ab176748

Cell Viability Assay Kit (Fluorometric – Blue Ex 405 nm)

Instructions for Use

For the detection of cell viability in a variety of studies for adherent and suspension cells.

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

Version: 1 Last Updated: 6 February 2019

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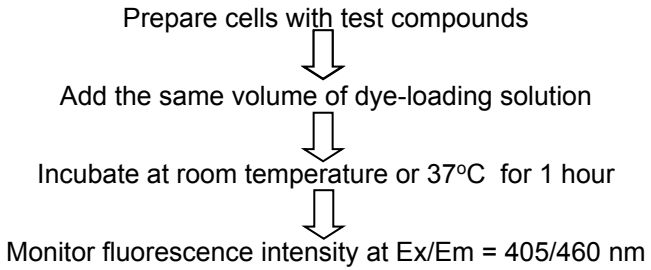
1. Overview

Abcam's Cell Viability Assay Kit (Fluorometric - Blue Ex 405 nm) (ab176748) uses a proprietary cell viability dye whose fluorescence is strongly enhanced upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The weakly fluorescent CytoCalcein Violet 450, AM is hydrolyzed by intracellular esterase to generate a strongly fluorescent hydrophilic product that is well-retained in the cell cytoplasm. The esterase activity is proportional to the number of viable cells, and thus directly related to the fluorescence intensity of the product generated from the esterase-catalyzed hydrolysis of the fluorogenic substrate. Cells grown in black wall/clear bottom plates can be stained and quantified in less than two hours. The assay is more robust than tetrazolium salt or Alarmar Blue® based ones. It can be readily adapted for many different types of fluorescence platforms such as microplate assays, fluorescence microscope, and flow cytometry. The kit is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. It provides all the essential components with an optimized cell-labeling protocol and can be used for both suspension and adherent cells.

Kit Key Features

- **Robust** – Higher maximum signal with lower variation across the plate
- **Convenient** – Formulated to have minimal hands-on-time
- **Rapid dye loading** – Dye loading at RT for 30 min to 1 hour special requirements for waste treatment
- **Versatile application** – Compatible with many cell lines and targets

2. Summary



3. Kit Components

Item	Quantity	Storage upon arrival	Storage after use/ reconstitution
CytoCalcein Violet 450, AM	5 vials	-20°C	-20°C
DMSO	200 µL	-20°C	-20°C
Assay buffer	50 mL	-20°C	-20°C

4. Storage and Stability

Upon arrival, store the kit at-20°C and protected from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- 96 or 384-well black plate with clear flat bottoms
- Multi-well spectrophotometer (ELISA reader)
- 1 X Hanks salt solution
- 20mM Hepes buffer (HHBS) or buffer of choice

6. Assay Protocol

1. Reagent preparation

a) CytoCalcein Violet 450, AM stock solution:

Add 20 µL of DMSO into the vial of CytoCalcein Violet 450, AM and mix well.

Note: 20 µL of CytoCalcein Violet 450, AM stock solution is enough for one plate. Unused CytoCalcein Violet 450, AM stock solution could be aliquoted and stored at <--20°C for one month if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles.

b) CytoCalcein Violet 450, AM dye-loading solution for one cell plate:

Add the whole content (20 µL) of CytoCalcein Violet 450, AM stock solution (Step 1a) into 10 mL of Assay Buffer and mix them well. This working solution is stable for at least 2 hours at room temperature.

Note: If the cells such as CHO cells contain organic-anion transporters which promote the leakage of the fluorescent dye over time, a probenecid stock solution should be prepared and added to the loading buffer at a final in-well working concentration ranging from 1 to 2.5 mM. Aliquot and store the unused probenecid stock solution at <-20°C.

2. Sample Preparation

Plate 100 to 10,000 cells/well in a tissue culture microplate with black wall and clear bottom, and add test compounds into the cells. Incubate for a desired period of time (such as 24, 48 or 96 hours) in a 37 °C, 5% CO2 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 25 µL for a 384-well plate.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for proliferation or cytotoxicity induction. For proliferation assays, use fewer cells; for cytotoxicity assays, use more cells to start with.

