LIVE/DEAD® Funga*Light™ Yeast Viability Kit*

Quick Facts

Storage upon receipt:

- <–20ºC
- Desiccate
- Protect from light

Ex/Em:

480/500 nm (SYTO® 9 stain)
490/635 nm (propidium iodide)

Introduction

LIVE/DEAD® *Funga*Light™ Yeast Viability Kit (L34952) allows researchers to easily, reliably and quantitatively distinguish live and dead yeast in minutes. The LIVE/DEAD® *Funga*Light™ Yeast Viability Kit contains solutions of our SYT0® 9 green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, propidium iodide. These stains differ both in their spectral characteristics and in their ability to penetrate healthy yeast cells. When used alone, the SYTO® 9 stain generally labels all yeast in a population — those with intact membranes and those with damaged membranes. In contrast, propidium iodide penetrates only yeast with damaged membranes, causing a reduction in the SYTO® 9 stain fluorescence by fluorescence resonance energy transfer (FRET) when both dyes are present. Thus, with an appropriate mixture of the SYTO® 9 and propidium iodide stains, yeast with intact cell membranes stain fluorescent green, whereas yeast with damaged membranes stain fluorescent red. The excitation/emission maxima for these dyes are 480/500 nm for SYTO® 9 stain and 490/635 nm for propidium iodide. The background remains virtually nonfluorescent. Furthermore, this kit also accommodates fine-tuning of the dye combinations so that optimal staining of yeast can be achieved under a variety of experimental conditions.

A common criterion for yeast viability is the ability of a yeast cell to reproduce in suitable nutrient medium. Under certain conditions, however, yeast having compromised membranes may be able to recover and reproduce — such yeast may be scored as "dead" in this assay. Conversely, some yeast with intact membranes may be unable to reproduce in nutrient medium, and yet these may be scored as "live."

Materials

Kit Contents

- SYTO® 9 dye, 3.34 mM (Component A), 300 µL solution in DMSO
- Propidium iodide, 20 mM (Component B), 300 µL solution in DMSO

Each kit contains sufficient material to perform ~200 tests by flow cytometry.

Storage and Handling

Upon receipt, the kit should be stored frozen at ≤–20˚C, desiccated and protected from light. Stored properly, the kit components should remain stable for at least one year. Allow the compounds to warm to room temperature before opening the vials. DMSO solutions absorb water, which can cause a loss of dye activity. DMSO stock solutions of SYTO® 9 dye should be stored desiccated and used within a short period of time.

Caution: SYTO® 9 stain and propidium iodide bind to nucleic acids. Propidium iodide is a potential mutagen, and we have no data addressing the mutagenicity or toxicity of the SYTO® 9 stain. The DMSO stock solutions should be handled with particular care, as DMSO is known to facilitate the entry of organic molecules into tissues. Please dispose of the stains in compliance with all pertaining local regulations.

General Considerations

Washing Yeast Cultures

Care should be taken to remove traces of growth medium before staining yeast with these kit reagents. The nucleic acids and other media components can bind the SYTO® 9 and propidium iodide dyes in unpredictable ways, resulting in unacceptable variations in staining. A single wash step is usually sufficient to remove significant traces of interfering media components from the yeast suspension. Phosphate wash buffers are not recommended because they may decrease staining efficiency. Sterilize buffers using 0.2 µm filtration

Staining Optimization

The two dye components provided with the LIVE/DEAD® *Funga*Light™ Yeast Viability Kit have been balanced so that a 1:1 mixture provides good live/dead discrimination in most applications. The protocol set out below was developed using

cultures of *Saccharomyces* spp. However, the proportions of the two dyes may need to be adjusted for optimal discrimination in specific applications. For example, if green fluorescence is too low in the preparation, we suggest that you try either increasing the concentration of SYTO® 9 stain (by using more of Component A) or by decreasing the concentration of propidium iodide (by using less of Component B). To thoroughly optimize the staining, we recommend experimenting with a range of dye concentrations. These SYTO® 9 dye and propidium iodide solutions can be blended at different ratios, and then the mixtures applied to mixtures of live and killed yeast suspensions.

Experimental Protocols

Preparing Yeast Suspensions

This section describes the preparation of the yeast samples for staining. We recommend preparing washed cell suspensions of untreated, killed, and experimental cells for single-color controls and instrument setup.

