Funga*Light™ CFDA, AM/Propidium Iodide Yeast Vitality Kit*

Quick Facts

Storage upon receipt:

- <–20ºC
- Desiccate
- Protect from light

Ex/Em:

492/517 nm (CFDA, AM dye)
490/635 nm (propidium iodide)

Introduction

Molecular Probes *Funga*Light™ CFDA, AM/Propidium Iodide Yeast Vitality Kit combines a cell permeable esterase substrate with a membrane integrity indicator to evaluate the vitality of yeast cells by flow cytometry or microscopy. The acetoxymethyl ester (AM) of the esterase substrate 5-carboxyfluorescein diacetate (CFDA) allows this reagent to permeate cell membranes. Once inside the cell, the lipophilic blocking and diacetate groups are cleaved by nonspecific esterases, resulting in a fluorescent, charged form that leaks out of cells very slowly. In contrast, the membrane integrity indicator, propidium iodide, penetrates yeast with damaged membranes. With an appropriate mixture of the CFDA, AM and propidium iodide stains, esterase-active yeast with intact cell membranes stain fluorescent green, whereas yeast with damaged membranes stain fluorescent red. The excitation/emission maxima for these dyes are 492/517 nm for CFDA, AM and 490/635 nm for propidium iodide.

Materials

Kit Contents

- **5-carboxyfluorescein diacetate, acetoxymethyl ester (CFDA, AM)** (Component A), three vials each containing 1 mg of dry powder
- **Propidium iodide**, 20 mM (Component B), 300 µL solution in DMSO
- **Dimethylsulfoxide (DMSO), anhydrous** (Component C), 500 µL

Each kit provides sufficient material for ~200 flow cytometry assays.

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 six months. 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 CFDA, AM should be stored desiccated and used within a short period of time.

Caution: Propidium iodide binds to nucleic acids, and is a potential mutagen. 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

A single wash step using an isotonic solution such as phosphate-buffered saline (PBS) is usually sufficient to remove significant traces of interfering media components from a yeast suspension. Additives such as 0.1% Pluronic® F127 (P3000MP) or 0.01% Tween® 20 may assist dye entry into the cells and serve to separate yeast from other debris. Sterilize staining buffers using 0.2 µm filtration.

Staining Optimization

The two dye components provided with the *Funga*Light™ CFDA, AM/Propidium Iodide Yeast Vitality Kit have been optimized using *Saccharomyces* spp. and balanced so that a 1:1 mixture should provide good vital/dead discrimination in most applications. 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 increasing the concentration of CFDA, AM stain by using more of Component A. To thoroughly optimize the staining for a particular application, we recommend testing a range of concentrations of both dyes using different ratios on a standard mixture of live and killed yeast.

Experimental Protocols

Dye Preparation

For convenience, the CFDA, AM dye in this kit is provided in three separate vials. Each vial provides sufficient material for staining approximately 100 yeast samples. However, once reconstituted, the DMSO solutions of the dyes are somewhat unstable, especially if exposed to moisture.

1.1 Bring one vial of CFDA, AM (Component A), the vial of propidium iodide (Component B), and the vial of anhydrous DMSO (Component C) to room temperature before removing the caps.

1.2 Add 100 µL of DMSO to the vial of CFDA, AM. Vortex to mix.

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 2.1–2.5). Potentially higher background levels will necessitate careful instrument setup and population analysis.

2.1 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.

2.1.1 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.

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

2.2 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.

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

2.4 Resuspend samples in 1 mL of appropriate buffer.

2.5 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 2.4) 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 2.5 (i.e., killed, untreated, and experimental yeast suspensions).

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

3.2 Single-color CFDA, AM dye controls. Add 1 µL of CFDA, AM dye (Component A, prepared in step 1.2) to one tube of untreated cells and to one tube of killed cells.

3.3 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.

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

3.5 After stain is added, each tube should be vortexed gently. Incubate all samples at room temperature or 37˚C 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.

4.1 Instrument configuration. Stained yeast can be assayed in a flow cytometer equipped with a 488 nm argon laser. Fluorescence from CFDA, AM–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.

4.2 Forward and side-scatter amplification settings.

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

4.2.2 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).

4.2.3 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.

