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Probes for Yeast Viability

L-7009 LIVE/DEAD® Yeast Viability Kit

- F·7030 FUN® 1 cell stain
- F-13150 FUN® 2 cell stain

Quick Facts

Storage upon receipt:

- -20°C
- Desiccate
- Protect from light

Introduction

Molecular Probes' LIVE/DEAD[®] Yeast Viability Kit combines a novel two-color fluorescent probe for yeast viability, FUN[®] 1, with a fluorescent fungal surface labeling reagent of a third color, CalcofluorTM White M2R. Calcofluor is an ultraviolet–excitable dye that has long been used as a marker of fungal cell walls.¹⁻⁵ Our FUN 1 viability stain can be used alone or together with Calcofluor to determine the metabolic activity of fungal cells by fluorescence microscopy or through other instrumental techniques. In addition, either kit component can be used for detecting fungi in complex mixtures or in pure cultures. The FUN 1 cell stain (F-7030), as well as FUN 2 cell stain (F-13150), a shorter-wavelength analog of FUN 1 cell stain, are available as separate products.

The FUN 1 and FUN 2 viability stains exploit normal endogenous biochemical processing mechanisms that appear to be well conserved among different species of yeast and other fungi.6 The conversion of FUN 1 and FUN 2 cell stains from a diffusely distributed pool of green fluorescent intracellular stain to a compact form consisting of orange-red or yellow-orange fluorescent intravacuolar structures, respectively, requires both plasma membrane integrity and metabolic capability. Only metabolically active cells are marked clearly with fluorescent intravacuolar structures, while dead cells exhibit extremely bright, diffuse, green-yellow fluorescence. Cells with intact membranes but with little or no metabolic activity have diffuse green cytoplasmic fluorescence and lack fluorescent intravacuolar bodies. Figure 1, panels A and B, shows the fluorescence emission of a suspension of FUN 1 dye-stained, actively respiring Saccharomyces cerevisiae cells as a function of time. Figure 1C illustrates a similar experiment in which FUN 2 stain was used. Biochemical processing of the FUN 1 and FUN 2 stains allows the viability of individual fungal cells to be directly assessed with high accuracy and sensitivity by fluorescence microscopy. Moreover,

the relative metabolic activity of fungal cell suspensions can be measured in a fluorometer or fluorescence microplate reader, and potentially in the flow cytometer. Our LIVE/DEAD Yeast Viability Kit allows researchers to easily, reliably and quantitatively distinguish live and dead fungal cells — generally in less than 30 minutes — whether in pure or mixed cultures, body fluids or environmental samples.

The LIVE/DEAD Yeast Viability Kit has been thoroughly tested with several species of yeast and fungi under many different conditions. The LIVE/DEAD Yeast Viability Kit is intended as a research tool and our Technical Assistance Department welcomes any feedback on the performance of this kit, especially if the fungal species or the environmental conditions differ significantly from those described in this product information sheet.

Materials

Kit Contents

- **FUN 1 cell stain** (Component A), 300 µL of a 10 mM solution in anhydrous dimethylsulfoxide (DMSO)
- **Calcofluor White M2R** (Component B), 500 µL of a 5 mM solution in water
- Assay protocols and suggestions for use

At the typical reagent dilutions and volumes, this kit contains sufficient material to perform at least 1000 individual tests on yeast in 96-well assay plates and many more preparations for analysis by fluorescence microscopy.

FUN 1 and FUN 2 Cell Stains

When purchased separately, both FUN 1 and FUN 2 cell stains are supplied in a 100 μL unit size as a 10 mM solution in anhydrous DMSO.

Storage and Handling

Both DMSO and aqueous stock solutions should be stored frozen at -20°C and protected from light. Allow reagents to warm to room temperature and centrifuge briefly before opening the vials. Before refreezing, seal all vials tightly. When stored properly, these stock solutions are stable for at least one year. The Calcofluor dye solution may show some precipitate after repeated freezing and thawing. After the vial of Calcofluor White M2R is thawed and shaken gently, it should be centrifuged for 3 minutes at $10,000 \times g$ in a microfuge to clear the solution before labeling cells.

