Product Information

Yeast Viability Staining Kit

Catalog Number: 31063

Unit Size: 1000 assays

Kit contents

Component	31063-1 (Green/Red)	31063-2 (Red/Blue)	31063-3 (Far-Red/Red)
Fixable Dead Cell Stain	32006A 4 vials	32003A 4 vials	32005A 4 vials
Concanavalin A (ConA)	29016-1mg 5 vials	29017-1mg 5 vials	29019-1mg 5 vials
Anhydrous DMSO	99953-1 250 uL	99953-1 250 uL	99953-1 250 uL

Storage and Handling

Store the solid dyes and anhydrous DMSO at -20°C, desiccated and protected from light.

Live-or-Dye™: When stored as directed, solid dye is stable for at least 6 months from the date of receipt. Once reconstituted in anhydrous DMSO, leftover dye can be stored at -20 °C for at least one month.

ConA: When stored as directed solid dye is stable for at least 12 months from date of receipt. Reconstituted dye solution can be stored at 2-6°C with the addition of 2 mM sodium azide. For longer storage, store aliquots at ≤ -20°C and avoid repeated freeze-thaw cycles.

Spectral Properties

Component	Catalog No.	Abs/Em maxima
Live-or-Dye™ 594/614	32006A	561/624 nm
Live-or-Dye™ 405/452	32003A	408/452 nm
Live-or-Dye™ 568/583	32005A	562/583 nm
CF™488A-ConA	29016-1mg	490/515 nm
CF™594-ConA	29017-1mg	593/614 nm
CF™640R-ConA	29019-1mg	642/662 nm

Product Description

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

Live-or-Dye™ Fixable Dead Cell Stains are cell membrane impermeable 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.

Lectins are versatile probes for detecting glycoconjugates in microscopy and flow cytometric applications. Concanavalin A (Con A) selectively binds to α-mannopyranosyl and α-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 dyes, live cells display bright fluorescent cell wall staining (ConA), while dead cells will have both cell wall staining and cytoplasmic staining (Live-or-Dye™).

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.

ConA dye reconstitution, 40X stock

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.

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™ 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.

1. In a clear buffer suitable for imaging, such as PBS or HBSS, dilute the ConA conjugate 1:40 to prepare a 1X staining solution (prepare enough to stain all of your samples, 100 uL/sample). Vortex to mix well, then briefly spin down to pellet any residual dye aggregates (which may cause increased background during staining).
2. Culture cells in the appropriate growth medium. Spin down and resuspend in the 1X ConA staining solution from step 1.
3. Prepare a working solution of 100X Live-or-Dye by diluting the stock 1:5 in DMSO. Add 1 uL to 100 uL cells in ConA staining solution.
4. Incubate for 15-30 minutes at room temperature, rocking, protected from light.
5. Image on a fluorescence microscope using appropriate emission filters.

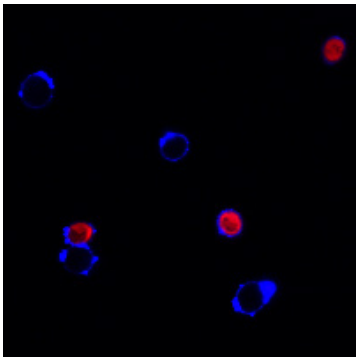

Figure 1. *Saccharomyces cerevisiae* stained with 1X Live-or-Dye™ 568/583 (red) and 1X CF640R-ConA (blue). Dead cells are stained with both dyes while live cells are outlined in blue. Imaged on a Zeiss LSM700 confocal microscope using Cy5® and Texas Red® imaging settings.

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
31064	Yeast Live-or-Dye™ Fixable Live/Dead Staining Kit	Thiazole Orange to stain all cells, 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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