Note: Yeast cultures may be stained and analyzed without washing (washing described in steps 1.1 – 1.5). Potentially higher background levels will necessitate careful instrument setup and population analysis.

1.1 Prepare one killed control sample. Collect ~1 mL samples of the yeast culture in microcentrifuge tubes, and centrifuge samples at 10,000 × g for 1–3 minutes to pellet the cells. Remove the supernatants.

1.1.1 To prepare an alcohol-killed control, resuspend one pellet in 1 mL of 70% isopropyl alcohol and incubate the sample at room temperature for 30–60 mintues, mixing every 15 minutes.

1.1.2 To prepare a heat-killed control, resuspend one pellet in 1 mL of appropriate buffer and place loosely capped in a 70–80ºC waterbath for 10 minutes.

1.2 Prepare untreated control and experimental samples. Collect ~1 mL samples of the yeast cultures in microcentrifuge tubes. Centrifuge all samples, including killed control, at 10,000 × g for 1–3 minutes to pellet the cells. Remove the supernatants.

1.3 Wash all samples in 1 mL of appropriate buffer and pellet again by centrifugation at 10,000 × g for 1–3 minutes.

1.4 Resuspend samples in 1 mL of appropriate buffer.

1.5 Dilute samples to ~10^6 cells/mL in appropriate buffer. (For dense cultures of *Saccharomyces* spp., ~200 µL of washed yeast cell suspension (from step 1.4) diluted in 10 mL of buffer is usually sufficient.)

Staining Yeast Samples

For the staining procedure, prepare the required number of flow tubes, each containing 1 mL of yeast suspension at ~10^6 cells/mL. Prepare tubes for each yeast suspension from step 1.5 (i.e., killed, untreated, and experimental yeast suspensions).

2.1 Unstained controls. Place 1 set of tubes aside without adding dye.

2.2 Single-color SYTO® 9 dye controls. Add 1 µL of SYTO® 9 dye (Component A) to one tube of untreated cells and to one tube of killed cells.

2.3 Single-color propidium iodide controls. Add 1 µL of propidium iodide (Component B) to one tube of untreated cells and to one tube of killed cells.

2.4 Experimental samples. Add 1 µL of Component A and 1 µL of Component B to each tube of experimental samples.

2.5 After stain is added, each tube should be vortexed gently. Incubate all samples at room temperature or 37ºC protected from light for 15–30 minutes. Cells can be analyzed by flow cytometry without washing.

Adjusting the Flow Cytometer and Analyzing the Samples

In the flow cytometer, yeast are identified solely on the basis of their size and staining. **It is best to inspect each sample by fluorescence microscopy to confirm that the particles detected are indeed yeast cells** (see *Analyzing the Stained Yeast by Fluorescence Microscopy*). In addition, with the long data-acquisition times required for very dilute yeast samples, the number of noise events acquired in the yeast frame may become significant.

Instrument capabilities may vary considerably, but the techniques and parameters established here should aid considerably in setting up similar analyses in the majority of flow cytometers now in use. The unstained and single-color controls prepared as described above can be used to locate cell populations and determine compensation settings.

3.1 Instrument configuration. Stained yeast can be assayed in a flow cytometer equipped with a 488 nm argon laser. Fluorescence from SYTO® 9 dye–stained samples may be collected using a 530/30 bandpass filter. Fluorescence from propidium iodide–stained controls may be collected with a ≥ 610 longpass filter.

3.2 Forward and side-scatter amplification settings.

3.2.1 Set amplifiers to logarithmic amplification. Use forward or side scatter as the acquisition trigger parameter.

3.2.2 With an unstained control, set the amplification of the signals from forward and side scatter so that the yeast are in the middle of the data space (Figure 1A).

3.2.3 Adjust the acquisition trigger level (also named "threshold level" on some instruments) to minimize electronic noise appearing on the monitor. To check for exclusion of electronic noise, briefly interrupt the sample flow; if the instrument is correctly adjusted, the signal rate should drop to nearly zero. To avoid coincidence error, maintain flow rate at ≤1000 events/second.

3.3 Fluorescence amplification settings. **NOTE: Compensation is not necessary to resolve live from dead populations.**

3.3.1 Set amplifiers to logarithmic amplification of the green-fluorescence channel detector so that the signals from the untreated yeast control stained with the SYTO® 9 dye stain appear in the middle to top range of the signal axis (Figure 1B). If necessary, adjust the compensation settings to remove the signal from the opposite axis.

