Revised: 14–March–2001

LIVE/DEAD® Reduced Biohazard Viability/Cytotoxicity Kit #1 (L-7013)

Quick Facts

Storage upon receipt:
- –20°C
- Protect from light

Note: Stock solutions are in DMSO — HANDLE WITH CARE.

Introduction

The LIVE/DEAD® Reduced Biohazard Viability/Cytotoxicity Kit #1 (L-7013) is a two-color fluorescence assay for animal cell viability that is designed to reduce the risk associated with handling potential biohazards such as viral, bacterial or protozoan pathogens. The test is simple, fast and can be carried out using fluorescence microscopes, flow cytometers or fluorescence microplate readers. The basis for the viability test is differential permeability of live and dead cells to a pair of fluorescent stains. SYTO® 10, a green fluorescent nucleic acid stain, is a highly membrane-permeant dye and labels all cells, including those with intact plasma membranes. DEAD Red™ is a cell-impermeant red fluorescent nucleic acid stain that labels only cells with compromised membranes. The emission spectra for the dyes are shown in Figure 1. The dye concentrations and their relative affinities are balanced so that a cell population exposed simultaneously to both dyes becomes differentially stained — live cells appearing fluorescent green and dead cells, fluorescent red.

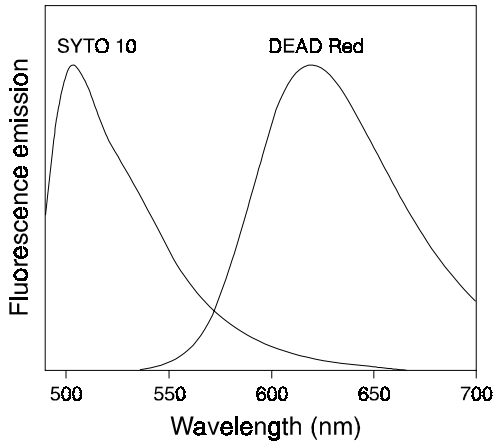

Figure 1. *Normalized fluorescence emission spectra of SYTO 10 and DEAD Red nucleic acid stains excited at 490 nm in the presence of dsDNA.*

In general, rigorous precautions are necessary during analysis of biohazardous specimens.[1] Fixation procedures that inactivate cells while producing minimal distortion of their analytical characteristics are therefore highly advantageous.[2,3] Glutaraldehyde is well known for its ability to inactivate cells and viruses while preserving their overall morphology.[4] For example, glutaraldehyde has been found to irreversibly inhibit the activity of human retroviruses and to be more effective and faster acting than formaldehyde in this regard.[5] By using this kit, viability staining can take place while the potentially pathogenic sample is well contained; subsequent treatment with 4% glutaraldehyde allows for safer handling during analysis, without disrupting the distinctive staining pattern.

Materials

Kit Contents

- SYTO 10 green fluorescent nucleic acid stain (Component A), 50 µL solution in DMSO
- DEAD Red (ethidium homodimer-2) nucleic acid stain (Component B), 50 µL solution in 20% DMSO

The kit contains sufficient material for at least 100 tests when used according to the protocol below. Components A and B should be stored at -20°C and protected from light. Allow the reagents to warm to room temperature and centrifuge briefly before opening the vials. The contents of each vial should be mixed thoroughly to ensure homogeneity of the solution.

Caution: Because both Components A and B bind to nucleic acids, they should be treated as mutagens and used with appropriate care. The stock solutions in DMSO should be handled with particular caution, as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling these stock solutions. As with all nucleic acid stains, solutions containing these reagents should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dyes.

Materials Required but Not Provided

- HEPES-buffered saline solution (HBSS): 135 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1.8 mM $CaCl_2$, 10 mM HEPES, pH 7.4
- 4% Glutaraldehyde in HBSS, freshly prepared from recently acquired, reagent-grade glutaraldehyde (50%)

Experimental Protocols

The following protocols are provided to assist the researcher in the rapid development of procedures for use of the LIVE/DEAD

Reduced Biohazard Viability/Cytotoxicity Kit. Excellent cell staining and maximal conservation of kit reagents can be achieved by performing cell staining and fixation in small containers, such as 1.5 mL microcentrifuge tubes. With small tubes, a smaller sample of the cell culture is required, less reagent is used and cells can be pelleted more quickly and conveniently. Suspensions of 4×10^5 cells in 200 µL (2×10^6 cells/mL) can be handled easily and will provide sufficient material for most flow cytometric analyses. Upon centrifugation, the pellets will be clearly visible; supernatant solutions can be pipetted off easily.