3. Run the cell viability assay

a) Treat cells with test compounds as desired.

Note: It is not necessary to wash cells before adding compounds. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before adding compounds. Add 100 µL/well (96-well plate) and 25 µL/well (384-well plate) of 1X Hank's salt solution and 20 mM Hepes buffer (HHBS) or the buffer of your choice after aspiration. Alternatively, cells can be grown in a serum-free media.

b) Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of dye-loading solution (Step 1a).

c) Incubate the dye-loading plate at room temperature or 37°C for 1 hour, protected from light. (The incubation time could be from 15 minutes to overnight. We got the optimal results with the incubation time less than 4 hours.)

Note 1: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

Note 2: DO NOT wash the cells after loading.

Note 3: For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after incubation.

d) Monitor the fluorescence intensity at Ex/Em = 405/460 nm.

7. Data Analysis

The fluorescence in blank wells with the growth medium is subtracted from the values for those wells with cells treated with the test compounds. The background fluorescence of the blank wells may vary depending on the sources of the microtiter plates or the growth medium.

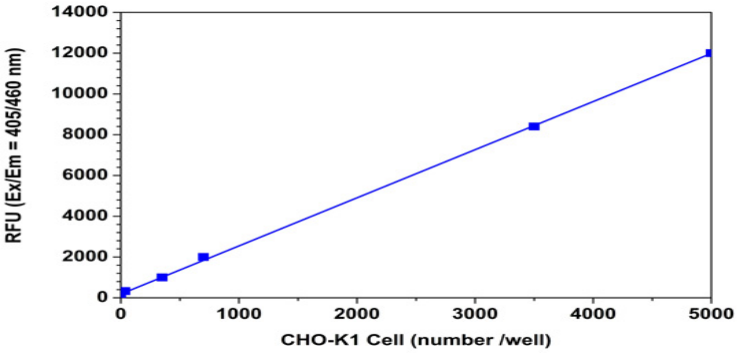


Figure 1. CHO-K1 cell number response was measured with Abcam's Cell Viability Assay Kit (Fluorometric - Blue Ex 405 nm) (ab176748). CHO-K1 cells at 0 to 5,000 cells/well/100 µL were seeded overnight in a black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of CytoCalcein Violet 450, AM dye-loading solution for 1 hour at room temperature. The fluorescence intensity was measured at Ex/Em = 405/460 nm. The fluorescence intensity was linear (R2 = 1) to the cell number as indicated. The detection limit was 70 cells/well (n=6).

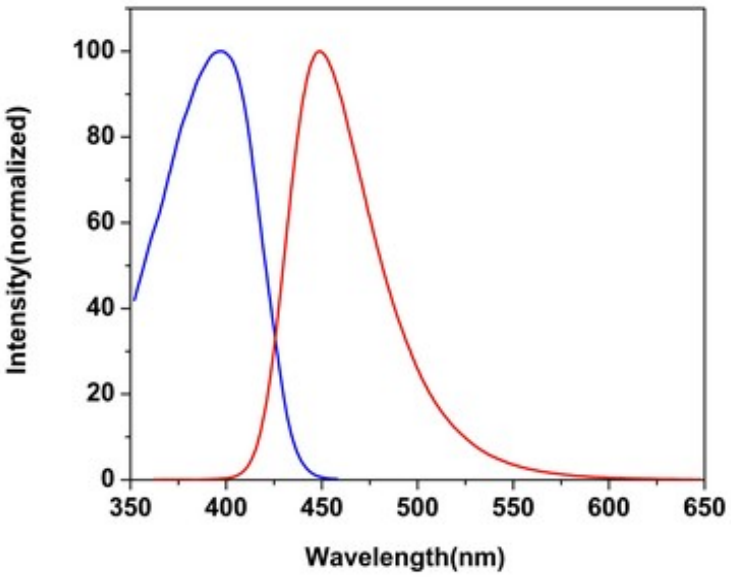


Figure 2. Excitation and Emission Spectra for Cell Viability Assay Kit (Fluorometric - Blue Ex 405 nm) (ab176748)

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)***
	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)

Problem	Reason	Solution
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

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp