MycoLight™ Flow Cytometric Live Bacteria Assay Kit

Catalog number: 22407
Unit size: 100 Tests

Component	Storage	Amount
Component A: MycoStain It™ 520	Freeze (<-15 °C), Minimize light exposure	2 vials
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)
Component C: DMSO	Freeze (<-15 °C)	1 vial (200 µL)

OVERVIEW

The MycoLight Flow Cytometric Live Bacteria Assay Kit provides an easy and convenient methodfor evaluating bacterial vitality as a function of the intracellular esterase activity. MycoLight™ 520 is non-fluorescent esterase substrate that diffuse into both Gram positive and Gram-negative bacteria. Upon hydrolysis by bacterial intracellular non-specific esterase, a green fluorescent product is produced and accumulated within bacteria. Compared to the commonly used esterase substrate CFDA and CFDA-AM, this kit provides brighter and more stable signal with lower background and easier staining protocol.

AT A GLANCE

Protocol summary

1. Prepare 100X dye stock solution.
2. Prepare bacteria samples.
3. Incubate bacteria samples with MycoLight™ 520 at 37°C for 5-10 minutes or room temperature for 60 minutes in dark.
4. Analyze sample by flow cytometry with FITC channel.

Important
Thaw one of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm filter
Instrument specification(s):	FITC channel

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.

1. MycoLight™ 520 stock solution (100X):
Add 100 µL of DMSO (Component C) into one vial of MycoLight™ 520 (Component A) to make 100X stock solution.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare bacteria sample with concentration in range of 10^6 to 10^8 cells/ml. Grow bacteria into late log phase in appropriate medium. Remove medium by centrifugation at 10,000 x g for 10 minutes and re-suspend the pellet in Assay Buffer (Component B).

   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. Treat cells with test compounds as desired. Remove treatments by centrifugation at 10,000 x g for 10 minutes and re-suspend the pellet in appropriate amount of Assay Buffer (Component B) so the concentration of bacteria in the treated sample is the same as the live.

   Note Determine the concentration of the bacterial culture before starting the treatment.

   Note Dead bacteria can serve as negative control, it is recommended to kill bacteria with 70% ethanol for 30 min followed by 60 min of boiling.

3. Example of Live/Dead bacterial mixtrue preparation: Mix seven different propotions of the bacterial suspensions as in Table 1 for a total volume of 200 µL for each sample.

4. Add 2 µL of the 100X MycoLight™ 520 stock solution to 200 µL of the bacterial sample in Assay Buffer.

5. Mix well and incubate in dark for 5-10 min at 37°C or 60 min at RT for optimum staining results.

6. Add 300 µL of Assay Buffer (Component C) to increase volume before analyzing the cells with a flow cytometer.

7. Monitor fluorescence of bacteria with a flow cytometer through FITC channel (488/530 nm).

   Note To exclude debris, it is recommended to set the threshold of the flow cytometer as the following: FSC >10,000, SSC>5,000.

   Note The efficiency of MycoLight™ 520 is highly strain dependent and the staining conditions should be optimized accordingly.

Table 1. Example Volumes of Live and Dead samples to mix to achieve various Live/Dead ratios.

Ratio of Dead:Live Cells	µL of Dead Sample	µL of Live Sample
0:100	0	200
10:90	20	180
30:70	60	140
50:50	100	100
90:10	180	20
100:0	200	0

EXAMPLE DATA ANALYSIS AND FIGURES

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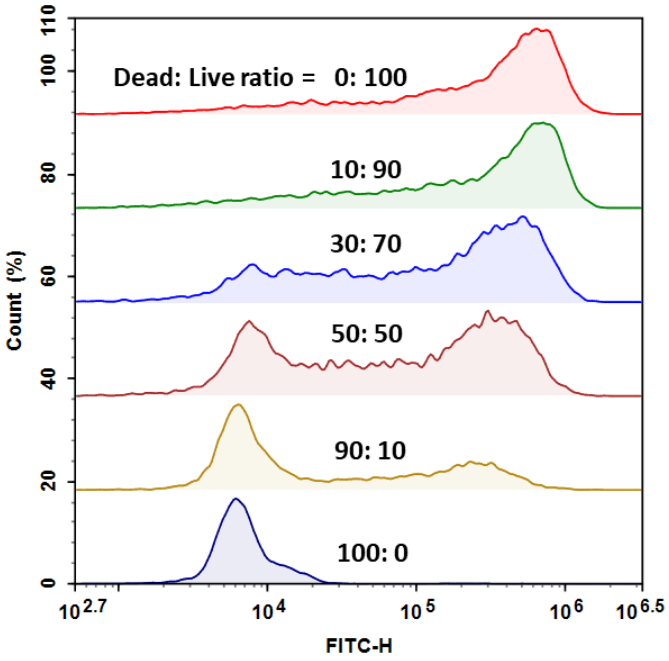

Figure 1. Relative viability of *E.coli* suspension was analyzed using ACEA NovoCyte flow cytometer in FITC channel. The readings (Count(%)) were obtained from various Live/Dead *E.coli* mixtures (Table 1). The live and dead population in each mixture can be easily distinguished by the two distinct peaks.