LIVE/DEAD® Cell Vitality Assay Kit (L34951)

with C_{12}-Resazurin and SYTOX® Green Stain

Quick Facts

Storage upon receipt:

- ≤–20°C, Components A, B, C
- 2–6°C, Component D
- Protect from light, Component B
- Desiccate, Components A, B, C

Abs/Em of reaction product: 563/587 nm

Assays: 1000

Introduction

The LIVE/DEAD® Cell Vitality Assay kit provides a simple, two-color fluorescence assay that distinguishes metabolically active cells from injured cells and dead cells. The assay is based on the reduction of C_{12}-resazurin to red-fluorescent C_{12}-resorufin in metabolically active cells and the uptake of the cell-impermeant, green-fluorescent nucleic acid stain, SYTOX® Green dye, in cells with compromised plasma membranes (usually late apoptotic and necrotic cells). As seen in Figure 1, dead cells emit mostly green fluorescence whereas the healthy, metabolically active cells emit mostly red fluorescence. The injured cells have lower metabolic activity and, consequently, reduced red fluorescence emission; because they possess intact membranes, however, injured cells accumulate little SYTOX Green dye and, therefore, emit very little green fluorescence.

Nonfluorescent resazurin (R12204), which can be reduced by viable cells to red-fluorescent resorufin, has been extensively used to detect the metabolic activity of many different cell types, from bacteria to higher eukaryotes.[1-3] Resazurin is nontoxic and stable in culture media, allowing researchers to continuously monitor proliferating cells [4] and to investigate cytotoxicity in both conventional [5] and high-throughput applications.[6] The LIVE/DEAD Cell Vitality kit includes a lipophilic version of resazurin, C_{12}-resazurin, which is more permeable to live cells and, after reduction to C_{12}-resorufin, is better retained than the nonlipo-philic resorufin. These characteristics result in brighter signals and better detection limits.

Materials

Kit Contents

- **C_{12}-Resazurin** (MW = 398, Component A), 5 vials, each containing 40 µg (dried material)
- **SYTOX Green stain** (Component B), 100 µL of a 10 µM solution in DMSO
- **Dimethylsulfoxide (DMSO), anhydrous** (Component C), 1.5 mL of high-quality anhydrous DMSO
- **10X Phosphate-buffered saline** (Component D), 100 mL

Storage and Handling

Upon receipt, store Components A, B, and C at ≤–20°C and Component D at 2–6°C. When stored properly, the kit components should be stable for at least 6 months. Prior to opening, warm vials A, B, and C to room temperature. Reseal these vials tightly before returning to storage.

Caution: No data are available addressing the mutagenicity or toxicity of SYTOX Green stain. Because this reagent binds to nucleic acids, it should by treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Please dispose of stains in compliance with all pertaining local regulations.

Experimental Protocol for Flow Cytometry

The assay has been optimized using Jurkat cells. Some modifications may be required for use with other cell types. A negative control for necrosis should be prepared by incubating cells with 2 mM hydrogen peroxide for 4 hours at 37°C. Untreated cells should be used as a positive control for C_{12}-resazurin staining.

1. Prepare a 1 mM stock solution of C_{12}-resazurin. Dissolve the contents of the vial of C_{12}-resazurin (Component A) in 100 µL of DMSO (Component C). It may be necessary to agitate the solution in an ultrasonic water bath to fully dissolve the C_{12}-resazurin. The C_{12}-resazurin stock solution should be stable for 3 months if stored at ≤–20°C, protected from light. Prepare a fresh 50 µM working solution of C_{12}-resazurin by diluting 1 µL of the 1 mM C_{12}-resazurin stock solution in 19 µL of DMSO.

2. Prepare a 1 μM working solution of SYTOX Green stain. For example, dilute 5 μL of the 10 μM SYTOX Green stain stock solution (Component B) in 45 μL of DMSO (Component C). The unused portion of this working solution may be stored at ≤–20°C for up to 1 month.

