Cell Meter™ Colorimetric Cell Cytotoxicity Assay Kit

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: #22780 (1,000 assays), #22781-B (5,000 assays)	Keep in freezer and avoid light	Fluorescence microplate readers

Introduction

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 water-soluble dye that changes its absorption spectra upon cellular reduction. The absorption ratio change is directly proportional to the number of living cells. Our 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 can be readily adapted for a wide variety of instrument platforms. 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, non-radioactive and no-wash method made the kit suitable for high throughput screening of cell proliferation or cytotoxicity against a variety of compounds.

Kit Key Features

Non-Radioactive:	No special requirements for waste treatment.
Continuous:	Easily adapted to automation with no mixing or separation required.
Convenient:	Formulated to have minimal hands-on time.
Variety 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 Components

Component	**#22780** 1000 assays (96-well) 2000 assays (384-well)	**#22780-B** 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 microplate: Tissue culture microplate with black wall and clear bottom is recommended.
- Absorption microplate reader: Capable of detecting absorption from 550 nm to 650 nm.

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells with test compounds (100 µL /96-well plate or 50 µL/384-well plate)→ Add 1/5 volume Assay Solution (Component A) → Incubate at room temperature for 1-4 hrs → Read Absorbance ratio at 570 and 605 nm

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 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 total suggested 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 used to deliver test compounds [Hank's balance 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 by growth medium.

2. **Assay procedures:**

2.1 Warm up the Assay Solution (Component A) to 37°C upon thawing, and mix it thoroughly before starting the experiments.

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

2.3 Incubate the cells at 37 °C, 5% CO_2 for 1-24 hours. Protect the solution from the 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 at 570 nm and 605 nm, and 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 can be varied depending upon 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}.

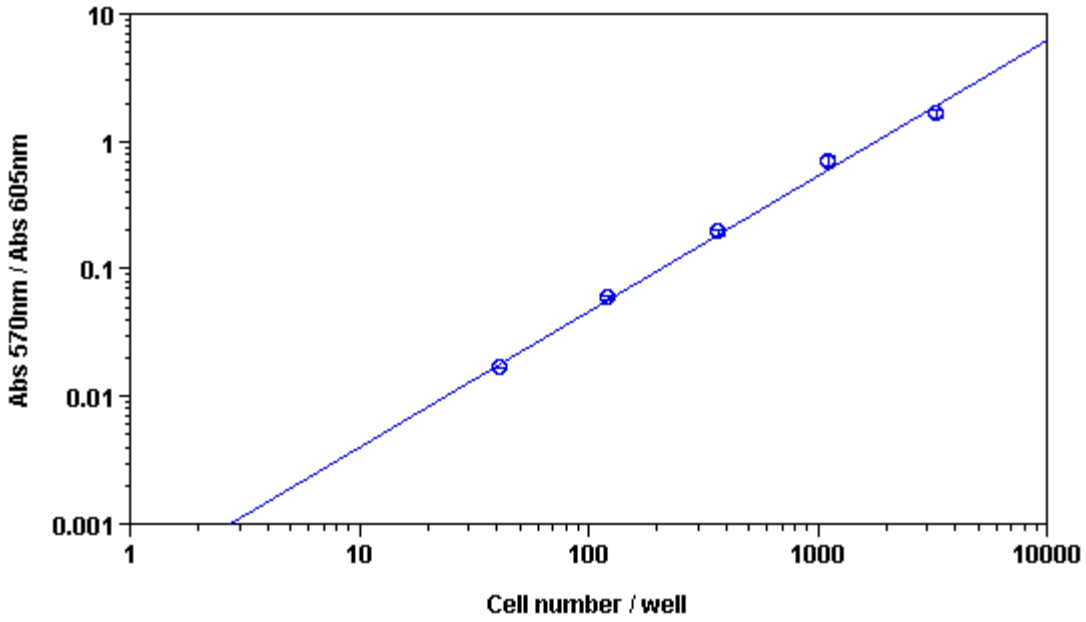

Figure 1. CHO-K1 cell number response was measured with Cell Meter™ Colorimetric Cell Cytotoxicity Assay Kit. CHO-K1 cells at 0 to 10,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom Costar plate. The cells were incubated with 20 µL/well of Component A for 3 hr at 37°C. The absorbance intensity was measured at 570 and 605 nm using 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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