

Product Information

Yeast Live-or-Dye™ Fixable Live/Dead Staining Kit

Catalog Number: 31064

Unit Size: 1000 assays

Kit contents

Component	Size
32005A: Live-or-Dye™ 568/583	4 vials
31064A: Thiazole Orange, 10 mM in DMSO (1000X)	100 uL
99953-1: Anhydrous DMSO	250 uL

Storage and Handling

Store at -20°C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Spectral Properties

Live-or-Dye™ 568/583 Ex/Em: 562/583 nm

Thiazole Orange Ex/Em: 512/533 nm (with DNA)

Product Description

The Yeast Live-or-Dye™ Fixable Live/Dead Staining Kit is designed for discrimination between live and dead cells during flow cytometry or microscopy.

Live-or-Dye™ 568/583 is a cell membrane impermeable amine-reactive red dye. The dye is able to enter into dead cells that have compromised membrane integrity and covalently label free amines on intracellular proteins. The dye labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells.

Thiazole Orange is a membrane permeable green dye. It stains both living and dead yeast cells. In unfixed yeast, the staining is cytoplasmic with nuclear accumulation. After fixation, the staining becomes diffuse throughout the cell.

When yeast are co-stained with the two dyes, live cells will fluoresce green, while dead cells will fluoresce green and red (and may appear yellow in a merged image).

Live-or-Dye™ dye reconstitution, 500X stock

Remove one vial of lyophilized Live-or-Dye dye and the anhydrous DMSO from the freezer and bring to room temperature. Add 50 uL of anhydrous DMSO to the vial, vortexing or pipetting up and down to ensure that all of the dye has dissolved. Once dissolved, the dye should be used within a few hours. Leftover dye solution can be aliquoted and stored desiccated at -20°C for at least 1 month.

Protocol for staining cells in liquid culture

This staining protocol was optimized using *Saccharomyces cerevisiae* yeast in pure culture. The optimal dye concentration may need to be determined experimentally for other organisms.

1. Culture cells in the appropriate growth medium. Spin down and resuspend in a clear buffer suitable for imaging, such as PBS or HBSS.

Note: Live-or-Dye™ is amine reactive, and therefore staining efficiency will be reduced if done in the presence of BSA or other protein, or in a Tris-based buffer.

2. Prepare a working solution of 100X Live-or-Dye by diluting the stock 1:5 in DMSO. Prepare a working solution of 100X Thiazole Orange by diluting the stock 1:10 in DMSO. Add 1 uL of each dye to 100 uL cells in buffer.
3. Incubate for 30 minutes at room temperature, rocking, protected from light.
4. Optional: Cells can be fixed in 4% paraformaldehyde.
5. Image on a fluorescence microscope using a Texas Red® or similar emission filter to visualize Live-or-Dye 568/583 and a FITC emission filter to visualize green cytoplasmic staining.

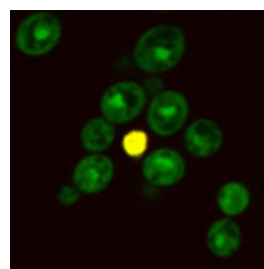


Figure 1. *Saccharomyces cerevisiae* stained with 1X Live-or-Dye 568/583 and 1X Thiazole Orange. Dead cells stained with both dyes appear yellow in the merged image. Imaged on a Zeiss LSM700 confocal microscope using FITC and Texas Red® imaging settings.

Related Products

Catalog number	Product	Function
29067	Calcofluor White, 5 mM in water	Cell wall/bud scar stain
29068	ViaVac™ Red/Green, 10 mM in DMSO	Yeast vital dye
40077	Thiazole Orange, 10 mM in DMSO	Live cell fixable cytoplasmic stain
31062	Yeast Vitality Staining Kit	Calcofluor White to stain the cell wall, ViaVac Red/Green as a vital dye
31063	Yeast Viability Staining Kit	ConA to stain cell walls, Live-or-Dye to stain dead cells
29015-29020; 29058	CF™ Dye Concanavalin A (Con A)	Cell wall stain in a variety of dye conjugates
29021-29029; 29059; 29064	CF™ Dye Wheat Germ Agglutinin (WGA)	Cell wall bud scar stain in a variety of dye conjugates
32002-32009	Live-or-Dye™ Fixable Viability Staining Kits	Dead-cell-specific viability stains in a variety of dye colors
23001	EverBrite™ Mounting Medium	Wet set mounting medium for microscopy

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