MycoLight™ Fluorimetric CTC Live Bacteria Quantification Kit

Catalog number: 22405
Unit size: 100 Tests

Component	Storage	Amount
Component A: CTC	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	100 mL

OVERVIEW

The CTC Flow Cytometric Live Bacteria Assay Kit provides an easy and convenient method for evaluating bacterial health and vitality as a function of the respiratory activity. CTC itself is non-fluorescent, once reduced by the electron transfer system of viable bacterial cell surface, red and insoluble fluorescent CTC formazan is formed and can be detected with a flow cytometer. Dead bacteria that are not respiring or unhealthy bacteria that respire at a lower rate will produce none or less red fluorescent with CTC staining, thus providing a semi-quantitative measure of health status of bacteria population.

AT A GLANCE

Protocol summary

1. Prepare 10X dye stock solution
2. Prepare bacteria samples
3. Incubate bacteria samples with CTC at 37°C for 30 minutes
4. Analyze sample by flow cytometry with PE-Texas Red 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:	610/20 nm filter
Instrument specification(s):	PE-Texas Red 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. CTC stock solution (10X):
Add 1 mL of distilled water into the vial of CTC (Component A) to make 10X stock solution.

Note Stock solution is stable at -20°C for 2 weeks. Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare bacteria sample with concentration of 10^6 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 appropriate amount of PBS.

   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. Add 10 µL of the 10X CTC stock solution to 90 µL of the bacterial sample.

4. Mix well and incubate in the dark for 30 min at 37°C.

5. Add 400 µL of Assay Buffer (Component B) to increase volume before analyzing the cells with a flow cytometer.

6. Monitor fluorescence of bacteria with a flow cytometer through PE-Taxes Red channel (Ex/Em = 488/615 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 CTC is highly strain dependent and the staining conditions would be optimized accordingly.

EXAMPLE DATA ANALYSIS AND FIGURES

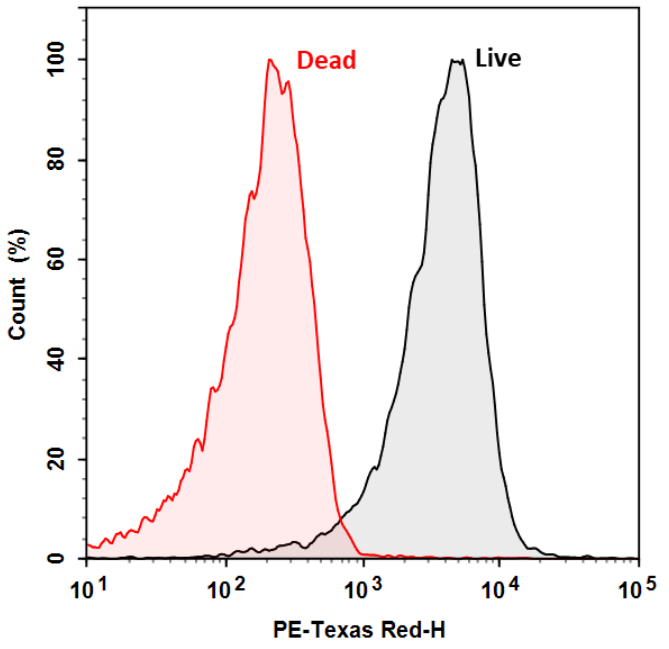

Figure 1. Live and dead (ethanol treated and boiled) *E.coli* were stained with 1X of CTC for 30 mins at 37°C. Samples were analyzed by flow cytometer with a 488 nm excitation and 615/24 nm bandpass filter. Live and dead bacteria population showed very distinct peaks.

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