BIOLUMINOR

MycoLight™ Rapid Fluorescence GramPositive Bacteria Staining Kit

Table 1 Contents and storage

Material	Amount	Storage	Stability
IF647-ConA	1 vial (200 µL)	• ≤ –20°C • Desiccate	
Assay Buffer	1 bottle (25 mL)	• Protect from light	
Spectral characteristic of the fluorescent probe: **Ex~650, Em~669**			

Introduction

The gram stain is an important and widely used method for the taxonomic classification of bacteria in clinical and research settings. The original method involves quite a few steps like heat fixation, two-steps staining protocol, alcohol extraction and counterstaining. These steps can create inconsistent staining. one-step kit overcomes the existing problems by eliminating the labor-intensive steps. The kit uses a fluorescently labeled Concanavalin A (ConA), which is a lectin that selectively binds to N-acetyl glucosamine exposed on the surface of gram-positive bacteria. When gram-negative and gram-positive bacteria are stained with the fluorescently labeled ConA conjugate, only gram-positive bacteria fluoresce red. Stained bacteria can be monitored fluorimeterically. Our kit is robust and convenient since the fluorescently labeled ConA conjugate used in our kit demonstrates higher brightness and photo stability over other existing dyes.

Guidelines for Use

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. IF647-ConA stock solution (100X): Add 100 µL of Assay Buffer (Component B) into the vial of IF647-ConA (Component A) and mix them well. Note Store stock solution at -20ºC, avoid light and store in smaller aliquots to avoid repeated freeze-thaw cycles.

Experiment procedure

Preparation of Bacterial Samples

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

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 5 minutes and re-suspend the pellet in Assay Buffer (Component B).

Staining Protocol

1. Add 1 µL of the IF647-ConA stock solution (100X) to 100 µL of the bacterial sample.

2. 2. Mix well and incubate in dark for 5-15 minutes at room temperature.

3. Centrifuge at 10,000 x g for 5 minutes and remove the IF647-ConA staining solution.

4. Resuspend in 100 µL of Assay Buffer (Component

B).

5. Monitor fluorescence of bacteria with a fluorescent microscope through Cy5 (Ex/Em = 650/669 nm) channel.

Note The protocol only provides a guideline, should be optimized with different bacterial strain or other specific needs. An optional washing step with Assay buffer (Component B) can be added before imaging if higher background is observed.

Fluorescence Data

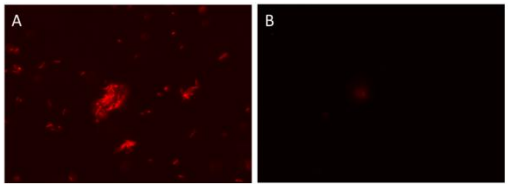

Figure 1. Bacillus subtilis (Gram-positive) (A) and Escherichia coli (Gram-negative) (B) was stained with MycoLight™ Rapid Fluorescence Gram-Positive Bacteria Staining Kit.