ab189818

Bacterial viability assay kit

Instructions for Use

For the measurement of viability of a bacterial culture

This product is for research use only and is not intended for diagnostic use.

Version 1b Last Updated 15 January 2025

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1. Introduction

The ability to measure the viability of a bacterial culture has importance in many aspects of microbiology. Whether dealing with uncharacterized species or pathogenic strains, the ability to measure the effects of different media or the effect of drug treatment on bacterial survival is highly advantageous in advancing knowledge of these organisms.

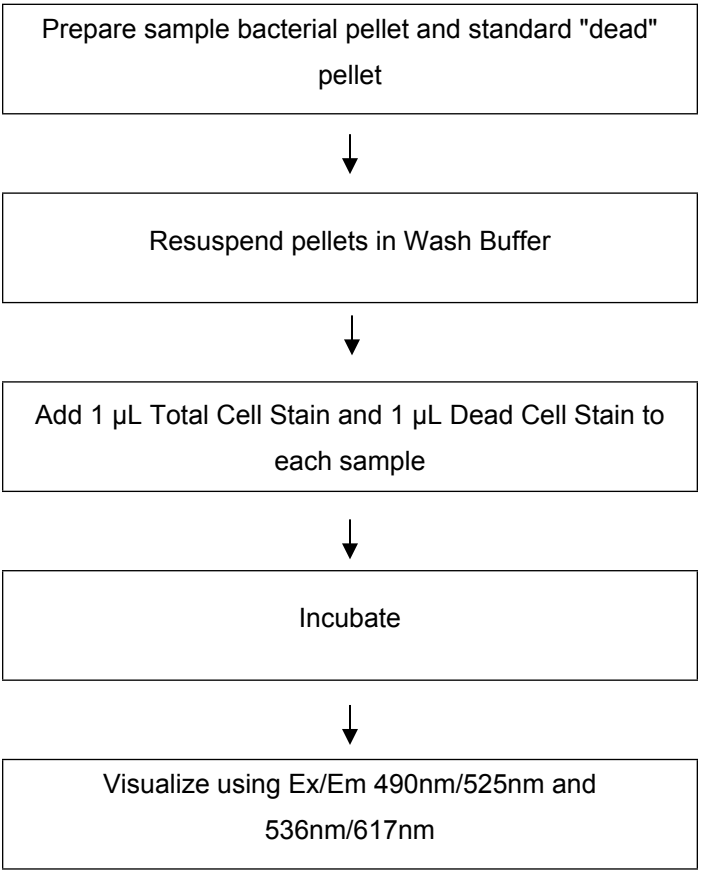
Abcam's Bacterial viability assay kit - (ab189818) utilizes two highly-specific, ultrasensitive fluorescent reagents to quickly and easily assess the percentage of live and dead cells within a bacterial culture. The total cell stain is permeant to all cells and thus all bacteria within the culture will be stained allowing the total number to be calculated. The dead cell stain is inpermeant to living cells and as such will only be able to enter and stain dead cells; this allows the number of dead cells to be calculated. The ratio of dead to live cells can then be quickly and easily calculated.

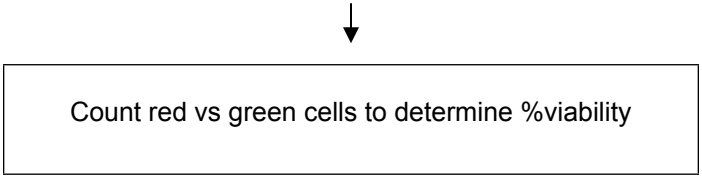
Although only validated with *E. coli*, results should be obtainable with a wide range of both gram positive and gram negative bacterial species including *Agrobacterium tumefaciens*, *Pseudomonas sp* and *Bacillus sp*.

This assay kit contains all of the reagents for testing up to 100 samples including dead cell controls. In addition, the kit also contains a detailed protocol.

