Cell Meter™ Colorimetric Cell Cytotoxicity Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22780 (1,000 assays), 22780-B (5,000 assays)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

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 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 tetrazolium based colorimetric assays (such as MTT and XTT). It comes with reagents sufficient to run 1000 assays (regular size) or 5000 assays (bulk package). 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.

Kit Key Features

Non-Radioactive:	No special requirements for waste treatment.
Continuous:	Easily adapted to automation without mixing or separation.
Convenient:	Formulated to have minimal hands-on time.
Wide Applications:	Cell proliferation and cytotoxicity.
Sensitive And Accurate:	As low as 300 cells can be accurately quantified.
Enhanced Value:	Less expensive than the sum of individual components

Kit Component

Component	**Amount**	
	Cat. # 22780	**Cat. # 22780-B**
	1,000 assays (96-well) 2,000 assays (384-well)	5,000 assays (96-well) 10,000 assays (384-well)
Component A: Assay Solution	20 mL	100 mL

Materials Required (but not provided)

- 96 or 384-well microplates: Tissue culture microplates with black wall and clear bottom are recommended.
- An absorption microplate reader: Capable of detecting absorption change in the range of 550 nm to 650 nm.

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells with test compounds (100 µL/well/96-well plate or 50 µL/well/384-well plate) → Add 1/5 volume of assay solution (Component A) → Incubate at 37 °C for 1-24 hours → Monitor absorbance ratio at 570 nm and 605 nm

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1. Prepare cells and test compounds:

1.1 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.

1.2 Set up the following controls at the same time.

- Positive control contains cells and known proliferation or cytotoxicity inducer.
- Negative control contains cells but no test compounds.
- Vehicle control contains cells and the vehicle used to deliver test compounds.
- Non-cell control contains growth medium without cells.
- 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.

2. Assay procedures:

2.1 Thaw and warm up the Assay Solution (Component A) to 37 °C. Mix it thoroughly before starting the experiments.

2.2 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.

2.3 Incubate the cells in a 37 °C, 5% CO_2 incubator for 1-24 hours, protected from light.
Note 1: 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.
Note 2: Extremely prolonged incubation time is not recommended since the indicator could be converted to colorless compound.

2.4 Monitor the absorbance change at 570 nm and 605 nm. 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}.

3. Perform data analysis:

3.1 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.

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

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

% 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 test compound.
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}.

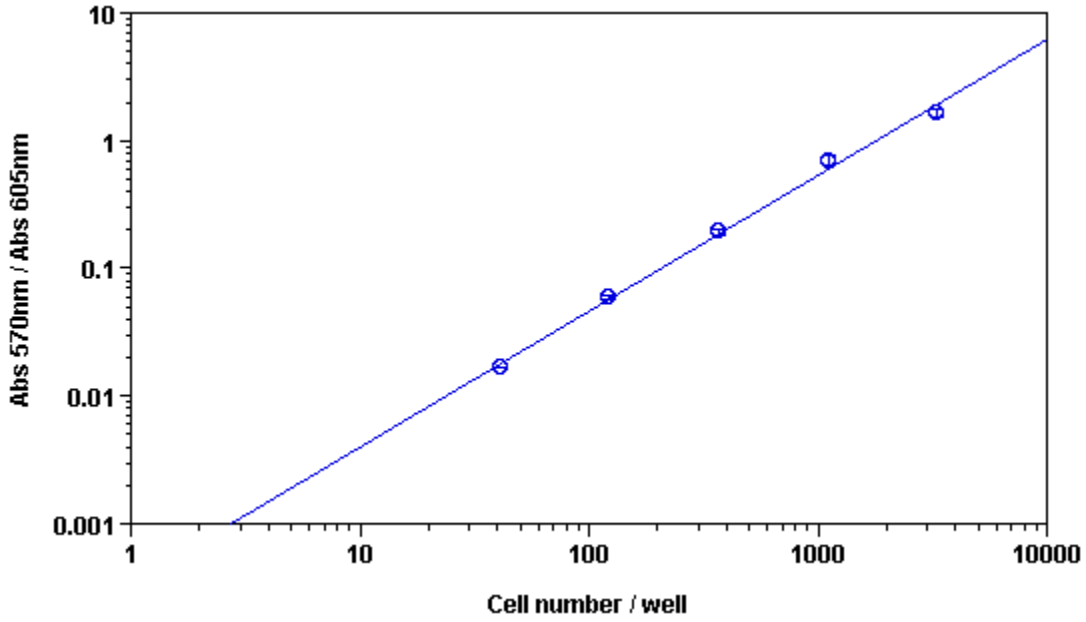

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Colorimetric Cell Cytotoxicity Assay Kit. CHO-K1 cells at 0 to 10,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 20 µL/well of Assay Solution (Component A)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

References

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