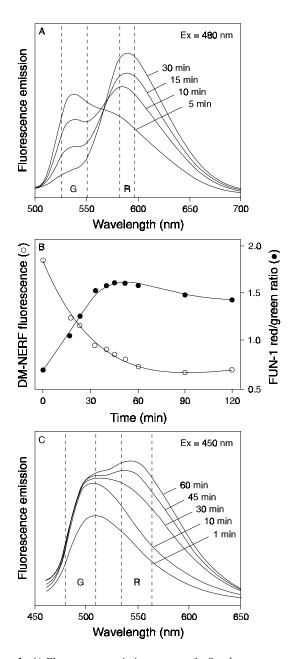


Figure 1. A) Fluorescence emission spectra of a Saccharomyces cerevisiae suspension that has been stained with FUN 1 cell stain, the principal component in our LIVE/DEAD Yeast Viability Kit. After FUN 1 dye was added to the medium, the suspension was excited at 480 nm in a fluorometer and its emission spectrum was recorded over a 30-minute time period. The shift from green to red fluorescence reflects processing of the FUN 1 dye by metabolically active yeast cells. The emission bands used to measure the green (G) and red (R) fluorescence of the FUN 1 dye-stained cells are marked by the vertical dashed lines in panel A. Glucose utilization and concomitant acidification of the extracellular solution is indicated by the reduction in fluorescence at 538 nm of the pH indicator DM-NERF. B) Yeast metabolic activity and FUN 1 dye processing, as measured in a fluorescence microplate reader. Processing of the FUN 1 cell stain into red fluorescent vacuolar structures is indicated by an increase in the red/green fluorescence ratio over time. C) Fluorescence emission spectra of an S. cerevisiae suspension that has been stained with the FUN 2 cell stain. After FUN 2 dye was added to the medium, the suspension was excited at 450 nm in a fluorometer and its emission spectrum was recorded over a 60-minute time period. The shift from green to orange fluorescence reflects processing of the FUN 2 dye by metabolically active yeast cells. Vertical dashed lines indicate green (G) and "red" (R) regions of the FUN 2 emission spectra that may be used to generate R/G ratios.

Caution: No data are available addressing the mutagenicity or toxicity of FUN 1 or FUN 2 cell stains. Because both reagents will bind to nucleic acids, they should be treated as potential mutagens and used with appropriate care. The DMSO stock solutions of FUN 1 and FUN 2 cell stains should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution. As with all nucleic acid stains, concentrated FUN 1 and FUN 2 reagent solutions should be poured through activated charcoal before disposal. The charcoal can then be incinerated to destroy the dyes.

Protocols and Suggestions for Use

In order to directly compare experimental techniques, S. cerevisiae, strain SF838-1Da, was used for each of the example protocols provided in this information sheet. The FUN 1 and FUN 2 cell stains will stain nucleic acids in most cell types, producing diffuse green to green-yellow cytoplasmic staining in live or membrane-compromised dead yeast cells. In metabolically active yeast stained with between 1 µM and 50 µM FUN 1 or FUN 2 reagent, Cylindrical IntraVacuolar Structures (CIVS) are produced in less than an hour (Figure 2). The structures are approximately 0.5–0.7 µm in diameter and range from one to several µm in length with FUN 1 stain and generally longer and less numerous with FUN 2 stain. 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 FUN 1 loaded cells, CIVS have distinct orange-red fluorescence when excited by light from about 470 nm to 590 nm; whereas CIVS in FUN 2 dye-loaded cells have distinct yellow-orange fluorescence when excited optimally at 450 nm. The development of CIVS in yeast is affected by several factors. When yeast cells are sampled from stationary cultures or other environments that are not optimal for growth, the formation of CIVS can usually be enhanced by allowing the cells to "recover" in a buffered solution containing dextrose or another readily metabolizable carbohydrate. The staining and extent of subsequent CIVS formation is also affected by the dye concentration. The cell density will also influence CIVS formation because under optimal loading conditions the cells will remove a large fraction of the FUN 1 and FUN 2 dye from the solution. This may be particularly problematic in the presence of a large proportion of dead cells because cells with compromised plasma membranes will usually accumulate more FUN 1 or FUN 2 stain than do live cells, limiting the availability of the stain. In general it is best to decide on either a single concentration of dye or a single cell density, and then adjust the other parameter for optimal CIVS formation. Temperature also plays a very important role in the rate of CIVS formation because this affects a number of enzymatic activities and physiological processes of the cell. The Q₁₀ (the magnitude of change for a 10°C change in temperature) of CIVS formation rate in S. cerevisiae is on the order of 3.5-fold.

