

ab112122

Cell Viability Assay Kit – Fluorometric Green

Instructions for Use

For detecting cell viability in suspension and adherent cells by using our proprietary green fluorescence probe

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

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1. Introduction

There are a variety of parameters that can be used to monitor cell viability. The CellGreen fluorescent dye used in the kit is a hydrophobic compound. It easily permeates intact live cells and gets enhanced fluorescence upon entering into live cells. The hydrolysis of the non-fluorescent substrate by intracellular esterases generates a strongly green fluorescent hydrophilic product that is well-retained in the cell cytoplasm. The green fluorophore generated by the non-fluorescent substrate used in the kit has the spectral properties of fluorescein at Ex/Em = ~490 nm/520 nm. When well excited with the Argon Laser at 488 nm, the fluorophore emits intense green fluorescence at ~520 nm.

ab112122 provides all the essential components with an optimized cell-labeling protocol for fluorescence microplate assays. It can also be used with a fluorescence microscope equipped with a FITC filter set.

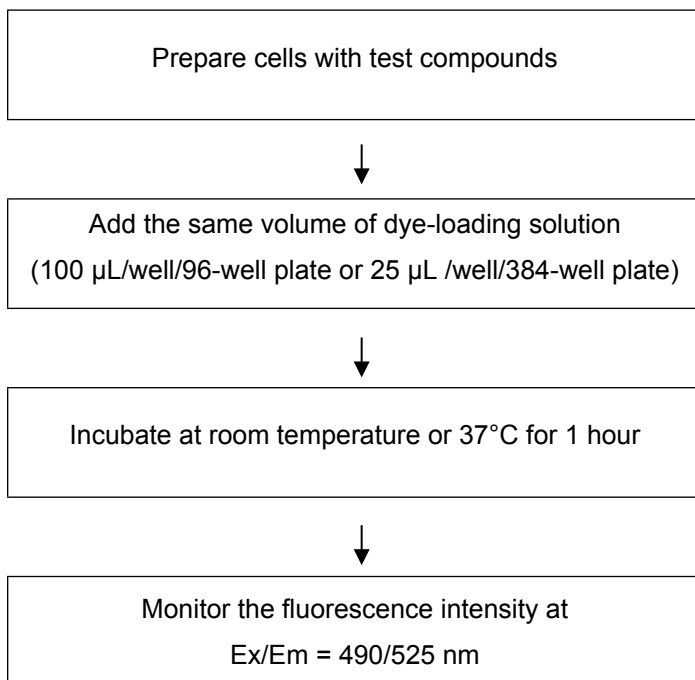
ab112122 provides an effective tool of labeling cells for fluorescence microplate and microscopic investigations of cellular functions. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. ab112122 is suitable for proliferating and non-proliferating 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.
- **Versatile Applications:** Compatible with many cell lines and targets.

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: CellGreen fluorescent dye	5 x vials
Component B: DMSO	200 μ L
Component C: Assay Buffer	50 mL

4. Storage and Handling

Keep at -20°C. Protect from light.

5. Assay Protocol

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

A. Preparation of cells

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.

B. Preparation of Dye-loading Solution

1. Thaw one of each kit component at room temperature before use.
2. Make up CellGreen fluorescent dye stock solution: Add 20 µL of DMSO (Component B) into the vial CellGreen fluorescent dye (Component A), and mix well.

Note: 20 μ L of CellGreen fluorescent dye stock solution is enough for one plate. Unused of Green Fluorescent stock solution could be aliquoted and stored at $< -20^{\circ}\text{C}$ for one month if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles.

3. Make CellGreen fluorescent dye-loading solution for one cell plate: Add the whole content (20 μ L) of CellGreen fluorescent dye stock solution (from Step 2) into 10 mL of Assay Buffer (Component C), 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 $< -20^{\circ}\text{C}$.

C. Run Cell Viability Assay:

1. Treat cells with test compounds as desired (from Step A).

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 μ L/well (96-well plate) and 25 μ L/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.

2. Add 100 μ L/well (96-well plate) or 25 μ L/well (384-well plate) of dye-loading solution (from Step B.3).
3. 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.

Monitor the fluorescence intensity at
Ex/Em = 490/525 nm.

6. 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 media.

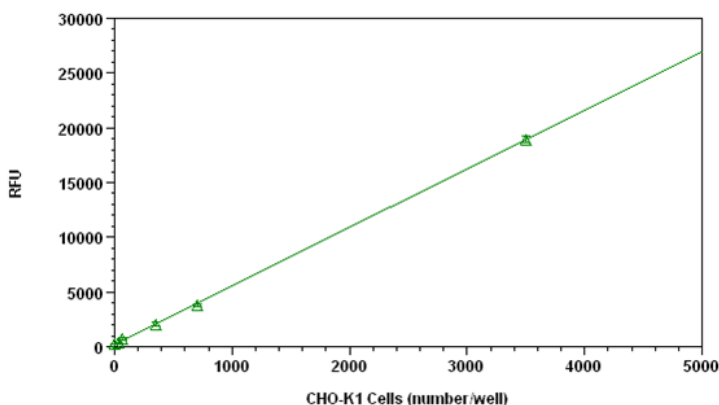


Figure 1. CHO-K1 cell number response was measured with ab112122. CHO-K1 cells at 0 to 5,000 cells/well/100 μ L were seeded overnight in a black wall/clear bottom 96-well plate. The cells were incubated with 100 μ L/well of CellGreen fluorescent dye-loading solution for 1 hour at room temperature. The fluorescence intensity was measured at Ex/Em = 490/525 nm. The fluorescence intensity was linear ($R^2 = 1$) to the cell number as indicated. The detection limit was 30 cells/well ($n=6$).

7. 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

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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