

MycoLight™ Red JJ94

 Catalog number: 24006
 Unit size: 100 uL

Component	Storage	Amount
MycoLight™ Red JJ94	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL) (2.5 mM in DMSO)

OVERVIEW

Green fluorescent protein (GFP) derivatives are widely used as fluorescent reporters to study biological processes at the cellular and molecular level, including labeling bacteria. Unfortunately, the GFP-based bacterial labeling is tedious, and several important organisms have remained difficult to modify for fluorescent protein expression. Complimentary to GFP-based fluorescence labeling of bacteria, SYTO-9 has been widely used for labeling bacteria in a more convenient mode, i.e., through a simple incubation. However, SYTO-9 is reported to significantly inhibit some bacterial growth. AAT Bioquest has developed MycoLight™ Red JJ94 that has minimal inhibition of bacterial growth compared to SYTO-9. In addition, MycoLight™ Red JJ94 can be used for multicolor detection of bacteria with the widely used GFP probes. It has an excitation and emission at ~637 and ~651 nm, respectively. This perfectly matches the He-laser excitation and Cy5 filter set that are commonly equipped in most fluorescence microscopes and flow cytometers. MycoLight™ Red JJ94 is a far-red fluorescent DNA-binding dye for straightforward live bacterial cell labeling, allowing subsequent infection studies. It has low intrinsic fluorescence, but upon binding DNA, its fluorescence emission intensity increases dramatically. MycoLight™ Red JJ94 readily stains live bacteria without compromising microbial growth. Under the same conditions, SYTO-9 inhibits bacterial growth substantially while MycoLight™ Red JJ94 is fully compatible with normal bacterial growth in liquid medium or on solid medium. The bacterial fluorescence labeling by MycoLight™ Red JJ94 is stable over several hours after shifting the bacteria into dye-free media.

AT A GLANCE

1. Stains live bacteria without affecting normal microbial growth
2. Uses Cy5 filter set and He-laser excitation, widely available in most flow cytometers and fluorescent microscope
3. Can be used for multicolor detection in bacteria
4. Stable labelling for several hours

Important Thaw the components at room temperature before starting the experiment.

KEY PARAMETERS

Flow cytometer

Excitation	640 nm laser
Emission	660/20 nm filter
Instrument specification(s)	APC channel

Fluorescence microscope

Excitation	630 nm
Emission	660 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Cy5 filterset

SAMPLE EXPERIMENTAL PROTOCOL

1. Dilute the MycoLight™ Red JJ94 with buffer of your choice. MycoLight™ Red JJ94 can be supplemented directly into bacterial culture medium to the final concentration of 2.5 µM.
2. Vortex samples to mix, and then incubate for at least 10 minutes.

3. Monitor sample fluorescent signal by fluorescent microscope or flow cytometer with Cy5 filter set.

Note The above protocol can be adapted for most bacterial strains. These conditions require adjustment for each strain and experimental system. Growth medium, cell density, the presence of other organisms and factors may influence staining. Residual detergent on glassware may also affect staining of many organisms, and cause brightly stained material to appear in solutions with or without cells present.

Note Use plastic tubes when diluting MycoLight™ Red JJ94, because the diluted stain adheres to glass. In general, the best results are obtained in buffers that do not contain phosphate.

Note Dye concentration may be optimized with different bacterial strain to obtain best results.

Note Since MycoLight™ Red JJ94 is fully compatible with bacterial growth, live bacteria can be incubated with MycoLight™ Red JJ94 for prolonged period of time.

EXAMPLE DATA ANALYSIS AND FIGURES

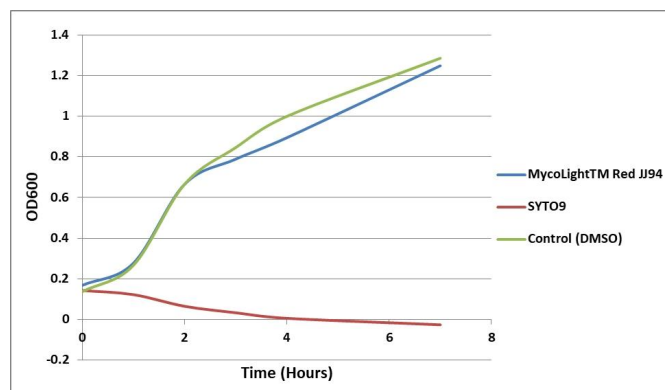


Figure 1. Optical density (OD600 nm) of *E. coli* LB cultures supplemented with 1% DMSO (control), MycoLight™ Red JJ94 or Syto-9™ over time. Syto-9™ inhibits bacterial growth substantially while MycoLight™ Red JJ94 is fully compatible with normal bacterial growth.

DISCLAIMER

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