

Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit

Catalog number: 22781, 22782
Unit size: 1000 Tests, 5000 Tests

Component	Storage	Amount	
		Cat No. 22781	Cat No. 22782
Component A: Assay Solution	Freeze (<-15 °C), Minimize light exposure	20 mL	100 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. The measurement of mitochondrial dehydrogenases (e.g. LDH) activity is a well-accepted assay to quantify cell numbers and monitor cell viability. This cell viability assay kit provides a fast, simple, accurate and homogeneous assay for the fluorometric detection of viable cells. This assay is based on the observation that blue and non-fluorescent resazurin is reduced to a pink fluorescent dye (resorufin) by accepting the electron from mitochondrial respiratory chain in live cells. The amount of resorufin produced is directly proportional to the number of living cells. The detection sensitivity of cell proliferation and cytotoxicity assays using this kit is higher than other assays such as MTT. Since the kit components are quite stable with minimal cytotoxicity, a longer incubation (such as 24 to 48 hours) is possible. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format. The characteristics of its high sensitivity (<100 CHO cells), non-radioactive and no-wash method made the kit suitable for high throughput screening of cell proliferation or cytotoxicity against a variety of compounds and adaptable for a wide variety of instrument platforms. The kit provides all the essential components with an optimized assay protocol. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells. Using 20 µl of reagents per well in a 96-well format, this kit provides sufficient reagents to perform 1000 assays. Using 10 µl of reagents per well in a 384-well format, this kit provides sufficient reagents to perform 2,000 assays.

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% CO₂ incubator for 1 - 24 hours
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important Thaw the 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 CELL SAMPLES

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

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% CO₂

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 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.

Note LDH in serum will contribute to background fluorescence.

- E. **Test compound control:** contains the vehicle 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, and mix it thoroughly before starting the experiments.
4. Add 20 µL/well (96-well plate) or 10 µL/well (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 resazurin could be converted to colorless dihydroresorufin.

6. Monitor the fluorescence intensity with a fluorescence microplate reader (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570nm).

EXAMPLE DATA ANALYSIS AND FIGURES

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

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

The fluorescence reading in each well indicates the cell number in that well.

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

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

F_{sample} is the fluorescence reading in the presence of the test compound.

F_{ctrl} is the fluorescence reading in the absence of the test compound (vehicle

control).

F_0 is the averaged background (non-cell control) fluorescence intensity.

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 baseline 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>

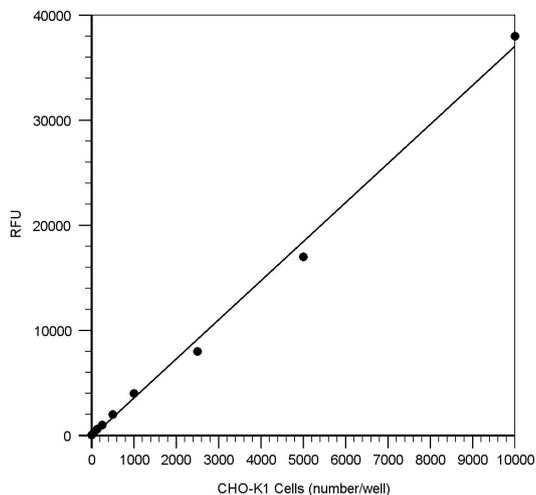


Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit. CHO-K1 cells at 0 to 10,000 cells/well/100 μ L were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 20 μ L/well of Assay Solution (Component A) for 3 hours at 37°C. The fluorescence intensity was measured at Ex/Em = 540/590 nm (Cutoff = 570 nm) with NOVOstar instrument (BMG Labtech). The fluorescence intensity was linear ($R^2 = 0.998$) to the cell number as indicated.

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