3. Prepare a 1X phosphate-buffered saline (PBS) solution. For example, for about 20 assays, add 2 mL of 10X PBS (Component D) to 18 mL of deionized water (dH_2O). Pass the 1X PBS through a 0.2 μm filter before use.

4. Harvest the cells and dilute as necessary to about 1×10^6 cells/mL using the 1X PBS. The cells may be washed with 1X PBS if desired.

5. Add the dyes to the cell suspension. Add 1 μL of the 50 μM C_{12}-resazurin working solution (prepared in step 1) and 1 μL of the 1 μM SYTOX Green stain working solution (prepared in step 2) to each 100 μL of cell suspension (final concentrations of 500 nM C_{12}-resazurin and 10 nM SYTOX Green dye).
Note: If the fluorescence intensity of the SYTOX Green dye is too low, the final dye concentration can be increased up to 50 nM.

6. Incubate the cells at 37°C in an atmosphere of 5% CO_2 for 15 minutes.

7. Dilute the cell suspension. After the incubation period, add 400 μL of the 1X PBS, mix gently, and keep the samples on ice.

8. Analyze the cell sample. As soon as possible, analyze the stained cells by flow cytometry, exciting at 488 nm and measuring the fluorescence emission at 530 nm and 575 nm. The population should separate into two groups: live cells with a low level of green and a high level of orange fluorescence and necrotic cells with a high level of green fluorescence and a low level of orange fluorescence (Figure 1). Confirm the flow cytometry results by viewing the cells with a fluorescence microscope, using filters appropriate for fluorescein (FITC) and tetramethylrhodamine (TRITC).

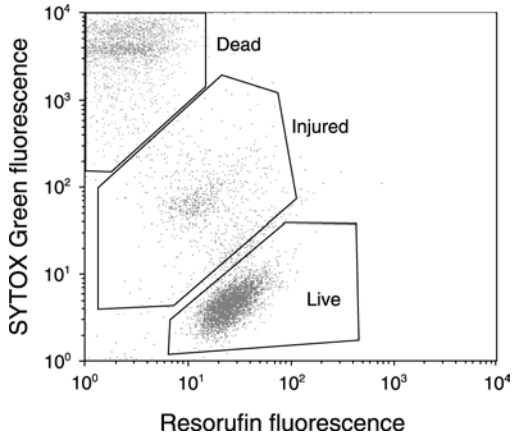

Figure 1. Analysis of Jurkat cells using the LIVE/DEAD Cell Vitality Kit. Jurkat cells (T-cell leukemia, human) were induced with 10 μM camptothecin for 4 hours at 37°C, 5% CO_2. The cells were incubated with the reagents in the LIVE/DEAD Cell Vitality Assay Kit and analyzed by flow cytometry. The dot plot of SYTOX Green fluorescence versus resorufin fluorescence shows resolution of live, injured, and dead cell populations.

The appropriate probe concentration for optimal staining will vary by application. The initial conditions suggested here are intended as guides but may need to be modified depending on the cell type and the permeability of the cells or tissues to the probe, among other factors. The 1 mM stock solution of C_{12}-resazurin (see step 1 of *Experimental Protocol*, above) may also be diluted in the desired culture medium or buffer. However, media containing thiols (e.g., dithiothreitol or cysteine) should be avoided to prevent reduction of the C_{12}-resazurin to fluorescent C_{12}-resorufin. For microplate-based assays, loading concentrations of 5–10 μM are recommended. For flow cytometry applications, the loading concentration should be reduced to 0.1–0.5 μM. For either type of application, incubate the cells for 15 minutes at 37°C in an atmosphere of 5% CO_2.

References

1. Appl Environ Microbiol 56, 3785 (1990); **2.** J Dairy Res 57, 239 (1990); **3.** J Neurosci Methods 70, 195 (1996); **4.** J Immunol Methods 210, 25 (1997); **5.** J Immunol Methods 213, 157 (1998); **6.** Antimicrob Agents Chemother 41, 1004 (1997).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
L34951	LIVE/DEAD® Cell Vitality Kit *with C_{12} resazurin and SYTOX® Green* *1000 assays*	1 kit

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