

ab115347 –

Live and Dead Cell Assay

Instructions for Use

[View kit datasheet: www.abcam.com/ab115347](http://www.abcam.com/ab115347)

(use www.abcam.cn/ab115347 for China, or www.abcam.co.jp/ab115347 for Japan)

Differential fluorescent labeling of live and dead cells

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

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1. Introduction

Cell viability is an important component of any in-vitro cell based assay. Culture conditions and experimental treatments can affect cell viability by directly or indirectly inducing cytotoxicity, apoptosis and/or necrosis. Cell viability itself can be an important experimental endpoint. In addition, it is important to be cognizant of cell viability when interpreting any experimental results since low cell viability can confound data interpretation.

A hallmark of viable cells is an intact plasma membrane and intracellular enzymatic activity. These two features form the basis of this Live and Dead Cell Assay. Live cells are identified on the basis of intracellular esterase activity (generating green fluorescence) and exclusion of the red dye. Dead cells are identified by the lack of esterase activity and non-intact plasma membrane which allows red dye staining

Protocols and sample data are included below for flow cytometry and fluorescent microscopy analysis of cell viability.

Principle: ab115347 is a one-step assay to differentially label live and dead cells with fluorescent dyes. It is useful for the rapid quantitation of cell viability using flow cytometry or fluorescent

microscopy. The provided Live and Dead Assay stain is sufficient for ~1000 assays.

The Live and Dead assay stain solution is a mixture of two highly fluorescent dyes that differentially label live and dead cells:

- The Live cell dye labels intact, viable cells green. It is membrane permeant and non-fluorescent until ubiquitous intracellular esterases remove ester groups and render the molecule fluorescent. The Excitation (max) and Emission (max) are 494nm and 515nm, respectively (similar to FITC).
- The Dead cell dye labels cells with compromised plasma membranes red. It is membrane-impermeant and binds to DNA with high affinity. Once bound to DNA, the fluorescence increases >30-fold. The Excitation (max) and Emission (max) are 528nm and 617nm, respectively.

The Live and Dead Cell Assay is a one-step staining procedure that is simple and fast. It can be used directly in cell culture media. Stained cells are not compatible with fixation.

Limitations:

- FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC PROCEDURES.
- Do not mix or substitute reagents from other lots or sources.
- Any variation in operator, pipetting technique, incubation time or temperature, and kit age can cause variation in binding.

2. Assay Summary

Culture (and treat) cells as desired



Label cells with the Live and Dead Dye

Add 1X Live and Dead Dye to cells

(e.g. mix 2X dye in PBS 1:1 with cells in culture media)

Incubate 10 min at room temperature



Analyze cells by flow cytometry or fluorescent microscopy

LIVE: Emission (max): 495nm, Excitation (max): 515nm

DEAD: Emission (max): 528nm, Excitation (max): 617nm

3. Kit Contents

- 1000X Live and Dead Cell stain in DMSO : 0.1 mL

4. Storage and Handling

Store the Live and Dead staining solution at -20°C. Avoid multiple freeze-thaw cycles. Allow the product to warm to room temperature before opening. Use diluted solutions of Live and Dead stain promptly.

5. Additional Materials Required

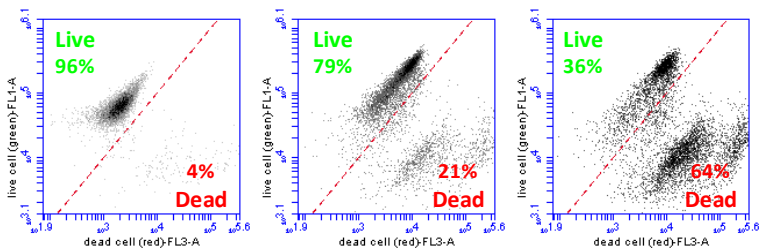
- Flow cytometer and/or fluorescence microscope (required excitation/emission wavelengths 495nm/515nm and 528nm/617nm)
- General tissue culture supplies
- PBS – sterile (1.4 mM KH₂PO₄, 8 mM Na₂HPO₄, 140 mM NaCl, 2.7 mM KCl, pH 7.3)

