BIOLUMINOR

KitMycoLight™ Ratiometric Bacterial Membrane Potential Kit *Red/Green Fluorescence

Table 1 Contents and storage

Material	Amount	Storage	Stability
MycoStain It™ Green	1 vial ((1 mL-100X)	• ≤ –20°C • Desiccate • Protect from light	
CCCP	1 vial (300 µL-500 µM in DMSO)		
PBS	2 bottle (Each 100 mL)		
Spectral characteristic of the fluorescent probe: **Ex~510/600nm, Em~530/660 nm**			

Introduction

MycoLight™ Ratiometric Bacterial Membrane Potential Kit uses a fluorescent sensor that exhibits green fluorescence in both gram-positive and negative bacterial cells in low concentration, but the fluorescence shifts toward red emission at higher cytosolic concentrations due to the dye molecule aggregation caused by larger membrane potentials. The magnitude of membrane potential varies with different bacterial species. For many gram-positive species, the red/green ratio tend to vary with the intensity of the proton gradient while in many gram-negative bacteria the response of the dye does not appear to be proportional to proton gradient intensity. This kit is designed to assay bacterial membrane potentials when the bacterial concentrations are in the range of 10^5 – 10^7 organisms per mL. 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.

Guidelines for Use

Important 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: Micrococcus luteus, Staphylococcus aureus, S. warnerii, Bacillus cereus, Klebsiella pneumoniae, Escherichia coli, and Salmonella choleraesuis.

Note Many bacteria do not show a proportional response to partial membrane depolarization with MycoStain It™ Green. The response of each bacterial system should be investigated and optimized. Occasionally the MycoStain It™ Green concentration and staining time must be adjusted for optimal detection of membrane potential. The following is the recommended protocol for bacterial staining. The protocol only provides a guideline, should be modified according to the specific needs.

Note Some common buffer components, such as Tween-20, Sodium Azide and thimerosal, can alter membrane potential, and should be avoided. Be sure to test buffer additives for their effect on membrane potential during optimization studies.

Experiment procedure

1. Grow bacteria in any appropriate medium. Best results for healthy bacteria are obtained from log-phase cultures. Dilute the bacterial culture to ~ 10^6 cells per mL in PBS (Component C) or equivalent sterile buffer. Bacteria may be diluted directly from the culture medium without washing. Prepare sufficient suspension to provide 500 mL per test.

2. Aliquot 500 µl of the bacterial suspension into a flow cytometry tube for each staining experiment to

be performed. Prepare two additional tubes for a depolarized control and an unstained control.

3. Add 10 µl of 500 µM CCCP (Component B) to the depolarized control sample and mix.

4. Add 5 µl of MycoStain It™ Green (100X) (Component A) to each flow cytometry tube and mix (do not add stain to the unstained control sample). Incubate samples at room temperature for 30 mins. Stained samples can be analyzed after 5 min, but signal intensity continues to increase until ~ 30 mins.

5. Stained bacteria can be assayed in a flow cytometer equipped with a laser emitting at 488 nm.Fluorescence is collected in the green (fluorescein filter) and red (Texas Red filter) channels. The forward scatter, side scatter, and fluorescence should be collected with logarithmic signal amplification.

6. Instrument adjustments are especially critical for detecting relatively small particles such as bacteria. Use the unstained control sample to locate bacterial populations in the forward and side scatter channels. Use the side scatter as the parameter for setting the acquisition trigger.

7. Apply the depolarized control sample after adjusting the flow cytometer as described above. Gate on bacteria using forward versus side scatter and adjust fluorescence photomultiplier tube voltages such that the green and red MFI values are approximately equal. Do not set compensation.

8. While the relative amount of red and green fluorescence intensity will vary with cell size and aggregation, the ratio of red to green fluorescence intensity can be used as a size-independent indicator of membrane potential. The data can also be processed by gating on bacteria using forward versus side scatter, and analyze gated populations with a dot plot of red versus green fluorescence reporting MFI values as linear values, not as channels.

9. On a ratiometric histogram, set markers around the peaks of interest and record the mean ratio values. For a dot plot of red versus green fluorescence, set regions around the populations of interest and record red and green mean fluorescence intensity (MFI) values for each. To evaluate the data, divide the red population MFI by the green population MFI.

10. In the flow cytometer, bacteria are identified solely on the basis of their size and stain ability. It is best to inspect each sample by fluorescence microscopy to confirm that the particles detected are indeed bacteria.

Fluorescence Data

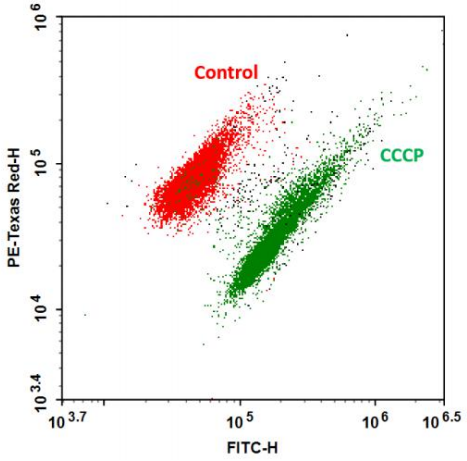

Figure 1.

Bacillus subtilis was cultured to log phase and diluted to the concentration of 1 x10^6cells per mL in PBS. Cells were then treated with 5 µM CCCP for 20 min andincubated with 1X MycoStain It™ Green for 30 min before flow cytometry analysis