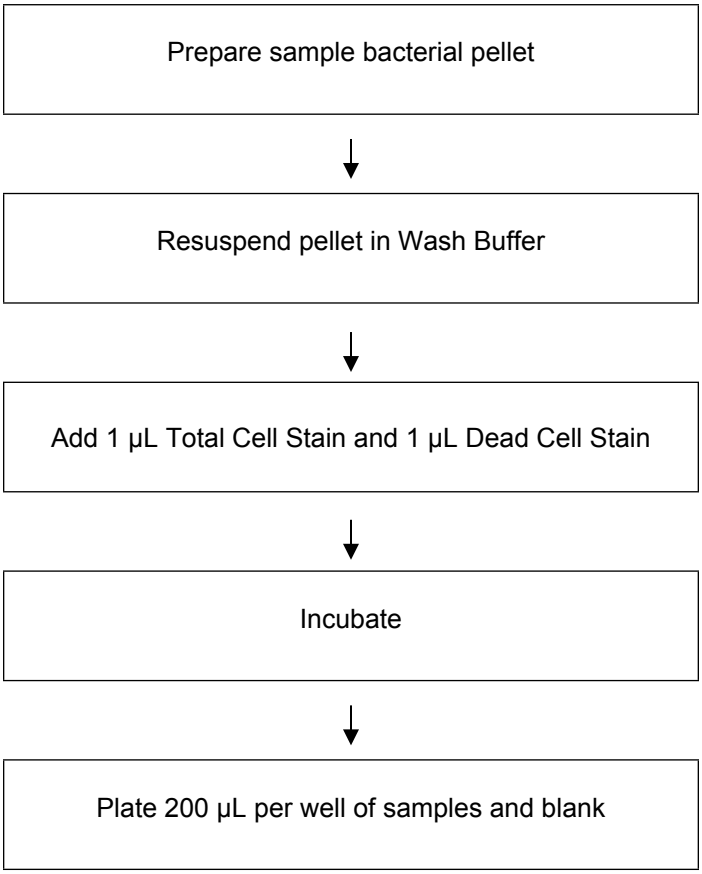
2. Protocol Summary

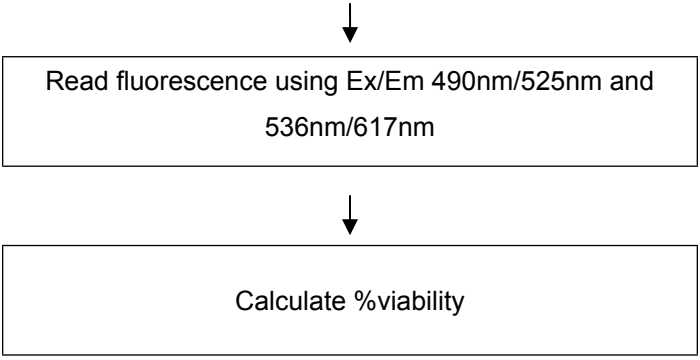
A. Microscopy Format





B. Microtiterplate Format





3. Kit Contents

Components	Amount	Storage
Wash Buffer*	2 x 30 mL	Store cold at 4°C
Total Cell Stain	0.2 mL	Store at or below -20°C Light sensitive
Dead Cell Stain	0.2 mL	Store at or below -20°C Light sensitive

*Note: Wash buffer is supplied as 40X concentrate and requires dilution to 1X prior to use.

4. Storage and Handling

Fluorescent reagents, should be handled with care, kept cold when not in use, and stored at -20ºC. In case of contact with skin or eyes, wash thoroughly with soap and cold water. Reagents should be stable for at least 6 months following purchase. High background fluorescence will indicate decomposition.

5. Additional Materials Required

- 96-well microplates
- Fluorescence microplate reader
- Fluorescence microscope
- Isopropanol

6. Assay Protocol

A. Sample Preparation

- Grow bacteria in 5 mL of appropriate growth media overnight.
- Harvest cells by centrifugation at 10000 x *g* for 10 minutes.
- Resuspend in 2 mL of 1X Wash Buffer.
- Add 1 mL of bacterial suspension to 5 mL of 1X Wash Buffer (live sample).
- If required, suspend the remaining 1 mL in 5 mL of 70% isopropanol (to produce a standard “dead” sample).
- Incubate both samples at RT for 1 hour mixing occasionally by inversion.
- Centrifuge at 10000 x *g* for 10 minutes.
- Resuspend both pellets in 5 mL of 1X Wash Buffer each.
- Centrifuge at 10000 x *g* for 10 minutes.
- Resuspend pellets in 1 mL of 1X Wash Buffer.
- If desired, measure OD_{670} to determine bacterial concentration and adjust concentration in accordance with your instrumentation. Recommendation is to use approx. 2×10^8 bacteria/mL (~0.06 OD_{670}).

B. Protocol for Microscopy

- To each 1 mL sample tube prepared in section A. above, add 1 µL Total Cell Stain and 1 µL Dead Cell stain.
- Incubate at room temperature in the dark for 15 minutes.
- Wet mount a small volume of each sample on a microscope slide or appropriate well of a culture dish.
- