MycoLight™ Rapid Fluorescence Gram-Positive Bacteria Staining Kit

Catalog number: 22415
Unit size: 100 Tests

Component	Storage	Amount
Component A: IF647-ConA	Freeze (< -15 °C), Minimize light exposure	2 vials
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (25 mL)

OVERVIEW

AAT Bioquest's MycoLight™ Rapid Fluorescence Gram-Positive Bacteria Staining Kit provides a novel one-step fluorescence assay for the determination of gram sign in living bacteria. 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. AAT Bioquest's 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.

AT A GLANCE

Protocol Summary

1. Prepare bacteria samples
2. Prepare and add IF647-ConA stock solution
3. Incubate bacteria samples with IF647-ConA for 5-15 minutes at room temperature in the dark
4. Remove IF647-ConA staining solution and resuspend in Assay Buffer
5. Analyze sample by fluorescence microscope with Cy5 filter set

Important Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Fluorescence microscope

Excitation	650 nm
Emission	669 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Cy5 filter

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.

IF647-ConA stock solution (100X)

Add 50 µL of Assay Buffer (Component B) into one vial of IF647-ConA (Component A) and mix them well.

Note Store stock solution at -20 °C, avoid light and store in smaller aliquots to avoid repeated freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

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.

3. 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. 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.

EXAMPLE DATA ANALYSIS AND FIGURES

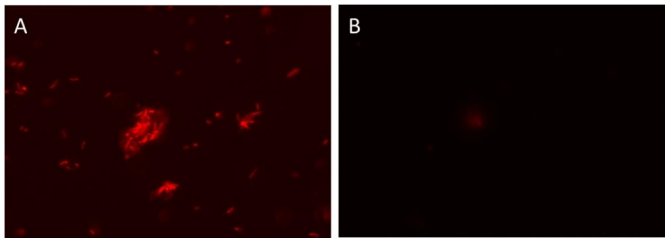

Figure 1. *Bacillus subtilis (Gram-positive)* (A) and *Escherichia coli (Gram-negative)* (B) was stained with MycoLight™ Rapid Fluorescence Gram-Positive Bacteria Staining Kit.

DISCLAIMER

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