LIFESCT
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LiFluor™ Yeast Cell Viability Kit

Cat. #: C0029 Size: 1000 rxns

Introduction

LiFlour™ Yeast Cell Viability Kit combines a novel two-color fluorescent probe for fungi/yeast viability, Yeast stain, with a fluorescent fungal surface labeling reagent of a third color, Lifluor™ White M2R. Lifluor™ is an ultraviolet–excitable dye that has long been used as a marker of fungal cell walls. The Yeast stain is used to determine the metabolic activity of fungal cells by fluorescence microscopy or through other instrumental techniques. The assay kit can be used for detecting fungi in complex mixtures or in pure cultures.

Package Information

Components	C0028
Yeast stain	300 µl, 10 mM
Lifluor™ White M2R	500 µl, 5 mM

Number of assays: 1,000 tests using a fluorescence microscope

Storage

Store at -20°C.

Suggestions for Use

In metabolically active yeast stained with between 1 µM and 50 µM Yeast stain reagent, Cylindrical Intra- Vacuolar Structures (CIVS) are produced in less than an hour. The structures are approximately 0.5–0.7 µm in diameter. They are frequently observed moving freely in the intravacuolar space where they increase in length until their movement is constrained by the vacuolar membrane. In Yeast stain loaded cells, CIVS have distinct orange-red fluorescence when excited by light from about 470 nm to 590 nm.

Protocols

General Considerations

The following protocols are provided as examples to guide researchers in the development of their own taining procedures.

Although best results are obtained if traces of growth medium are removed prior to staining of fungal cells with these kit reagents, staining can be achieved in a number of different suspension fluids such as blood, saliva, etc. Nucleic acids and other media components may bind Lifluor™ White M2R and the Yeast stain in unpredictable ways, resulting in low or variable cell staining or unacceptably high background fluorescence. Phosphate wash buffers are not recommended because they appear to decrease staining efficiency.

Selection of Optical Filters

The fluorescence from both live and dead fungal cells labeled with Yeast stain may be viewed with any standard filter set depending on the goals of the experiment. For general staining and viewing of fungal cells, fluorescein longpass filters are recommended. When viewing cells stained with Yeast stain, a fluorescein filter set with excitation about 480 nm and emission >530 nm should be appropriate. When staining fungal cells for quantitative ratiometric analysis, bandpass filters suitable for fluorescein and rhodamine are recommended. To view fungal cells stained with Lifluor™ White M2R, filters appropriate for DAPI are recommended.

Culture Conditions and Preparation of Yeast Suspensions

1.1 Grow yeast to late log phase (usually 10^7–10^8 cells/ml) in an appropriate nutrient medium such as Yeast extract Peptone Dextrose (YPD).

1.2 Add 50 µl of the yeast culture to 1 ml of sterile, 0.2 µm–filtered water containing 2% D-(+)-glucose and 10 mM Na-HEPES (pH 7.2) in a microfuge tube.

1.3 Concentrate by centrifugation for 5 minutes in a microcentrifuge at 10,000× g.

1.4 Remove the supernatant and resuspend the pellet in 1 ml of sterile, 0.2 µm–filtered water containing 2% D-(+)-glucose and 10 mM Na-HEPES (pH 7.2).

Staining Yeast for Fluorescence Microscopy

The ability of yeast to produce the red-shifted fluorescent CIVS is a function of metabolic activity so it may be necessary, when assessing the metabolic potential of yeast in a sample, to provide a substrate at a concentration that is adequate to support the bioconversion of the reagent. Moderately high concentrations of glucose and a pH buffer are provided in the staining solution to allow the yeast to recover from environmental stress.

2.1 Combine a yeast cell suspension (10^6–10^7 cell/ml) with Yeast stain and Lifluor™ White M2R staining solutions. The final concentration of Yeast stain should be 5–20 µM and the Lifluor™ White M2R should be at a final concentration of 25 µM. For optimization of the stain concentration, see the following section.