Staining Cells in Suspension

1.1 Prepare a working solution of the mixed dyes by pipetting 2 µL of Component A and 2 µL of Component B into a common 1 mL volume of HBSS (1:500 dilution of each). Mix thoroughly by vortexing or by pipetting up and down several times. This working solution can be used for up to five tests and may be stored for a few days at 4°C, protected from light.

1.2 Pipet 10^5–10^6 cells from a culture vessel to appropriate tubes (note **A**) and centrifuge at about 250 × g for 10 minutes.

1.3 Remove the culture medium completely. Components in the medium can interfere with the staining.

1.4 Loosen the cell pellet by tapping the tube sharply several times, add 200 µL of diluted dye, and complete the resuspension. The cell density should be between 5×10^5 and 5×10^6 cells/mL.

1.5 Incubate the tube in complete darkness for 15 minutes at room temperature.

1.6 Pellet the cells by centrifugation. Remove the supernatant solution carefully to avoid carry-over of dye.

1.7 Resuspend the cells in a minimal volume (e.g., 50 µL) of fresh HBSS. The cells will tend to aggregate if they are not resuspended in a minimal volume of HBSS prior to adding the fixative.

1.8 Add enough 4% glutaraldehyde in HBSS (freshly prepared) to yield a cell density of less than 10^6 cells/mL.

1.9 Incubate cells in fixative for at least 15 minutes before observation.

1.10 For improved and more persistent differential staining of the live and dead cell populations, leave the cells in the fixative for one hour, then centrifuge, remove the fixative and resuspend the pellet in the same volume of HBSS. When stored at 4°C, shielded from light, the cells will retain their staining pattern for several hours.

Note

[A] The material, surface treatment and geometry of the centrifuge tubes used for staining and fixing cells in suspension may affect the initial red-green color separation, as well as the longevity of the staining pattern. We have found that certain polypropylene centrifuge tubes work well in this application (e.g., VWR 1.7 mL tubes, cat #20172-698; Nunc 15 mL tubes, cat #374640; or Nalgene 15 mL tubes, cat #3103-0015); however, other polypropylene tubes do not (e.g., VWR 15 mL tubes, cat #21008-066), nor do polystyrene tubes (e.g., VWR 15 mL tubes, cat #21008-827). Other centrifuge tubes have not been evaluated in our tests.

Table 1. *Optical filter sets recommended for use with the LIVE/DEAD Rediced Biohazard Viability/Cytotoxicity Kit*

Optical Filters *		Notes
Omega	**Chroma**	
XF25, XF26, XF115	11001, 41012, 71010	Longpass and dual emission filters useful for simultaneous viewing of SYTO 10 and DEAD Red stains
XF22, XF23	31001, 41001	Bandpass filters for viewing SYTO 10 alone
XF32, XF43 XF102, XF108	31002, 31004 41002, 41004	Bandpass filters for viewing DEAD Red alone

* Catalog numbers for recommended bandpass filter sets for fluorescence microscopy. Omega® filters are supplied by Omega Optical Inc. (www.omegafilters.com). Chroma filters are supplied by Chroma Technology Corp. (www.chroma.com)

Staining Adherent Cells on Coverslips

2.1 Grow adherent cells on sterile glass coverslips.

2.2 Remove the culture medium covering the cells and replace it with an equal volume of HBSS.

2.3 Prepare a dilute mixture of Component A and Component B dyes by pipetting 2 µL of each into a common 1 mL volume of HBSS (1:500 dilution of each). Mix thoroughly by vortexing or by pipetting up and down several times.

