ab112119

Cell Cytotoxicity Assay Kit - Fluorometric

Instructions for Use

For monitoring Cell Cytotoxicity in a variety of cell lines using a Fluorometric dye

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

The measurement of mitochondrial dehydrogenases (e.g. LDH) activity is a well-accepted assay to quantify cell numbers and monitor cell viability. ab112119 Cell Cytotoxicity Assay Kit provides a fast, simple, accurate and homogeneous assay for the colorimetric or fluorimetric detection of viable cells. ab112119 is based on the observation that oxidized non-fluorescent blue 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.

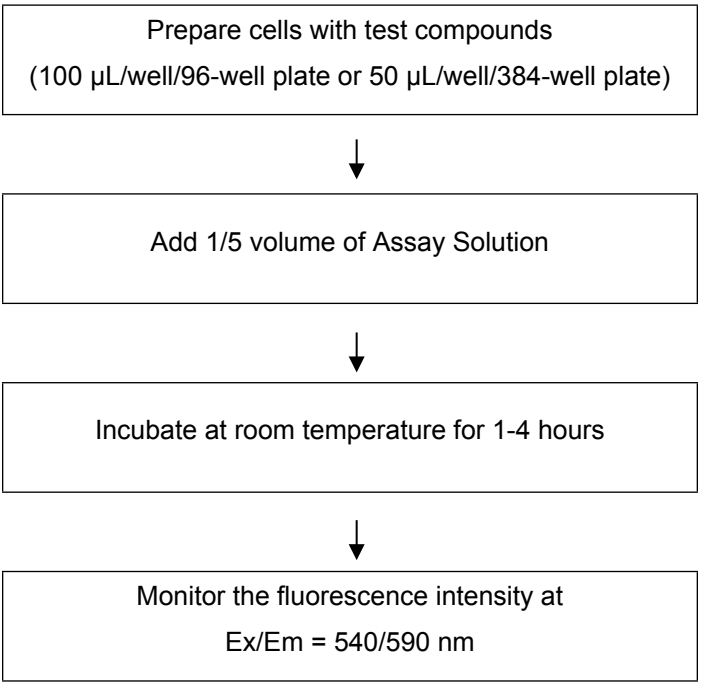
ab112119 is more sensitive for cell proliferation and cytotoxicity than other assays such as MTT. The kit components are quite stable with minimal cytotoxicity, thus a longer incubation time (such as 24 to 48 hours) is possible if required. The characteristics of its high sensitivity (<100 CHO cells), non-radioactivity and no-wash method make the kit suitable for high throughput screening of cell proliferation or cytotoxicity against a variety of compounds. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format with a filter set of Ex/Em = ~540/590 nm.

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

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount (1000 tests)	Amount (5000 tests)
Assay Solution	30 mL	100 mL

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Materials Required

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

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of Cells and Test Compounds

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 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.

- 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 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.

B. Run Assay:

1. Thaw and warm up the Assay Solution to 37°C, and mix it thoroughly before starting the experiments.

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

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 resazurin could be converted to colorless dihydroresorufin.

4. Monitor the fluorescence intensity (bottom read) at Ex/Em = 540/590 nm. Alternatively, read the O.D. at 570 nm (the reference wavelength should be 600 nm) to determine the cell viability in each well.

7. Data Analysis

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 may vary depending on the sources of the growth media or the microtiter plates.

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

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

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

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

   $\mathbf{F_{ctrl}}$ is the fluorescence reading in the absence of the test compound (vehicle control).

   $\mathbf{F_o}$ is the averaged background (non-cell control) fluorescence intensity.

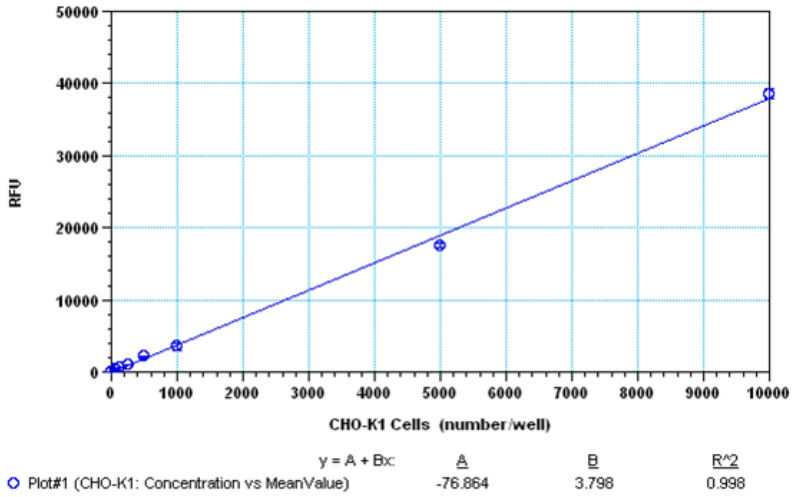


Figure 1. CHO-K1 cell number response was measured with ab112119. CHO-K1 cells at 0 to 10,000 cells/well/100 µL were seeded overnight in a black wall/clear bottom 96-well plate. The cells were incubated with 20 µL/well of Assay Solution for 3 hours at 37°C. The fluorescence intensity was measured at Ex/Em = 540/590 nm. The fluorescence intensity was linear (R^2 = 0.998) to the cell number as indicated. The detection limit was 60 cells/well (n=6). The insert shows the enlargement of the lower end of the cell number response.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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