2.2 Mix thoroughly and incubate at 30°C in the dark for 30 minutes.

2.3 Trap 5 µl of the stained yeast suspension between a slide and 18 mm × 18 mm coverslip.

2.4 Observe in a fluorescence microscope equipped with any of the filter sets listed above.

Optimization of Cell Number and Dye Concentration for Yeast Stain Loading

3.1 Grow yeast (*Saccharomyces cerevisiae*) cell cultures overnight at 30°C in 30–50 ml Yeast extract Peptone Dextrose (YPD) in 125 ml flasks, shaking at 200 rpm.

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3.2 Centrifuge 0.2 ml of cell suspension for 5 minutes at 10,000× g and resuspend the cells in 1 ml of sterile 2% D-(+)- glucose containing 10 mM Na-HEPES, pH 7.2 (GH solution).

3.3 Prepare 100 µl of a 200 µM solution of Yeast stain in GH solution from a 10 mM DMSO stock solution of Yeast stain.

3.4 Prepare serial twofold dilutions of the 200 µM Yeast stain solution in GH solution to yield 100, 50, 25, 12.5, 6.3, 3.1 and 1.6 µM Yeast stain solutions.

3.5 For each Yeast stain dilution, add 100 µl of Yeast stain solution to 100 µl of the yeast suspension prepared in step 3.2, resulting in final Yeast cell stain concentrations from 0.8–50 µM.

3.6 After incubating the yeast with the yeast stain reagent for 30 minutes at 30°C, trap 10 µl of the yeast suspension between a microscope slide and coverslip and seal with wax or other nontoxic sealant.

3.7 Examine the stained yeast by fluorescence microscopy using a filter set (see Selection of Optical Filters) and assess the size and number of orange-red intracellular structures.

Fluorescence Microplate Reader Measurements

4.1 Grow yeast (*S. cerevisiae*) cell cultures overnight at 30°C in 30–50 ml YPD in 125 ml flasks, shaking at 200 rpm.

4.2 Determine the optimal Yeast stain concentration for CIVS production in a 5 × 10^6 cells/ml yeast suspension by fluorescence microscopy as described above in Microscopic Optimization.

4.3 Centrifuge 5 ml of cell suspension (approximately 2× 10^7 cells/ml) for 5 minutes at 10,000× g and resuspend the cells in 10 ml of sterile GH solution, yielding a suspension with 1× 10^7 cells/ml.

4.4 Add 200 µl of water to all wells in rows A and H and in columns 1 and 12 in a flat-bottom 96-well microplate.

4.5 Aliquot 100 µl of yeast cell suspension (10^6 cells) into the wells in rows B–E, columns 2–11.

4.6 Pipet 100 µl of GH solution into the wells in rows F and G, columns 2–11. Tests of nine twofold serially diluted inhibitor solutions are carried out in quadruplicate (rows B–E). Rows F and G are reserved to allow correction for inhibitor fluorescence plus Yeast reagent's fluorescence in the absence of yeast cells.

4.7 Prepare Yeast cell stain and inhibitor solutions in a separate round-bottom 96-well plate as follows: Add 150 µl of a 4× concentrate of the highest desired concentration of inhibitor to the wells in column 2, rows B–G. Next, pipet 75 µl of GH solution into the wells in column 3–11, rows B–G. Finally, dilute the inhibitor solutions twofold consecutively from columns 2–10, rows B–G, by transferring 75 µl volumes sequentially and discarding the residual 75 µl from column 10. Then, add 75 µl of a 4× concentrated solution of Yeast cell stain in Na-HEPES-buffered water to the wells in columns 2–11 in rows B–G.

4.8 The two 96-well plates are then covered and cooled to 15°C for 30 minutes.

4.9 Add 100 µl of the solutions in rows B–G of the round-bottom well plate to the yeast suspensions in the flat-bottom plate using an 8-channel pipette.

4.10 Immediately read the fluorescence of the plate in an appropriate fluorescence microplate reader using two filter combinations: ~485 nm excitation/~530 nm emission (green) and ~485 nm excitation/~620 nm emission (red).

4.11 Subsequently incubate the plates at 30°C on a rotating shaker (300 rpm) and read every 10 minutes for 1 hour.

4.12 Calculate the red/green fluorescence ratio for each well by first subtracting the fluorescence of the wells containing dye and inhibitor (average of rows F and G) at each time point.

4.13 The inhibitor effect is observed as a dose–response relationship between the inhibitor concentration and the decrease in the rate of change in red/green fluorescence ratio.