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MycoLight™ Rapid Fluorescenc BacterialGram Stain Kit

Table 1 Contents and storage

Material	Amount	Storage	Stability
		• ≤ –20°C	
MycoLight™ Green	1 vial (100 µL)	• Desiccate	
MycoLight™ Red	1 vial (100 µL)	• Protect from light	
Spectral characteristic of the fluorescent probe:		**Ex~488/540 nm, Em~530/620 nm**	

Introduction

The MycoLight™ Rapid Fluorescence Bacterial Gram Stain Kit provides an easy and convenient way for determination of gram sign in live bacteria. Gram staining is a commonly used method in both clinical and research settings to taxonomically classify bacterial species into two large groups. Unfortunately, the traditional gram staining method is tedious and involves bacterial fixation which can be a significant drawback if the bacteria are to be characterized further. The MycoLight ™ Rapid Fluorescence Bacterial Gram Stain Kit provides a one-step gram staining assay for live bacteria that overcomes the problems inherent in the traditional gram staining assays. The MycoLight ™ Rapid Fluorescence Bacterial Gram Stain Kit utilizes two DNA dyes MycoLight ™ Green and MycoLight ™ Red with differential ability to stain gram positive and negative bacteria. MycoLight ™ Green stains both gram positive and negative bacteria while MycoLight ™ Red preferentially labels gram positive bacteria. The excitation/emission maxima for these two dyes are about 484/504 nm for MycoLight ™ Green and 518/600 nm for MycoLight ™ Red. Thus, when a mixture of gram positive and gram negative bacteria is stained with the dyes, gram positive bacteria will fluoresce red and gram negative bacteria will fluoresce green. The gram positive and negative staining can be monitored fluorimeterically with TRITC and FITC filter set respectively.

Experiment procedure

Preparation of Bacterial Samples

1. Prepare bacteria sample with concentration around 10^7 cells/ml. Grow bacteria into late log phase in appropriate medium.

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 10 minutes and re-suspend the pellet in ddH_2O, adjust bacteria concentration to ~ 10^7 cells/ml.

Staining Protocol

1. Add 2 µL MycoLight™ dye working solution to 100 µL of the bacterial suspension.
2. Mix well and incubate in dark for 15 min at room temperature.
3. Monitor fluorescence of bacteria with a fluorescent microscope through FITC (Ex/Em = 488/530 nm) channel for gram-negative bacteria and TRITC(Ex/Em=540/620nm)channel for gram-positive bacteria.

Note The protocol only provides a guideline, should be optimized with different bacterial strains or other specific needs.

Note Relative ratio of gram positive and gram negative bacteria in a population can also be estimated with fluorescence spectroscopy with this kit. A sample analysis is included with the figures.

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Fluorescence Data

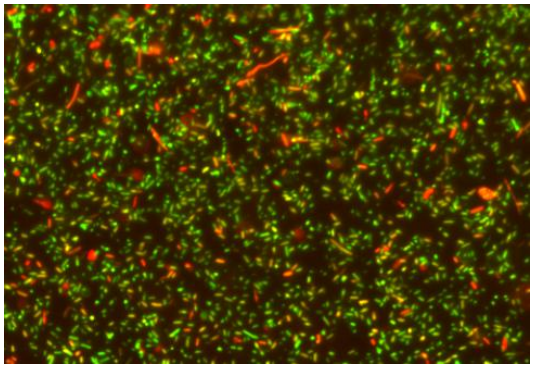

Figure 1. Mixture of Escherichia coli and Bacillus subtilis were stained with MycoLight™ Rapid Fluorescence Bacterial Gram Stain Kit. Red & Orange: Gram positive Bacillus subtilis cells; Green: gram-negative Escherichia coli cells.