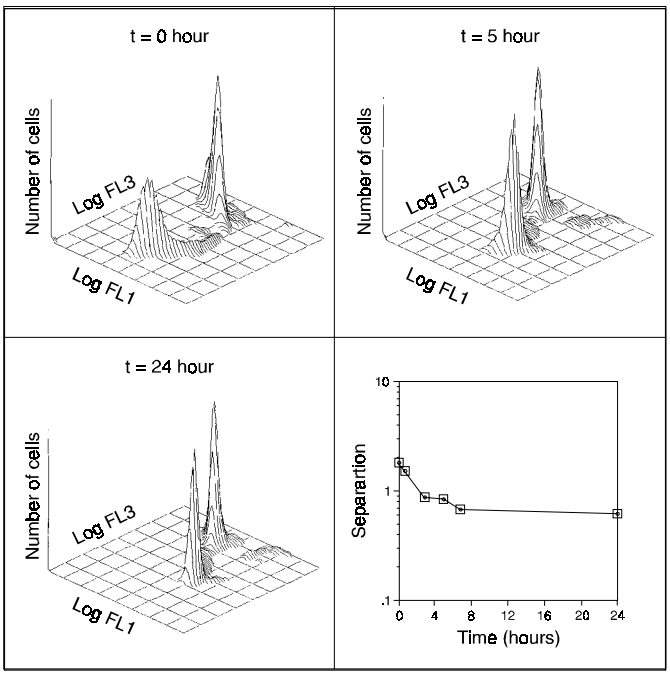

Figure 2. *Flow cytometric analysis of a mixed population of live and complement-treated goat lymphocytes stained with the LIVE/DEAD Reduced Biohazard Viability/Cytotoxicity Kit and monitored over a 24-hour period. Panels A–C represent the distribution of FL1 and FL3 fluorescence in lymphocytes at 0, 5 and 24 hours after fixation. Panel D illustrates the separation between the live and dead population peaks as a function of time.*

2.4 Remove the HBSS covering the cells and replace it with the diluted dye mixture; 200–500 µL should be sufficient. Incubate in darkness for 15 minutes at room temperature.

2.5 Remove the dye solution, and wash the cells with fresh HBSS.

2.6 Add 4% glutaraldehyde in HBSS (freshly prepared), and incubate for at least 15 minutes before observation.

2.7 For improved and more persistent differential staining of the live and dead cell populations, leave the cells in the 4% glutaraldehyde fixative for 1 hour, then remove the fixative and cover the cells with HBSS. When stored at 4°C, shielded from light, the cells will retain their staining pattern for several hours.

Optical Filter Selection for Fluorescence Microscopy

The fluorescence from both live and dead cells may be viewed simultaneously with any standard fluorescein longpass filter set or with appropriate multiband filter sets. However, when live and dead cells are viewed simultaneously, the fluorescence intensity of live (green fluorescent) cells will be significantly lower than that of dead (red fluorescent) cells. This is an unavoidable result of balancing the dye concentrations to optimally discriminate live and dead cells. Alternatively, the live and dead cells may be viewed separately with fluorescein and either rhodamine or Texas Red® bandpass filter sets. A summary of the fluorescence microscope filter sets recommended for use with the LIVE/DEAD Reduced Biohazard Viability/Cytotoxicity Kit is shown in Table 1.

Flow Cytometry Example

The protocol described in *Staining Cells in Susupension* for staining cells in suspension can be used to prepare cells for flow cytometry. Typically, cells are stained according to the protocol and diluted several-fold with HBSS for analysis in the flow cytometer. Figure 2 is provided to illustrate the use of the LIVE/DEAD Reduced Biohazard Viability/Cytotoxicity Kit in conjunction with flow cytometric analysis. In this example, prior to analysis, half of the lymphocytes were surface-labeled with the trinitrophenyl (TNP) hapten, exposed to anti-TNP IgG and then treated with complement to cause membrane damage. The kit clearly distinguishes between healthy cells and cells with compromised membranes.

References

1. Methods Cell Biol 42, 359 (1994); **2.** Methods Cell Biol 42, 295 (1994); **3.** Shapiro, H.M., *Practical Flow Cytometry, 3rd Edition*, Wiley-Liss (1995) pp. 250–251; **4.** *Manual of Clinical Microbiology, 5th Edition*, A. Balows, Ed., American Society for Microbiology (1991) pp. 183–200; **5.** J Infectious Diseases 152, 400 (1985).

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

Cat #	Product Name	Unit Size
L-7013	LIVE/DEAD® Reduced Biohazard Viability/Cytotoxicity Kit #1 *for animal cells* *100 assays*	1 kit

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