

# **Product Information**

# Yeast Viability Staining Kit

Catalog Number: 31063

#### **Kit Contents**

Component	<b>31063-1</b> (Green/Red)	<b>31063-2</b> (Red/Blue)	<b>31063-3</b> (Far-Red/Red)
Live-or-Dye™ Fixable Dead Cell Stain	32006A 4 vials	32003A 4 vials	32005A 4 vials
Concanavalin A	29016-1mg	29017-1mg	29019-1mg
(Con A)	5 x 1 mg	5 x 1 mg	5 x 1 mg
Anhydrous	99953-1	99953-1	99953-1
DMSO	1 x 250 uL	1 x 250 uL	1 x 250 uL

Unit Size: 1000 assays

## **Storage and Handling**

Store the solid dyes and anhydrous DMSO at -20°C, desiccated and protected from light. Live-or-Dye<sup>™</sup> is stable for at least 6 months from date of receipt when stored as recommended. Con A 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.

# Prepare 40X Con A Stock Solution

Stock solutions can be made at 2 mg/mL in water or in 0.1 M sodium bicarbonate pH 8.3. A small percentage of the conjugate may remain as a visible aggregate in solution. Reconstituted Con A solution can be stored at 4°C with the addition of 2 mM sodium azide. For longer storage, store aliquots at  $\leq$  -20°C and avoid repeated freeze-thaw cycles.

#### **Spectral Properties**

Cat. No.	Component	Ex/Em (nm)	Detection channel
32003A	Live-or-Dye™ 405/452	416/452	Pacific Blue®
32005A	Live-or-Dye™ 568/583	562/584	Cy®3 or PE
32006A	Live-or-Dye™ 594/614	593/615	Texas Red®
29016-1mg	CF®488A-Con A	490/516	FITC
29017-1mg	CF®594-Con A	593/615	Texas Red®
29019-1mg	CF®640R-Con A	642/663	Cy®5

#### **Product Description**

The Yeast Viability Staining Kits are designed to discriminate between live and dead cells during flow cytometry or microscopy.

Live-or-Dye<sup>™</sup> Fixable Dead Cell Stains are cell membrane-impermeant amine-reactive dyes. The dyes are 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.

Lectin conjugates of fluorescent dyes are versatile probes for detecting glycoconjugates in microscopy and flow cytometric applications. Concanavalin A (Con A) selectively binds to  $\alpha$ -mannopyranosyl and  $\alpha$ -glucopyranosyl residues found in the cell wall of yeast and fungi, and the cell membrane of mammalian cells.

When yeast are co-stained with the two probes, live cells will display bright fluorescent cell wall staining (Con A), while dead cells will have both cell wall staining and cytoplasmic staining (Live-or-Dye<sup>™</sup>).

## 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<sup>™</sup> 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.
- Prepare the 1X Con A staining solution. Use a protein- and amine-free buffer, such as PBS or HBSS, to dilute the Con A stock 1:40. For example, add 2.5 uL of Con A stock solution to 97.5 uL of buffer. Prepare enough for 100 uL of 1X Con A staining solution per sample. Vortex to mix well, then briefly spin down to pellet any residual dye aggregates, which may cause increased background during staining.
- 3. Prepare a working solution of 100X Live-or-Dye<sup>™</sup> by diluting the stock 1:5 in anhydrous DMSO.
- Pellet cells by centrifugation and remove the supernatant. Resuspend the cell pellet in 100 uL of the 1X Con A staining solution from step 2.
- 5. Add 1 uL 100X Live-or-Dye<sup>™</sup> working solution from step 3 to each sample containing 100 uL of Con A staining solution and immediately mix well.
- 6. Incubate for 15-30 minutes at room temperature, rocking and protected from light.
- 7. Wash cells once by briefly centrifuging, removing supernatant, and resuspending in 100 uL PBS or HBSS.
- 8. Analyze by microscopy or flow cytometry in the appropriate channel (see Spectral Properties).

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates and reactive dyes, membrane and cell surface stains, cell viability assays, and apoptosis kits.

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#### **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		
31064	Yeast Live-or-Dye™ Fixable Live/Dead 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		