FunXite™-1 *10 mM DMSO solution*

Catalog number: 22470
Unit size: 100 uL

Component	Storage	Amount (Cat No. 22470)
FunXite™-1	Freeze (< -15 °C), Minimize light exposure	1 vial (100 uL)

OVERVIEW

FunXite™-1 is chemically same molecule to FUN® 1 (FUN® 1 is the trademark of ThermoFisher). It is a unique two-color fluorescent viability probe for yeast and fungi. FunXite-1 diffuses into a variety of cells and initially stains the cytoplasm with a diffusely distributed green fluorescence. In some yeasts and fungi, the stain reacts with some cellular substances (e.g., glutathione) to generate distinct vacuolar structures that exhibit a striking red fluorescence, accompanied by a reduction in the green cytoplasmic fluorescence. Formation of the intravacuolar structures requires both plasma membrane integrity and metabolic capability. Dead yeast and fungus cells fluoresce bright yellow-green, with no discernable red structures.

AT A GLANCE

Storage and Handling

It is recommended to freeze DMSO stock solutions at -20°C while protecting them from light. Before using, allow reagents to reach room temperature, then briefly centrifuge vials prior to opening. Before freezing, ensure all vials are tightly sealed. By following these guidelines, the stock solutions can maintain stability for a minimum of one year.

KEY PARAMETERS

Fluorescence microscope

Emission	FITC Filter Set/TRITC Filter Set
Excitation	FITC Filter Set/TRITC Filter Set
Recommended plate	Black wall/clear bottom

Flow cytometer

Emission	530/30 nm and 575/26 nm
Excitation	488 nm laser
Instrument specification(s)	FITC Channel and PE Channel

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol is intended to be used as a guideline to assist researchers in developing their own staining procedures.

1. Grow yeast (*S. cerevisiae*) cell cultures overnight at 30 °C in 30-50 mL of an appropriate nutrient medium such as Yeast extract Peptone Dextrose (YPD).

2. Centrifuge 0.2 mL of cell suspension at 10,000 × g for 5 minutes. Then, resuspend the cells in 1 mL of sterile 2% D-(+)-glucose supplemented with 10 mM Na-HEPES at pH 7.2.

3. Prepare a 100 µL solution of FunXite™-1 reagent by diluting a 10 mM DMSO stock solution of FunXite™-1 cell stain in GH-solution to a final concentration of 200 µM.

4. Prepare serial two-fold dilutions of the 200 µM FunXite™-1 dye in GH-solution to obtain 100, 50, 25, 12.5, 6.3, 3.1, and 1.6 µM FunXite™-1 dye solutions.

5. For each FunXite™-1 cell stain dilution, combine 100 µL of FunXite™-1 dye solution with 100 µL of the yeast suspension prepared in step 3.2. This will yield final FunXite™-1 cell stain concentrations ranging from 0.8 to 50 µM.

6. Following a 30-minute incubation of yeast with the FunXite™-1 reagent at 30°C, place 10 µL of the yeast suspension between a microscope slide and coverslip, then seal it using wax or other safe sealants.

7. Examine the FunXite™-1 dye–stained yeast by fluorescence microscopy using a FITC filter set (dead/metabolically inactive cells) and a TRITC filter set (live, metabolically active cells). Evaluate the size and quantity of orange-red intracellular structures.

DISCLAIMER

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