6. Assay Procedure

For Flow Cytometry analysis:

1. Culture cells normally.
2. Generate single cell suspension.
 - a. For suspension cell lines, gently pipette up and down to dissociate any cell aggregates.
 - b. For adherent cell lines, trypsinize cells to dissociate from plate and quench with media.
3. Stain cells with the Live and Dead Dye diluted to 1X concentration.
 - a. Dilute Live and Dead Dye to 2X concentration in PBS. *E.g. 1mL PBS + 2 μ L 1000X Live and Dead Dye.*
 - b. Mix equal volumes of single cell suspension and 2 X Live and Dead Dye solutions. *E.g. 50 μ L cells + 50 μ L 2X dye; resulting Live and Dead Dye is 1X concentration.*
4. Incubate cells for 10min at room temperature in the dark.
5. Analyze cells by flow cytometry.

- a. Both dyes are excited by the 488nm laser and can be detected with 530/30 and 670LP detectors (as shown below).
- b. Establish Forward and Side Scatter gates to exclude debris.
- c. Establish a green dye vs. red dye dot plot (typically FL1 vs. FL3) to analyze live and dead cells. The sample dot plots below demonstrate varying ratios of live and dead cells.



6. Use a healthy cell population to draw a diagonal gate along the lower right border of live (green) cells.

More green = upper left = live cells; more red = lower right = dead cells.

For fluorescent microscopy analysis:

1. Culture cells normally.
 - a. *For adherent cells:* Culture cells on surface that is compatible with the available imaging system (e.g. coverslip, chamber slides, clear-bottom optical multi-well plates, etc).
 - b. *For suspension cells or adherent cells that have been converted to a single cell suspension by trypsinization:* Follow the steps above to stain cells for flow cytometry. At step 5, pipette a small volume (e.g. 5-10 μ L) of the cell suspension onto a slide and cover with a glass coverslip. Image immediately.
 - c. It may be necessary to concentrate the cells in the staining solution before imaging by gently centrifuging cells and removing 90% of the supernatant. Gently resuspend the cell pellet in the remaining solution and transfer to the slide.
 - d. Typically microscopy requires the Live and Dead Assay Stain to be used at 5X concentration instead of 1X. See note in Step 2b below.
2. Stain cells with the Live and Dead Dye
 - a. Dilute Live and Dead dye in PBS and overlay to cells in media to a final dye concentration of 5X.

Alternatively, to conserve the amount of dye used, it may be preferable to aspirate media and add 5X dye in PBS directly to cells.

- b. Fluorescent microscopy imaging of the Live and Dead dye may require staining with a higher concentration of the dye. Using a 5X dye solution is the recommended starting concentration and users should determine the optimal dye dilution based on the cell lines used and imaging systems available.

3. Image cells.

- a. Green = live cells; Red = dead cells. See Figure 1 below.

ADDITIONAL GUIDELINES:

1. The Live and Dead Dye is provided at 1000X. We recommend using it at 1X concentration for flow cytometry analysis and at 5X for fluorescent microscopy. Users should determine the optimal staining intensity for individual cell lines and equipment used.
2. There is flexibility in how the dye is added to cells. For example, a 2X solution of dye in PBS can be mixed 1:1 with cells in culture media. Another option is make one volume of 10X dye and add 9 volumes of cells in culture media. Alternatively, 1 volume of cells could be added to 9 volumes of 1.1X dye in PBS.

3. For uniform staining it is important that the dye is mixed well with the cells. For this reason it is not recommended to pipette directly 1000X dye to a cell solution. It is preferable that the dye is first pre-diluted in PBS and then further diluted 2 to 10-fold into the cell solution. Dye should be used promptly after dilution.
4. It is recommended to analyze the cells as quickly after staining as possible. The staining solution should not be exchanged for another buffer before data acquisition. Stained cells are stable for at least one hour at room temperature after staining. Live and Dead dye staining is lost if cells are fixed.