General Considerations

The following protocols are provided as examples to guide researchers in the development of their own staining procedures. Researchers at Molecular Probes have used the procedures described here and found them to be simple and reliable. Centrifugation steps in the following protocols can be accomplished either by using large-volume tubes in the appropriate rotor (e.g., SS-34 rotor) or by aliquotting the solution into microfuge tubes and centrifuging in a microcentrifuge. 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 Calcofluor White M2R and the FUN 1 (or FUN 2) cell 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 either FUN 1 or FUN 2 cell 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 FUN 1, a fluorescein filter set with excitation about 480 nm and emission \geq 530 nm should be appropriate. A Lucifer Yellow filter set with excitation about 430 nm and emission \geq 515 nm is recommended for general viewing of fungal cells stained with FUN 2. When staining fungal cells for quantitative ratiometric analysis, bandpass filters suitable for fluorescein and rhodamine are recommended. To view fungal cells stained with Calcofluor White M2R, filters appropriate for DAPI are recommended. In the case where fungal cells are stained with either FUN 1 or FUN 2 and with Calcofluor White M2R, we recommend using multipass filter sets appropriate for viewing DAPI, fluorescein and rhodamine. More detailed information for suitable optical filters can be found in Molecular Probes' Handbook of Fluorescent Probes and Research Chemicals, at our web site (www.probes.com) or by contacting our Technical Assistance Department.

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).⁷

1.2 Add 50 μ L of the yeast culture to 1 mL of sterile, 0.2 μ m–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 $10,000 \times g$.

1.4 Remove the supernatant and resuspend the pellet in 1 mL of sterile, 0.2μ m-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 $(10^6-10^7 \text{ cell/mL})$ with FUN 1 cell stain (or FUN 2 cell stain) and, if desired, Calcofluor White M2R staining solutions. The final concentration of FUN-1 cell stain should be 5–20 μ M and the Calcofluor White M2R should be at a final concentration of 25 μ M. 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 (up to 60 minutes with FUN 2).

2.3 Trap 5 μ L of the stained yeast suspension between a slide and 18 mm \times 18 mm coverslip.

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

Note on Utility

The LIVE/DEAD Yeast Viability Kit has been tested on the following fungi: *S. cerevisiae* (five different strains), *Candida pseudotropicalis, Neurospora crassa* and *Aspergillus nidulans.* A good correlation between the results obtained with

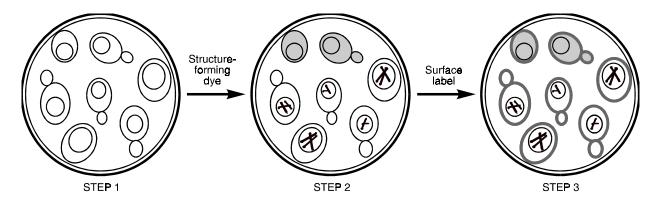


Figure 2. Schematic diagram of the LIVE/DEAD Yeast Viability assay. STEP 1 illustrates several unstained yeast cells in a typical microscope field. In STEP 2, the yeast cells have been incubated with the structure-forming FUN 1 cell stain; the shaded cells represent dead cells that exhibit a diffuse yellow-green fluorescence while the unshaded cells represent metabolically active yeast cells that contain cylindrical, red-fluorescent structures in their vacuoles. STEP 3 shows FUN 1 dye-stained cells that have been subsequently incubated with the surface label Calcofluor White M2R, which stains the cell walls fluorescent blue.

the LIVE/DEAD Yeast Viability Kit and those obtained with standard plate counts was achieved with both *S. cerevisiae* and *C. pseudotropicalis*. Tests have been performed on both logarithmically growing cultures and cells that have undergone different forms of environmental stress.

Optimization of Cell Number and Dye Concentration for FUN 1 or FUN 2 Cell Stain Loading

Overview

Dye loading can be optimized either by fluorescence microscopy or by fluorometry. The microscopy procedure is simpler to perform and interpret but the fluorometric optimization may be desired if cell suspensions are to be assayed. The protocols, as written, are for using FUN 1 cell stain; however, with minor modifications, the same protocols apply for using FUN 2 cell stain.

Microscopic Optimization

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.

3.2 Centrifuge 0.2 mL of cell suspension for 5 minutes at $10,000 \times g$ 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 μ L of a 200 μ M solution of the FUN 1 reagent in GH solution from a 10 mM DMSO stock solution of FUN 1 cell stain.

3.4 Prepare serial twofold dilutions of the 200 μ M FUN 1 dye solution in GH solution to yield 100, 50, 25, 12.5, 6.3, 3.1 and 1.6 μ M FUN 1 dye solutions.

