# Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit

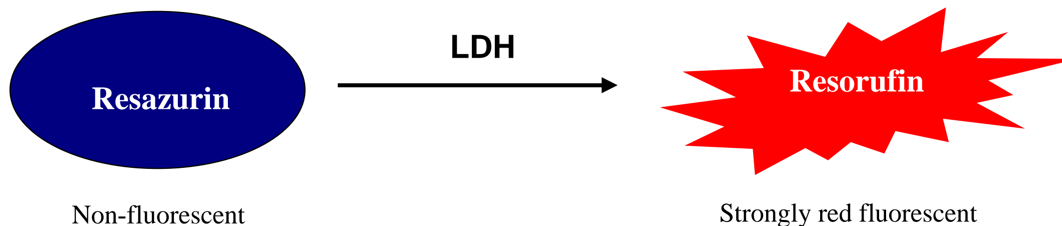
***Ordering Information:***
Product Number: #22781 (1000 assays); #22781-B (10,000 assays)

***Instrument Platform:***
Fluorescence microplate readers

***Storage Conditions:***
Keep in freezer and protect from light.

## Introduction

The measurement of mitochondrial dehydrogenases (e.g. LDH) activity is a well-accepted assay to quantify cell numbers and monitor cell viability. The Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit provides a fast, simple, accurate and homogeneous assay for the colorimetric or fluorimetric detection of viable cells. This assay is based on the observation that oxidized, blue and non-fluorescent resazurin is reduced to a red fluorescent dye (resorufin) by accepting an 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.




### 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 100 cells can be accurately quantified.
***Enhanced value:*** Less expensive than the sum of individual components.

## Kit Components

| Materials | #22781<br>1000 assays (96-well)<br>2000 assays (384-well) | #22781-B<br>10,000 assays (96-well)<br>20,000 assays (384-well) |
|---|---|---|
| Component A: Assay solution | 20 ml | 100 ml X 2 bottles |

## Materials Required (but not provided)

- 96 or 384-well microplate: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader: Capable of detecting emission at 590 nm with excitation at 530-560 nm.

## Assay Protocol (for 1 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 → Incubate at room temperature for 1-4 hrs**
**→ Read Fluorescence at Ex 540/Em 590**

**1. Prepare cells and test compounds**

1.1 Plate $1X10^{2-5}$ cells per well in a microplate. 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.
  *Note: LDH in serum will contribute to background fluorescence.*
- 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$^{o}$C, 5% $CO_2$ for 1-24 hours. Protect from the light.
*Note1:* *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.*
*Note2:* *Extremely prolonged incubation time is not recommended since resazurin could be converted to colorless dihydroresorufin.*

2.4 Monitor the fluorescence intensity (bottom read) at Ex/Em=530-560 nm/590 nm. Alternatively, read the O.D. at 560 nm (the reference wavelength should be 600 nm) to determine the cell viability in each well.

**3. Perform data analysis**

3.1 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 can be varied depending upon the sources of the growth media or the microtiter plates.*

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

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

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

$F_{sample}$: 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).
$F_o$: is the averaged background (non-cell control) fluorescence intensity.

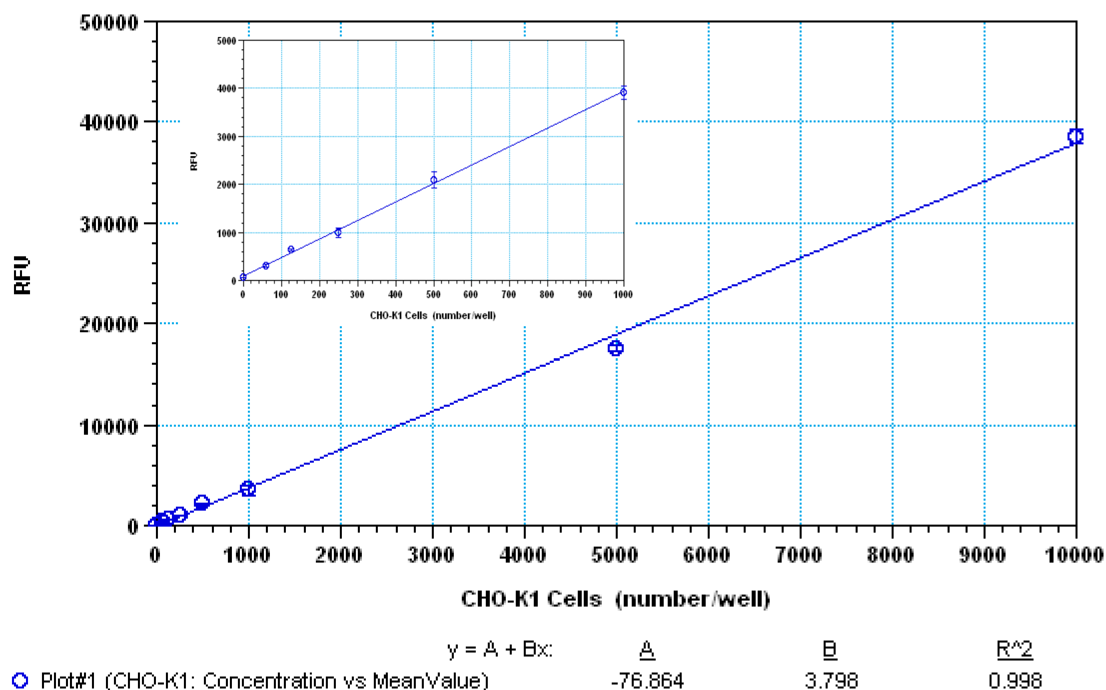



Figure 1. **CHO-K1 cell number response was measured with Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit.** CHO-K1 cells at 0 to 10,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom Costar plate. The cells were incubated with 20 µL/well of Component A for 3 hr at 37°C. The fluorescence intensity was measured at Ex 540/Em 590 using NOVOstar instrument (from BMG Labtech). The fluorescence intensity was linear ($R^2$ = 0.998) to the cell number as indicated. The detection limit from the blank controls was 60 cells/well (n=6). The small graph shows the enlargement of the lower end of the cell number response.

## References: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