Mycolight™ Bacterial Viability Assay Kit*Green/Red Fluorescence*

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22400	Keep in freezer	Fluorescence microscope Flow cytometry

Introduction

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.

Kit Components

Components	**Amount**
Component A: MycoLight™ Green	200 µl
Component B: Propidium iodide	200 µl

Assay Protocol for Bacteria Staining

Note1: Thaw kit components at room temperature and centrifuge briefly before starting your experiment. Note 2: 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 eurilytica, Lactobacillus sp., Mycoplasma hominus, Propionibacterium sp., Proteus mirabilis and Zymomonas sp. Note 3: The following is the recommended protocol for bacterial staining. The protocol only provides a guideline, should be modified according to the specific needs.*

1. Grow bacteria in any appropriate medium. Best results for healthy bacteria are obtained from log-phase cultures. Dilute the bacterial culture to ~ 10^6c to 10^8 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. Mix equal volume of MycoLight™ Green (Component A) and propidium iodide (Component B) in a microfuge tube to have 250x staining dye mixture.
3. Add 4 µL of the 250x staining dye mixture (from step 2) to each mL of the bacterial suspension. Mix well and incubate at room temperature for 15 minutes. Protect from light.
4. The stained bacterial cells can be analyzed by a fluorescence microscope, fluorescent microplate reader or flow cytometry.
5. 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 fluorescein and Texas Red filter sets.

References

1. Cheryl M. Davies. 1991. A comparison of fluorochromes for direct viable counts by image analysis. Lett Appl Microbiol 13, 58.
2. G.A. McFeters, A. Singh, S. Byun, P.R. Callis, S. Williams. 1991. Acridine orange staining reaction as an index of physiological activity in *Escherichia coli*. J Microbiol Methods 13, 87
3. A.S. Kaprelyants and D.B. Kell. 1992. Rapid assessment of bacterial viability and vitality by rhodamine 123 and flow cytometry J Appl Bacteriol 72, 410.