTAL PROTOCOL

1. Plate 100 to 10,000 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 corresponding amount of compound buffer. The suggested total volume is 100 µL for a 96-well plate and 50 µL for a 384-well plate.

2. Set up the following controls at the same time.

   A. **Positive control:** contains cells and known proliferation or cytotoxicity inducer.

   B. **Negative control:** contains cells but no test compounds.

   C. **Vehicle control:** contains cells and the vehicle used to deliver test compounds.

   D. **Non-cell control:** contains growth medium without cells.

   E. **Test compound control:** contains the vehicle control used to deliver test compounds [Hank's balance salt solution (HBSS) or phosphate-buffered saline (PBS)] and test compound. Some test compounds have strong autofluorescence and may give false positive results.

   Note: Match the total volume of all the controls to 100 µL for a 96-well plate or 50 µL for a 384-well plate with growth medium.

3. Warm up the Assay Solution (Component A) to 37°C. Mix it thoroughly before starting the experiments.

4. Add 20 µL (96-well plate) or 10 µL (384-well plate) of Assay Solution (Component A) into each well. Mix the reagents by shaking the plate gently for 30 seconds.

5. Incubate the cells in a 37°C, 5% CO2 incubator for 1 - 24 hours, protected from light.

   Note: The appropriate incubation time depends on the metabolism rate of the individual cell type and cell concentration used. Optimize the incubation time for each experiment. Extremely prolonged incubation time is not recommended since the indicator could be converted to colorless compound.

6. Monitor the absorbance increase with an absorbance plate reader at OD ratio A_{570nm}/A_{605nm}. The ratio of OD_{570} to OD_{605} is used to determine the cell viability in each well.

   Note: The cell viability is proportional to increased OD_{570} and decreased OD_{605}.

EXAMPLE DATA ANALYSIS AND FIGURES

The background absorbance reading from the non-cell control well is subtracted from the values for those wells containing the cells.

Note: The background absorbance of the blank wells may vary depending on the sources of the growth media or the microtiter plates.

The absorbance reading in each well indicates the cell number in the well.

Calculate the percentage of cell viability for samples and controls based on the following formula:

$$\% \text{ Cell viability} = 100 \times (R_{sample} - R_o)/(R_{ctrl} - R_o)$$

R_{sample} is the absorbance ratio of OD_{570}/OD_{605} in the presence of the testcompound.

R_{ctrl} is the absorbance ratio of OD_{570}/OD_{605} in the absence of the test

compound(vehicle control).

R_o is the averaged background (non-cell control) absorbance ratio of OD_{570}/OD_{605}.

The reading (Abs) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Cell number/well 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-TeI:

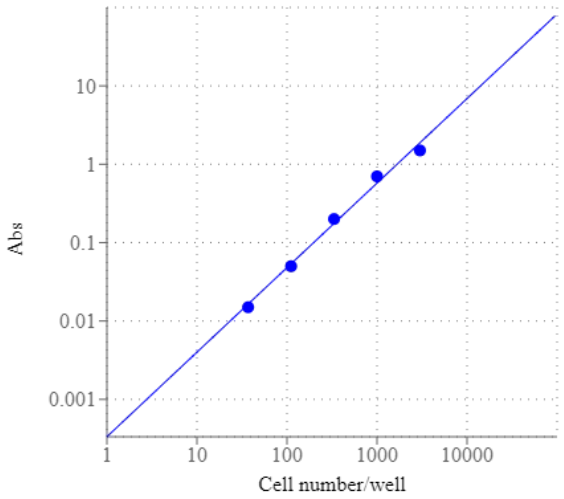

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Colorimetric Cell Cytotoxicity Assay Kit. CHO-K1 cells at 0 to 10,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96- well plate. The cells were incubated with 20 µL/well of Assay Solution (Component A) for 3 hours at 37 °C. The absorbance intensity was measured at 570 nm and 605 nm with SpectraMax plus (Molecular Devices). The ratio of OD570/OD605 is proportional to the number of cells as indicated.

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