4.3 Fluorescence amplification settings. **NOTE: Compensation is not necessary to resolve vital from nonvital populations.**

4.3.1 Set amplifiers to logarithmic amplification. Adjust the green-fluorescence channel detector so that the signals from the untreated yeast control stained with the CFDA, AM

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.

4.3.2 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.

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

4.5 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 vital and nonvital populations as shown.

Analyzing the Stained Yeast by Fluorescence Microscopy

Yeast stained using *Funga*Light™ CFDA, AM/Propidium Iodide 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 membrane-compromised (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 *Funga*Light™ CFDA, AM/Propidium Iodide Yeast Vitality Kit.

Omega® Filters *	Chroma Filters *	Notes
XF25, XF26, XF115	11001, 41012, 71010	Longpass and dual emission filters useful for simultaneous viewing of CFDA, AM and propidium iodide
XF22, XF23	31001, 41001	Bandpass filters for viewing CFDA, AM 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).

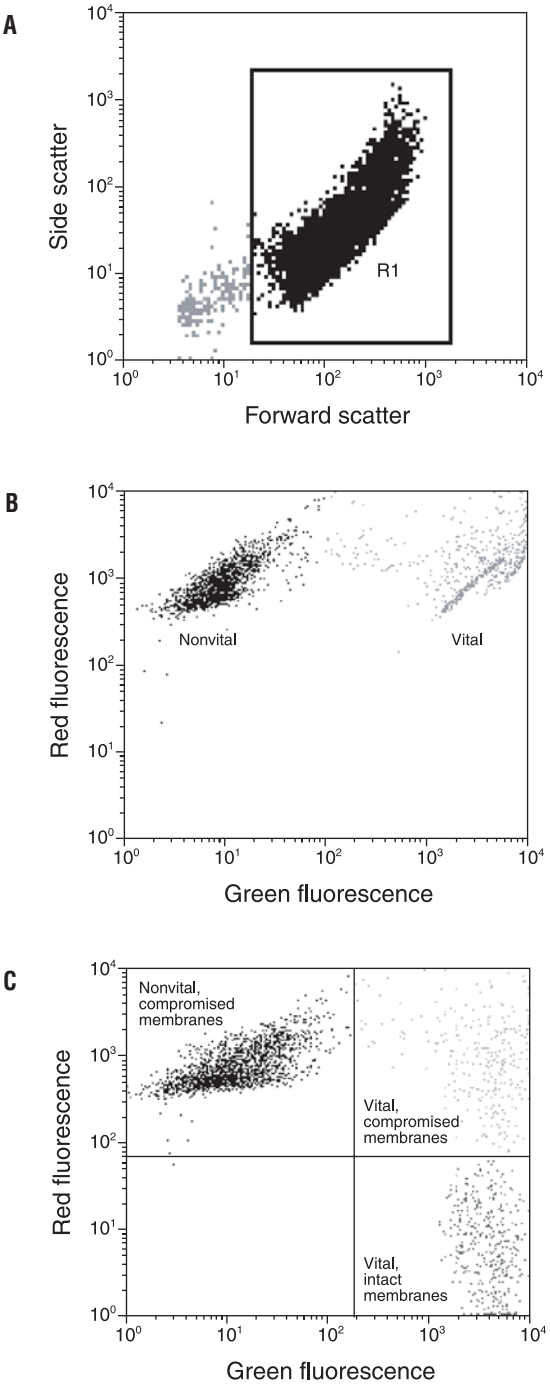

Figure 1. *Saccharomyces* spp. cell suspensions stained with CFDA, AM dye and propidium iodide and analyzed using a BD FACSCalibur™ flow cytometry system (Becton Dickinson and Co.). Panel A shows the dot plot of forward scatter vs. side scatter of an untreated, unstained *Saccharomyces* culture. Region R1 includes events of the appropriate size and scatter for yeast cells, and is used to set the instrument to exclude debris in the sample. Panel B shows the staining pattern obtained following analysis of a yeast sample containing a mixture of both live and alcohol-killed cells. The R1 gate (as in panel A) was applied to these events, but no compensation was applied; panel C shows the same sample with compensation applied. Vital cell events in panel B are distinguished from nonvital events, but events in panel C are further differentiated by membrane integrity

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

Cat #	Product Name	Unit Size
F34953	*Funga*Light™ CFDA, AM/Propidium iodide Yeast Vitality Kit	1 kit

Contact Information

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