MycoLight™ Rapid Fluorescence Bacterial Gram Stain Kit

Catalog number: 22413
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22413)
Component A: MycoLight™ Green	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)
Component B: MycoLight™ Red	Freeze (< -15 °C), Minimize light exposure	1 vial

OVERVIEW

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 650/669 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 Cy5 and FITC filter set respectively.

AT A GLANCE

Protocol Summary

1. Prepare bactrerial samples
2. Prepare and add MycoLight™ dye working solution to bacteria samples
3. Incubate with MycoLight™ dye working solution at room temperature in dark for 15 minutes
4. Analyze sample by fluorescence microscope or fluorescence spectroscopy with FITC and TRITC filter sets

Important Note

Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Fluorescence microscope

Emission	530/669 nm
Excitation	488/650 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	FITC/Cy5 filters

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

MycoLight™ Red stock solution

Add 100 µL of ddH2O into one vial of MycoLight™ Red (Component B) and mix them well.

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

PREPARATION OF WORKING SOLUTION

MycoLight™ dye working solution

Mix equal volume of MycoLight™ Green (Component A) and MycoLight™ Red stock solution in a tube and mix them well.

SAMPLE EXPERIMENTAL PROTOCOL

Preparation of Bacterial Samples

1. Prepare bacteria sample with concentration around 10^7cells/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. Remove the working solution by centrifugation at 10000 g for 10 minutes.
4. Resuspend the bacteria pallate in ddH2O.
5. Monitor fluorescence of bacteria with a fluorescent microscope through FITC (Ex/Em = 488/530 nm) channel for gram-negative bacteria and Cy5 (Ex/Em = 650/669 nm) 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.

EXAMPLE DATA ANALYSIS AND FIGURES

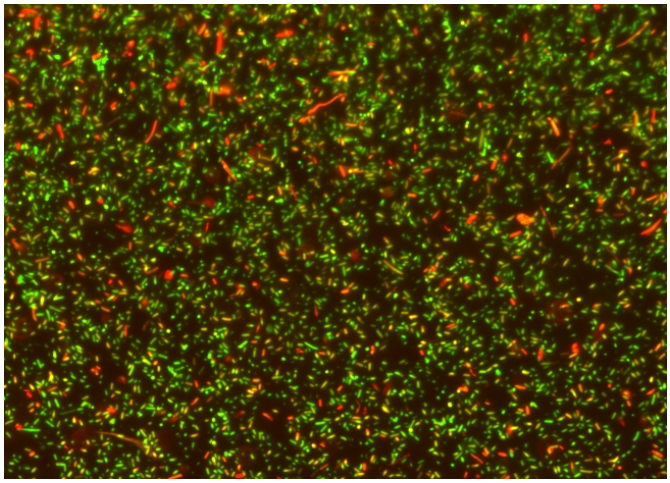

Figure 1. Mixture of *Escherichia coli* and *Bacillus subtilis* were stained with MycoLight™ Rapid Fluorescence Bacterial Gram Stain Kit. Red & Orange: Gram positive *Bacillus subtilis* cells; Green: gram-negative *Escherichia coli* cells.

DISCLAIMER

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