7. Sample Data

Live and Dead assay by fluorescent microscopy:

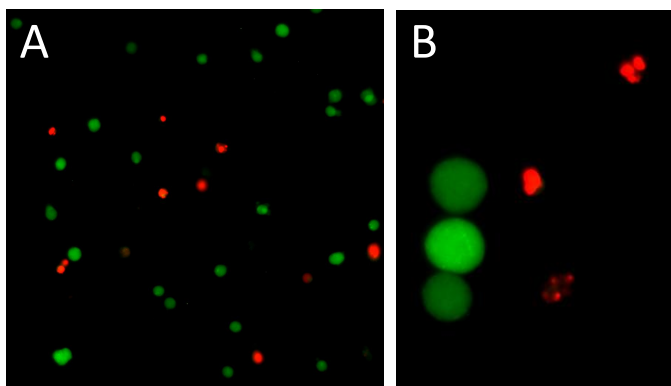


Figure 1. Jurkat cells stained with the live and dead assay kit.

Jurkat cells treated with Etoposide were labeled with the live and dead assay stain. Live cells (with esterase activity) stain green and dead cells (compromised plasma membrane) stain red. (A) Field of cells following 10 minute staining in media of live and dead stain. (B) Magnified view showing that in live cells the whole cell is stained green whereas in dead red cells it is the fragmented nuclear DNA that is stained.

Live and Dead assay by flow cytometry:

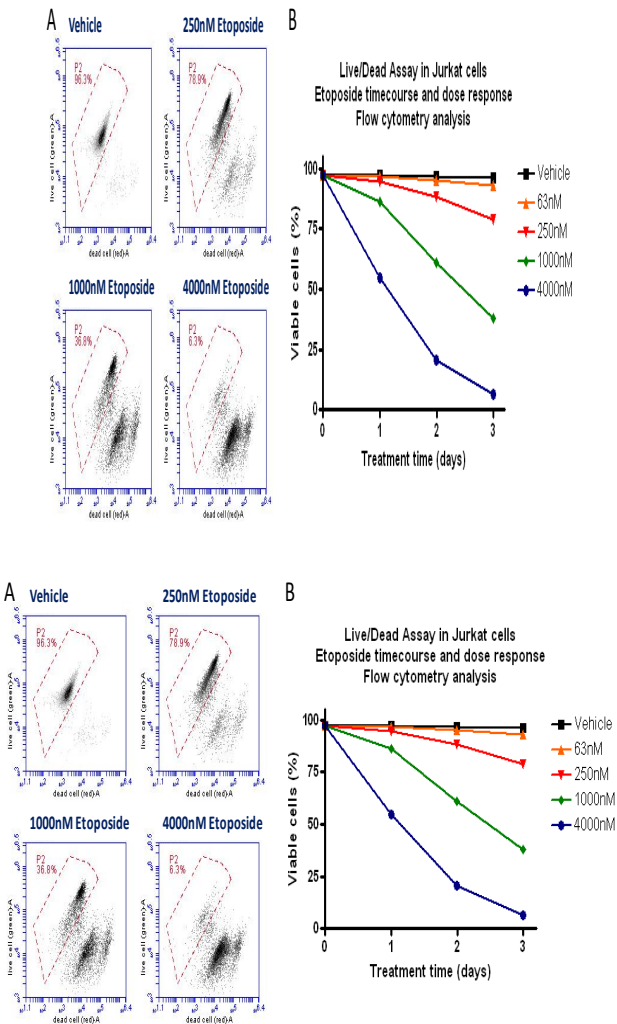


Figure 2. Flow cytometry analysis using the Live and Dead assay stain. Jurkat cells were treated with a dose response of Etoposide on day 0 and analyzed using the live and dead assay stain on days 1, 2 and 3 using flow cytometry. At each time point a small aliquot of cells was removed from the culture for analysis. (A) Dot plots showing live and dead analysis of day 3 samples. Live cells are on the y-axis and dead cells are on the x-axis. The red polygon gate identifies live cells and the number indicates the percent of live cells. (B) Graphical representation of the data presented in (A) with additional time points. % viable cells are plotted relative to time, with respect to Etoposide concentration.

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