3.5 For each FUN 1 cell stain dilution, add 100 μ L of FUN 1 dye solution to 100 μ L of the yeast suspension prepared in step 3.2, resulting in final FUN 1 cell stain concentrations from 0.8–50 μ M.

3.6 After incubating the yeast with the FUN 1 reagent for 30 minutes at 30°C, trap 10 μ L of the yeast suspension between a microscope slide and coverslip and seal with wax or other non-toxic sealant.

3.7 Examine the FUN 1 dye–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. If FUN 2 cell stain is used in place of FUN 1 cell stain, the intracellular structures will be yellow-orange.

Fluorometric Optimization

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 Centrifuge 4 mL of cell suspension for 5 minutes at $10,000 \times g$ and resuspend the cells in 10 mL of sterile GH solution.

4.3 Add 1.5 mL aliquots of this cell suspension to each of six fluorometer cuvettes (1 cm pathlength) containing 7 mm magnetic stir bars.

4.4 Prepare 4 mL of a 60 μ M FUN 1 reagent solution in GH solution from a 10 mM DMSO stock solution of FUN 1 cell stain.

4.5 Prepare serial twofold dilutions of the 60 μ M FUN 1 reagent solution in GH solution to yield 60, 30, 20, 10, 5, and 2.5 μ M FUN 1 cell stain solutions.

4.6 For each FUN 1 dye dilution, add 1.5 mL of FUN 1 reagent solution to each 1.5 mL aliquot of yeast suspension, resulting in final FUN 1 cell stain concentrations from 1.25– 30μ M.

4.7 Record the fluorescence emission spectrum of each FUN 1 dye–stained yeast suspension in a fluorometer equipped with a stirred, thermoregulated cuvette holder held at a constant $30 \pm 0.2^{\circ}$ C. Using excitation at ~470 nm, acquire emission spectra from 500–700 nm every 5 minutes for a total period of 60 minutes. (For cells loaded with FUN 2 cell stain, use excitation of ~450 nm and acquire spectra from 460–650 nm.)

4.8 At the end of the experiment, determine the red/green fluorescence ratio at each time point by dividing the integrated fluorescence at 560–610 nm (red) by that at 510–560 nm (green). The greatest rate of change in the red/green fluorescence ratio indicates the most efficient conversion of dye from green uniform stain to red CIVS structures. Confirm the results of the fluorometric ratio determination by observing the CIVS in the yeast cells using a fluorescence microscope fitted with a fluorescein longpass filter set or a DAPI/fluorescein/Texas Red[®] triple-band filter set. For cells loaded with FUN 2 cell stain, use 535–565 nm for red fluorescence and 480–510 nm for green fluorescence.

Temperature Optimization

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

5.2 Centrifuge 4 mL of cell suspension for 5 minutes at $10,000 \times g$ and resuspend the cells in 10 mL of sterile GH solution.

5.3 Add 1.5 mL aliquots of this cell suspension to each of six fluorometer cuvettes (1 cm pathlength) containing 7 mm magnetic stir bars.

5.4 Prepare a 10 μ M FUN 1 dye solution in GH solution from a 10 mM DMSO stock solution of FUN 1 cell stain.

5.5 After pre-equilibrating aliquots of the 10 μ M FUN 1 cell stain solution at appropriate temperatures, add an aliquot to each cuvette (also pre-equilibrated at the same temperature) to begin the experiment. The increase in red/green fluorescence ratio with time of FUN 1 dye exposure will be determined at a series of temperatures in 10°C intervals from 10–50°C.

5.6 Record the fluorescence emission spectrum of each FUN 1 dye–stained yeast suspension in a fluorometer equipped with a stirred, thermoregulated cuvette holder held at a constant temperature for the duration of each experiment. The temperature of the cuvette should be monitored continuously. Using excitation at ~470 nm, acquire emission spectra from 500–700 nm every 5 minutes for a total period of 60 minutes. For cells loaded with FUN 2 cell stain, use excitation of ~450 nm and acquire spectra from 460–650 nm.

5.7 Determine the red/green fluorescence ratio at each time point by dividing the integrated fluorescence at 560–610 nm (red) by that at 510–560 nm (green). For cells loaded with FUN 2 cell stain, use 535–565 nm for red fluorescence and 480–510 nm for green fluorescence.

5.8 The greatest rate of change in red/green fluorescence ratio indicates the most rapid conversion of dye from green diffuse stain to compact, bright red structures. Confirm the presence of these structures by microscopic observation of the yeast cells using a fluorescein longpass filter set or a DAPI/fluorescein/ Texas Red triple-band filter set.

