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ab112118

Cell Cytotoxicity Assay Kit - Colorimetric

Instructions for Use

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

View kit datasheet: www.abcam.com/ab112118
(use www.abcam.cn/ab112118 for China, or www.abcam.co.jp/ab112118 for Japan)

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

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Table of Contents

1.	Introduction	3
2.	Protocol Summary	5
3.	Kit Contents	6
4.	Storage and Handling	6
5.	Materials Required	6
6.	Assay Protocol	7
7.	Data Analysis	10
4.	Troubleshooting	12

1. Introduction

Monitoring cell cytotoxicity is one of the most essential tasks for studying cellular functions. Abcam Cell assay kits are a set of tools for monitoring cell viability. There are a variety of parameters that can be used. ab112118 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 ab112118 suitable for high throughput screening of cell proliferation or cytotoxicity against a variety of compounds.

ab112118 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. The kit components are quite stable with minimal cytotoxicity, thus a longer incubation time (such as 24 to 48 hours) is possible if required.

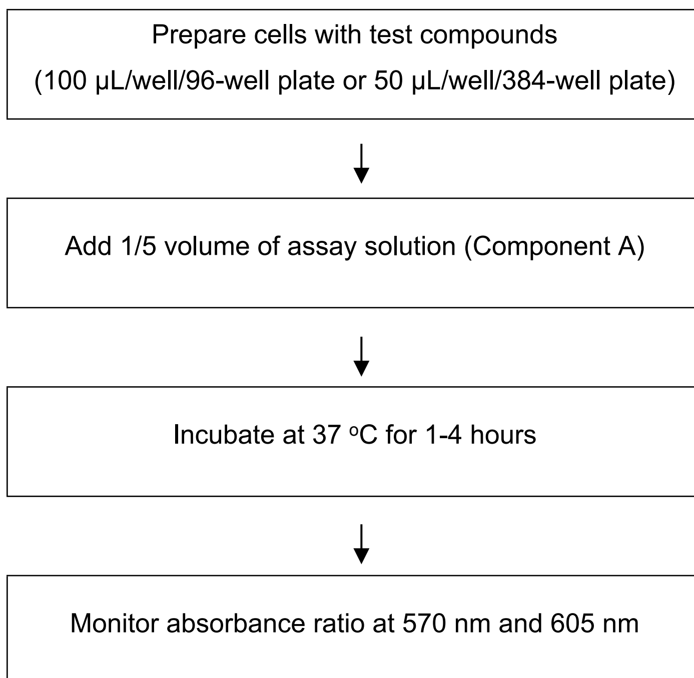
ab112118 is robust and convenient to use. It can be readily adapted for a wide variety of instrument platforms. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

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

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
Component A: Assay Solution	20 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.
- An absorption microplate reader: Capable of detecting absorption change in the range of 550 nm to 650 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 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.

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 auto fluorescence 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. Assay Procedure:

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

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.

3. Incubate the cells in a 37 ^{o}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.

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}.

7. Data Analysis

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.

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

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

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

   $\mathbf{R_{sample}}$ is the absorbance ratio of OD_{570}/OD_{605} in the presence of the test compound.

   $\mathbf{R_{ctrl}}$ is the absorbance ratio of OD_{570}/OD_{605} in the absence of the test compound (vehicle control).

   $\mathbf{R_o}$ is the averaged background (non-cell control) absorbance ratio of OD_{570}/OD_{605}.

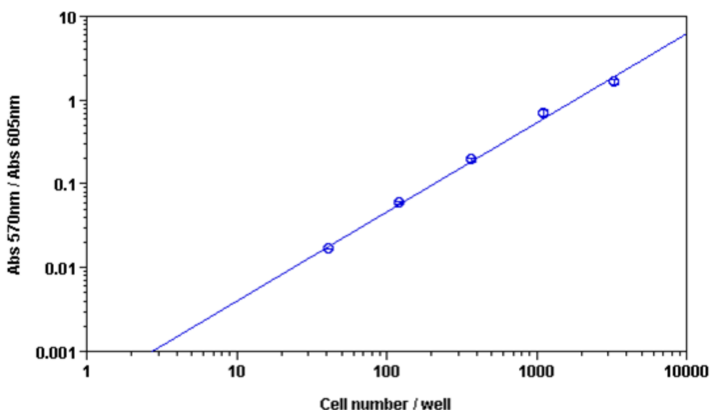


Figure 1. CHO-K1 cell number response was measured with ab112118. CHO-K1 cells at 0 to 10,000 cells/well/100 µL were seeded overnight in a black wall/clear bottom 96-well plate. The cells were incubated with 20 µL/well of Assay Solution (Component A) for 3 hours at 37 °C. The absorbance intensity was measured at 570 nm and 605 nm. The ratio of OD_{570}/OD_{605} is proportional to the number of cells as indicated.

4. 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	Unsuitable sample type	Refer to datasheet for details about incompatible samples

readings	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

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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