Live or Dead™ Yeast CFDA-AM/Propidium Iodide Vitality Kit

Catalog number: 22476
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22476)
Component A: CFDA-AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg/vial)
Component B: Propidium iodide	Freeze (< -15 °C), Minimize light exposure	1 vial (200 µL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

Live or Dead™ Yeast CFDA-AM/Propidium Iodide Vitality Kit is a convenient way to test viability of yeasts. The kit uses CFDA-AM and Propidium Iodide. CFDA-AM is a cell permeable esterase substrate. The acetoxymethyl (AM) group of the substrate will allow CFDA to penetrate cell membranes. Once inside the cells, lipophilic blocking and diacetate groups are cleaved by esterases, resulting in green fluorescence. In contrast, Propidium Iodide only stain the yeasts with damaged membranes resulting in red fluorescence.

AT A GLANCE

Protocol summary

1. Prepare yeast samples
2. Add 1 µL CFDA-AM dye working solution
3. Add 2 µL propidium iodide dye
4. Vortex gently, 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

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

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

CFDA-AM dye working solution

1. Add 100 µL of DMSO to the vial of CFDA-AM (Component A). Vortex to mix.

   Note Store stock solution in -20°C in single use aliquots. Avoid repeated freeze-thaws.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol should only be used as a guideline.

1. Prepare yeast samples containing 1 mL of suspension at ~10^6 cells/mL.
2. Add 1 µL of CFDA-AM dye working solution 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 CFDA-AM controls, add 1 µL of CFDA-AM 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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