# ab112121

# Cell Viability Assay Kit – Fluorometric Dual Green/Red

Instructions for Use

For detecting cell viability in suspension and adherent cells by using dual proprietary green and red fluorescence probes

View kit datasheet: www.abcam.com/ab112121
(use www.abcam.cn/ab112121 for China, or www.abcam.co.jp/ab112121 for Japan)

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

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# 1. Introduction

Abcam Cell Viability assay kits are a set of tools for monitoring cell viability and cellular functions. ab112121 uses two non-fluorescent indicators: The CellGreen fluorescent Dye for viable cells and a cell-impermeable DNA-binding dye for the cells with compromised membranes. The non-fluorescent CellGreen fluorescent Dye can easily permeate intact live cells and is hydrolyzed by intracellular esterase to generate the strongly fluorescent hydrophilic CellGreen fluorescent Dye which is well-retained in the cell cytoplasm. The esterase activity is proportional to the number of viable cells. The DNA-binding dye is quite polar and impermeable for viable cells that have intact membranes. It becomes fluorescent only upon binding to the DNA of dead cells. Cells grown in black-wall plates can be stained and quantified in less than two hours.

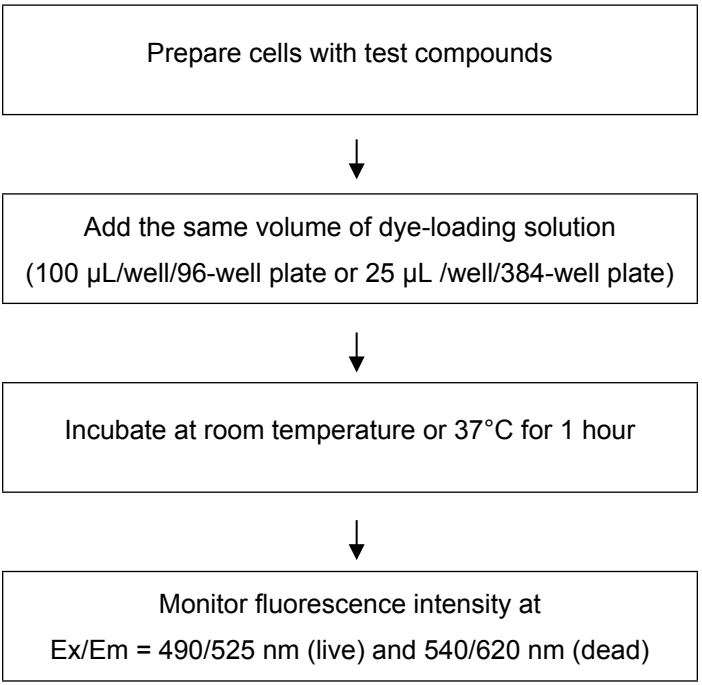
ab112121 is more robust and accurate than the other viability assays. It can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, fluorescence microscope, and flow cytometry. The kit provides all the essential components with an optimized assay protocol. It is suitable for both proliferating and non-proliferating cells (either suspension or adherent cells). ab112121 comes with sufficient reagents to perform either 200 assays (96-well format) or 800 assays (a 384-well format).

**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 | 2 x vials |
| Component B: Propidium Iodide | 40 µL |
| Component C: DMSO | 100 µL |
| Component D: Assay Buffer | 20 mL |

## 4. Storage and Handling

Keep at -20°C. Avoid exposure to 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% $CO_2$ 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 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 C) into the vial CellGreen fluorescent Dye (Component A), and mix well.

*Note: 20 µL of CellGreen fluorescent Dye stock solution is enough for one plate. Unused of CellGreen fluorescent dye 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.*

**3.** Make CellGreen fluorescent dye/ Propidium Iodide dye-loading solution for one cell plate: Add the whole content (20 µL) of CellGreen fluorescent Dye stock solution

(from Step 2) and Propidium Iodide (20 µL, Component B) 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 1: 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.*

*Note 2: As the optimal staining conditions may vary depending on different cell types, it's recommended to determine the appropriate concentration of Component A and B individually*

**C. Run Cell Viability Assay with plate reader or fluorescence microscope:**

**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 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.*

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

**3.** Incubate the dye-loading plate at room temperature or 37$^{o}$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.*

4. Monitor the fluorescence intensity at Ex/Em = 490/525 nm (FITC filter) for live cells, and 540/620 nm (TRITC filter) for dead cells.

**D. Run the cell viability assay with a flow cytometer:**

1. Treat cells with test compounds for a desired period of time.

2. Centrifuge the cells to get 1-5 × $10^5$ cells/tube.

3. Resuspend cells in 500 µL of CellGreen fluorescent dye/ Propidium Iodide dye-loading solution (from Step B.3).

4. Incubate at room temperature or 37°C for 10 to 30 minutes, protected from light.

   *Optional: Wash the cells with HHBS or buffer of your choice. Resuspend cells in 500 µL of HHBS to get 1-5 × $10^5$ cells per tube*

5. Monitor the fluorescence intensity at Ex/Em = 490/525 and 620 nm with a flow cytometer (using FL1 and FL2 channels).

# 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.

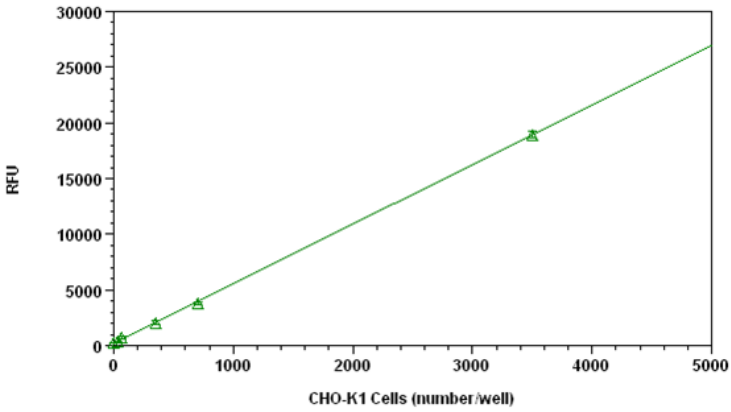



**Figure 1.** CHO-K1 cell number response was measured with ab112122. CHO-K1 cells at 0 to 5,000 cells/well/100 µL were seeded overnight in a black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of Green Fluorescent dye-loading solution for 1 hour at room temperature. The 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);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>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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