Detection of Cells in an Unknown Mixture

Overview

The presence of viable and nonviable yeast cells may be determined in samples of various biological fluids. As an example of this type of procedure, the protocol below describes use of FUN 1 cell stain for the detection of yeast in a blood sample. This protocol can be adapted for use with other types of unknown samples and, with minor modifications, can be used with FUN 2 cell stain in place of FUN 1 cell stain.

Example Protocol

6.1 Draw venous blood aseptically into a heparinized Vacutainer (Becton Dickinson and Co., Rutherford, New Jersey) and hold at 25°C until analysis is performed.

6.2 Add *Candida pseudotropicalis* or *S. cerevisiae* cells to a final density of 10⁶ cells/mL of blood, which corresponds to about one cell per field (40X objective lens, 10X ocular) in a standard wet-mount preparation.

6.3 Aliquot 250 μ L of a balanced salt solution (e.g., Hanks' Balanced Salt Solution) containing 4% D-(+)-glucose and 10 mM Na-HEPES (final pH 7.2) into a 1.5 mL microfuge tube and then add 5 μ L of a 10 mM DMSO stock solution of FUN 1 cell stain.

6.4 Mix the resulting solution thoroughly by repipetting and add $750 \,\mu$ L of whole blood, followed again by vigorous mixing.

6.5 Incubate the suspension at 30°C for 30 minutes.

6.6 Remove a $5-10 \mu L$ aliquot and trap between a microscope slide and coverslip.

6.7 Observe the fluorescence of the cells either by using a multiple-band filter set or by alternating between excitation with a standard fluorescein bandpass filter set and a standard Texas Red filter set. Live, actively metabolizing fungal cells are marked by very short cylindrical to spherical orange-red intracellular structures while dead cells are stained uniformly green-yellow. Leukocytes exhibit primarily green nuclear fluorescence but some cytoplasmic signal is also seen. Erythrocytes are virtually nonfluorescent.

Fluorescence Microplate Reader Measurements

Overview

This example protocol illustrates the use of the fluorescence microplate reader to measure the effect of a putative metabolic inhibitor on the generation of CIVS in yeast cell suspensions. The protocol, as written, is for using FUN 1 cell stain; however, with minor modifications, the same protocol applies for using FUN 2 cell stain.

Example Protocol

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

7.2 Determine the optimal FUN 1 dye concentration for CIVS production in a 5×10^6 cells/mL yeast suspension by fluorescence microscopy as described above in *Microscopic Optimiza-tion*.

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

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

7.5 Aliquot 100 μ L of yeast cell suspension (10⁶ cells) into the wells in rows B–E, columns 2–11.

7.6 Pipet 100 μ L 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 FUN 1 reagent's fluorescence in the absence of yeast cells.

7.7 Prepare FUN 1 cell stain and inhibitor solutions in a *sepa-rate round-bottom 96-well plate* as follows: Add 150 μ L of a 4X concentrate of the highest desired concentration of inhibitor to the wells in column 2, rows B–G. Next, pipet 75 μ L 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 μ L volumes sequentially and discarding the residual 75 μ L from column 10. Then, add 75 μ L of a 4X concentrated solution of FUN 1 cell stain in Na-HEPES– buffered water to the wells in columns 2–11 in rows B–G.

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

7.9 Add 100 μ L 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 pipettor.

7.10 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). For cells loaded with FUN 2 cell stain, use filter combinations ~450 nm/~500 nm for green fluorescence and ~450 nm/~550 nm for red fluorescence.

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

7.12 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.

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7.13 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.

References

1. J Cell Biol 123, 1821 (1993); **2.** J Cell Biol 114, 111 (1991); **3.** J Cell Biol 114, 101 (1991); **4.** J Cell Biol 111, 143 (1990); **5.** Biochim Biophys Acta 1035, 206 (1990); **6.** Appl Environ Microbiol 63, 2897 (1997); **7.** *Methods in Yeast Genetics*, F. Sherman, G.R. Fink and J.B. Hicks, Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, p. 61 (1983).

Product List	Current prices ma	y be obtained from our Web site or from our Customer Service Department
	ourront prices ma	

Cat #	Product Name	Unit Size
F-7030	FUN [®] 1 cell stain *10 mM solution in DMSO*	100 μL
F-13150	FUN [®] 2 cell stain *10 mM solution in DMSO*	100 μL
L-7009	LIVE/DEAD [®] Yeast Viability Kit *1000 assays*	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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