3.3.2 Adjust the red-fluorescence channel detector so that the signals from the killed yeast control stained with propidium iodide appear in the top range of the signal axis (Figure 1B). If necessary, adjust the compensation settings to remove the signal from the opposite axis.

3.4 After adjusting the flow cytometer as described above, apply experimental samples containing stained yeast.

3.5 Process the data by setting a gate on the desired population using forward and side scatter (Figure 1A). Using a gated fluorescence plot (Figure 1B), set regions on live and dead populations as shown.

Analyzing the Stained Yeast by Fluorescence Microscopy

Yeast stained using LIVE/DEAD® *Funga*Light™ Yeast Viability Kit may be viewed using most standard epifluorescence microscopes with the appropriate filters. Fluorescence from both live and dead yeast may be viewed simultaneously with any standard fluorescein longpass filter set. Alternatively, the live (green fluorescent) and dead (red fluorescent) cells may be viewed separately with fluorescein and Texas Red® bandpass filter sets. A summary of the fluorescence microscope filter sets recommended for use with the LIVE/DEAD® *Funga*Light™ Yeast Viability Kit shown in Table 1.

To analyze any of the samples using fluorescence microscopy, trap 5 µL of the stained yeast suspension between a slide and an 18 mm square coverslip and observe in a fluorescence microscope equipped with any of the filter sets listed in Table 1.

Table 1. Characteristics of common filters suitable for use with the LIVE/DEAD® *Funga*Light™ Yeast Viability Kit.

Omega® Filters*	Chroma Filters*	Notes
XF25, XF26, XF115	11001, 41012, 71010	Longpass and dual emission filters useful for simultaneous viewing of SYTO® 9 and propidium iodide stains
XF22, XF23	31001, 41001	Bandpass filters for viewing SYTO® 9 alone
XF32, XF43, XF102, XF108	31002, 31004, 41002, 41004	Bandpass filters for viewing propidium iodide 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).

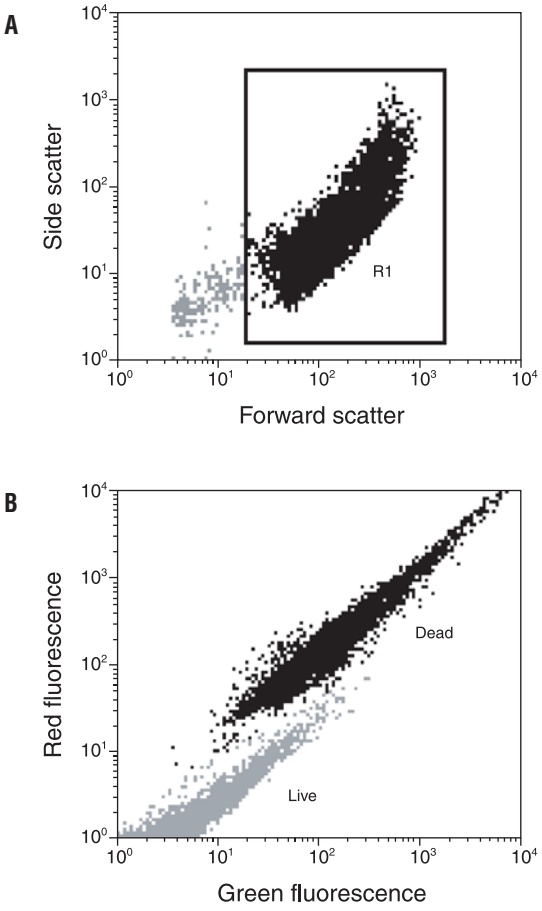

Figure 1. *Saccharomyces* spp. cell suspensions stained with SYTO® 9 dye and propidium iodide and analyzed using a BD FACSCalibur™ flow cytometry system (Becton Dickinson and Co.). Panel A shows the dot plot of forward scatter vs. side scatter of an untreated *Saccharomyces* culture, washed and stained with SYTO® 9 dye and propidium iodide as described in the protocol. The region R1 contains particles of the appropriate size for yeast cells; the forward scatter trigger is set to exclude debris in the sample. Panel B shows the R1-gated staining pattern obtained following analysis of a sample of yeast containing a mixture of both live and heat-killed cells.

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

Cat #	Product Name	Unit Size
L34952	LIVE/DEAD® *Funga*Light™ Yeast Viability Kit *for flow cytometry*	1 kit

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