

## Live or Dead™ Yeast Viability Assay Kit

 Catalog number: 22475  
 Unit size: 100 Tests

Component	Storage	Amount
MycLight™ Green JJ98	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)
Propidium iodide	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)

### OVERVIEW

The Live or Dead™ Yeast Viability Assay Kit is a reliable and robust assay that allows researchers to quantitatively distinguish live and dead yeast within minutes using flow cytometry. This kit contains two ready-to-use nucleic acid stains: MycoLight™ Green JJ98, a replacement for SYTO™ 9, and propidium iodide. MycoLight™ Green JJ98 is a green fluorescent nucleic acid stain that labels all yeast populations - live yeast with intact membranes and dead yeast with damaged membranes. In contrast, propidium iodide only stains dead yeast with damaged membranes. In dead yeasts where both dyes are present, the fluorescence of MycoLight™ Green JJ98 is notably reduced due to FRET by propidium iodide. As a result, live yeast with intact membranes will stain fluorescent green, and dead yeast with damaged membranes will stain fluorescent red.

### AT A GLANCE

#### Protocol Summary

1. Prepare yeast samples
2. Add 1 µL MycoLight™ Green JJ98 dye
3. Add 2 µL propidium iodide dye
4. Gently vortex, and incubate at 37°C for 15-30 minutes
5. Analyze with flow cytometer using 530/30 nm and 576/26 nm filters

#### Important

Allow the components to warm to room temperature before opening the vials. Propidium iodide binds to nucleic acids, it should be 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.

### KEY PARAMETERS

#### Flow cytometer

Excitation	488 nm laser
Emission	530/30 nm, 575/26 nm filters
Instrument specification(s)	FITC channel, PE channel

### SAMPLE EXPERIMENTAL PROTOCOL

The following protocol should be used only as a guideline.

1. Prepare yeast samples containing 1 mL of suspension at  $\sim 10^6$  cells/mL.
2. Add 1 µL of MycoLight™ Green JJ98 (Component A) and 2 µL of propidium iodide (Component B) to each tube of experimental samples. For unstained controls, place 1 set of tubes aside without adding dye. For single color MycoLight™ Green JJ98 controls, add 1 µL of MycoLight™ Green JJ98 to one tube of untreated cells and to one tube of killed cells. For single color propidium iodide controls, add 1 µL of propidium iodide to one tube of untreated cells and to one tube of killed cells. Please note, depending upon your specific application the proportion of the two dyes may need to be adjusted for optimal response.

3. Vortex each tube gently. Then incubate all samples at room temperature for 15 to 30 minutes, protected from light.
4. Monitor the fluorescence increase using a flow cytometer equipped with a 488 nm laser, and a 530/30 nm filter and a 575/26 nm filter (FITC and PE channel).

**Note** To analyze the samples with fluorescence microscopy, trap 5 µL of the stained yeast suspension between a slide and a coverslip. Observe in a microscope using FITC and Cy3 filter set.

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