

MycoLight™ Bacterial Viability Assay Kit

Catalog number: 22400
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 22400)
Component A: MycoLight™ Green	Freeze (< -15 °C), Minimize light exposure	1 Vial (200 µL)
Component B: Propidium iodide	Freeze (< -15 °C), Minimize light exposure	1 Vial (200 µL)

OVERVIEW

AAT Bioquest's MycoLight™ Bacterial Viability Assay Kit provides two-color fluorescence assay of bacterial viability in both gram-positive and negative bacterial cell. The kit utilizes the mixture of our green fluorescent nucleic acid stain MycoLight™ Green and the red-fluorescent nucleic acid stain propidium iodide. When used alone, the MycoLight™ Green stain generally labels all bacteria (live and dead) in a population. In contrast, propidium iodide penetrates only bacteria with damaged membranes, causing a reduction in the MycoLight™ Green stain fluorescence when both dyes are present. Thus, with an appropriate mixture of the MycoLight™ Green and propidium iodide stains, live bacteria with intact cell membranes emits green fluorescence, whereas dead or dying bacteria with damaged membranes gives red fluorescence. The MycoLight™ Bacterial Viability Assay Kit is a robust tool for monitoring the viability of bacterial populations as a function of the membrane integrity of the cell. Stained cells can be monitored fluorimetrically at 510-530 nm (FITC filter) and 600-660 nm (Texas red filter) with excitation at 488 nm, the most common excitation light source.

AT A GLANCE

Important Note

Thaw kit components at room temperature and centrifuge briefly before starting your experiment.

Note: The Kit has been tested at logarithmically growing cultures of the following bacterial species: *Bacillus cereus*, *B. subtilis*, *Clostridium perfringens*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Mycobacterium phlei*, *Pseudomonas aeruginosa*, *P. syringae*, *Salmonella oranienburg*, *Serratia marcescens*, *Shigella sonnei*, *Staphylococcus aureus* and *Streptococcus pyogenes*. *Agrobacterium tumefaciens*, *Edwardsiella ictaluri*, *Eurioplasma eurylytica*, *Lactobacillus* sp., *Mycoplasma hominus*, *Propionibacterium* sp., *Proteus mirabilis* and *Zymomonas* sp.

Note: The following is the recommended protocol for bacterial staining. The protocol only provides a guideline and should be modified according to the specific needs.

KEY PARAMETERS

Fluorescence microscope

Emission	530/660 nm
Excitation	510/600 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	FITC/Texas Red filter sets

Flow cytometer

Emission	530/30 nm, 610/20 nm filter
Excitation	488 nm laser
Instrument specification(s)	FITC, PE-Texas Red channel

PREPARATION OF WORKING SOLUTION

Dye working solution (250X):

1. Add equal volume of MycoLight™ Green (Component A) and Propidium Iodide (Component B) in a microfuge tube and mix them well.

Note: Prepare the working solution fresh each time.

SAMPLE EXPERIMENTAL PROTOCOL

1. Grow bacteria in any appropriate medium. Best results for healthy bacteria are obtained from log-phase cultures. Dilute the bacterial culture to ~ 106 to 108 cells per mL in 0.85% NaCl or appropriate buffer. Prepare sufficient suspension to provide 500 µL per test for flow cytometry or 100 µL per test for 96-well plate.

Note: Remove traces of growth medium before staining bacteria. A single wash step is usually sufficient to remove significant traces of interfering media components from the bacterial suspension. Phosphate wash buffers are not recommended because they appear to decrease staining efficiency.

2. Add 4 µL of the dye working solution (250X) to each mL of the bacterial suspension. Mix well and incubate at room temperature for 15 minutes. Protect from light.
3. The stained bacterial cells can be analyzed by a fluorescence microscope, fluorescent microplate reader or flow cytometry.
4. The fluorescence from both live and dead bacteria may be viewed simultaneously with any standard fluorescein long pass filter set. Alternatively, the live (green fluorescent) and dead (red fluorescent) cells may be viewed separately with FITC and Texas Red filter sets.

EXAMPLE DATA ANALYSIS AND FIGURES

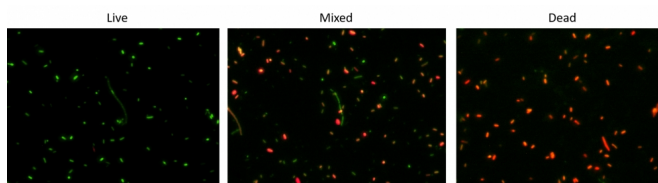


Figure 1. Fluorescence images of *E. coli* HST08 stained with Cell Meter™ Bacterial Viability Assay Kit (Cat#22400). Live bacteria with intact cell membranes showed green fluorescence (Left), while 70% alcohol-killed dead bacteria (Right) with compromised membranes showed red fluorescence. Live and dead *E.coli* bacterial cells were also visualized in a mixed population (Middle).

DISCLAIMER

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