MycoLight™ Fluorescence Live/Dead Bacterial Imaging Kit

Catalog number: 22411
Unit size: 100 Tests

Component	Storage	Amount
Component A: MycoLight™ 520	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Propidium iodide (100X)	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component D: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

AAT Bioquest's MycoLight™ Fluorescence Live/Dead Bacterial Imaging Kit provides two-color fluorescence assay for visualizing live and dead bacteria through fluorescent microscope. MycoLight™ 520 is a non-fluorescent esterase substrate that diffuses into both Gram positive and Gram-negative bacteria. Upon hydrolysis by bacterial intracellular non-specific esterases, a green fluorescent product is produced and accumulated within bacteria. In contrast, propidium iodide is a red-fluorescent nucleic acid stain that only penetrates bacteria with damaged membranes. Thus, with an appropriate mixture of the MycoLight™ 520 and propidium iodide stains, live bacteria with intact cell membranes emits green fluorescence, whereas dead bacteria with damaged membranes gives red fluorescence. The MycoLight™ Fluorescence Live/Dead Bacterial Imaging Kit is a robust tool for imaging Live/Dead bacteria. Stained cells can be monitored fluorimeterically (FITC filter set) and (TRITC filter set) for live and dead bacteria respectively.

AT A GLANCE

Protocol summary

1. Prepare 100X MycoLight™ 520 stock solutions
2. Prepare bacteria samples
3. Add MycoLight™ 520 and Propidium iodide
4. Incubate bacteria samples with MycoLight™ 520 and Propidium iodide at 37°C for 5-10 minutes or room temperature for 60 minutes in dark
5. Analyze sample by fluorescence microscope with FITC and TRITC filter sets

Important Thaw all the kit components at room temperature before starting the experiment

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	488 nm / 540 nm
Emission:	530 nm / 620 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	FITC / TRITC filter(s)

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

MycoLight™ 520 stock solution (100X):
Add 100 µL of DMSO (Component D) into the vial of MycoLight™ 520 (Component A).

Note Store stock solution at -20°C, avoid light and repeat freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

Preparation of bacterial sample

1. Prepare bacteria sample with concentration of 10^6 to 10^8 cells/mL. Grow bacteria into late log phase in appropriate medium.

   Note Measure the optical density of the bacterial culture at wavelength = 600 nm (OD600) to determine the cell number. For E. coli culture, OD600 = 1.0 equals 8 x 10^8 cells/ml.

2. Remove medium by centrifugation at 10,000 x g for 10 minutes and re-suspend the pellet in Assay Buffer (Component C).

3. Dead bacteria can be prepared by treating with 70% ethanol for 30 minutes followed by 60 minutes of boiling.

Staining protocol

The following is a suggested protocol and should be optimized with different bacterial strain or other specific needs. An optional washing step can be added before imaging if higher background is observed.

1. Add 1 µL of the 100X MycoLight™ 520 stock solutions and 1 µL of 100X Propidium iodide (Component B) to 100 µL of the bacterial sample in Assay Buffer.

2. Mix well and incubate in dark for 5-10 minutes at 37°C or 60 minutes at room temperature for optimum staining results.

3. Monitor fluorescence of bacteria with a fluorescent microscope through FITC (Ex/Em = 488/530 nm) and TRITC (Ex/Em = 540/620 nm) channel.

EXAMPLE DATA ANALYSIS AND FIGURES

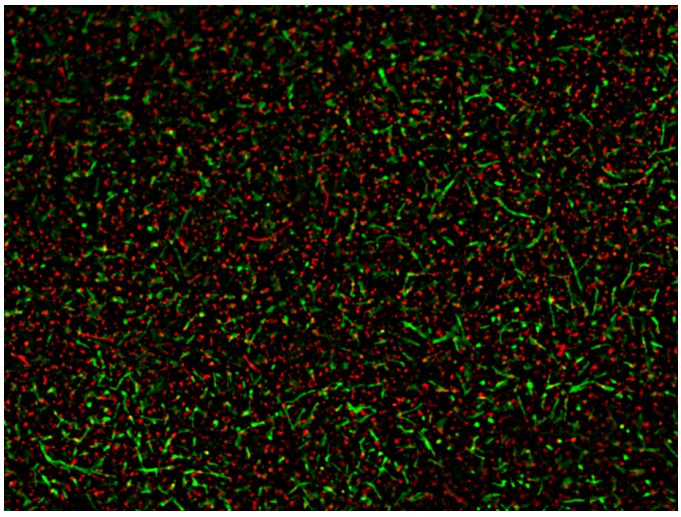

Figure 1. A mixed population of live and dead *Bacillus subtilis* was stained with MycoLight™ Fluorescence Live/Dead Bacterial Imaging Kit. Live bacteria with active intracellular esterase showed green fluorescence, while 70% alcohol-killed

dead bacteria with compromised membranes showed red fluorescence.

DISCLAIMER