

# **Product Information**

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

#### Catalog Number: 31064

#### **Kit Contents**

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

Unit Size: 1000 assays

## **Storage and Handling**

Store Live-or-Dye<sup>™</sup> 568/583 at -20°C, desiccated and protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Store Thiazole Orange at 4°C, protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Store DMSO at room temperature, 4°C, or -20°C, desiccated and protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

## Prepare 500X Live-or-Dye<sup>™</sup> Stock Solution

Bring one vial of lyophilized Live-or-Dye<sup>™</sup> and the anhydrous DMSO to room temperature. Add 50 uL of anhydrous DMSO to the vial, and vortex or pipette 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.

## Absorption/Emission:

Live-or-Dye™ 568/583: 562/583 nm Thiazole Orange: 515/531 nm (with DNA)

## **Product Description**

The Yeast Live-or-Dye<sup>™</sup> Fixable Live/Dead Staining Kit is designed to discriminate between live and dead cells during flow cytometry or microscopy.

Live-or-Dye <sup>™</sup> 568/583 is a cell membrane-impermeant 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-permeant 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).

## 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.

**Note:** Live-or-Dye  $^{\text{TM}}$  is amine-reactive, and therefore, staining efficiency will be reduced if done in the presence of BSA or other proteins, or in Tris-based buffer.

- 1. Culture cells in the appropriate growth medium. Pellet cells by centrifugation and remove the supernatant. Resuspend the cell pellet in 100 uL of a protein- and amine-free buffer, such as PBS or HBSS.
- Prepare a working solution of 100X Live-or-Dye<sup>™</sup> by diluting the stock 1:5 in anhydrous DMSO. Prepare a working solution of 100X Thiazole Orange by diluting the stock 1:10 in anhydrous DMSO. Add 1 uL of each dye working solution to each sample containing 100 uL cells in buffer and immediately mix well.
- 3. Incubate for 30 minutes at room temperature, rocking and protected from light.
- 4. Wash cells once by briefly centrifuging, removing supernatant, and resuspending in 100 uL PBS or HBSS.
- 5. Optional: Fix cells in 4% paraformaldehyde.
- Analyze by microscopy or flow cytometry. Use Texas Red® or a similar channel to detect Live-or-Dye<sup>™</sup> 568/583, and FITC or a similar channel to detect Thiazole Orange.

## **Related Products**

Cat. No.	Product
29067	Calcofluor White, 5 mM in Water
29068	ViaVac™ Red/Green (FUN®1)
40077	Thiazole Orange, 10 mM in DMSO
31062	Yeast Vitality Staining Kit
31063	Yeast Viability Staining Kit
32010	Live-or-Dye NucFix™ Red Staining Kit
29015 29136	Concanavalin A (Con A) CF® Dye Conjugates
29021 29128	Wheat Germ Agglutinin (WGA) Conjugates
32002 32018	Live-or-Dye™ Fixable Viability Staining Kits
30050 30139	ViaFluor® SE Cell Proliferation Kits
40107- 40113	BactoView™ Dead Stains
32019, 32020	BactoView™ Viability Kits
40101, 40102	BactoView™ Live
40119, 40120	BactoSpore™ Bacterial Stains
40013, 40019	PMA (Propidium Monoazide)
40060	RedDot™1 Far-Red Nuclear Stain, 200X in Water
40061	RedDot™2 Far-Red Nuclear Stain, 200X in DMSO
41033 41040	NucSpot® Nuclear Stains
40083	NucSpot® 470 Nuclear Stain
40085	NucSpot® Far-Red, 1000X in DMSO
40051	Ethidium Homodimer III (EthD-III), 1 mM in DMSO
70054 70082	MitoView™ Mitochondrial Dyes
70058 70086	LysoView™ Dyes
70065, 70069	LipidSpot™ Lipid Droplet Stains
30088- 30090	CellBrite® Fix Membrane Stains
30092 30104	MemBrite® Fix Cell Surface Staining Kits
00027 00064	Phalloidin Conjugates
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative

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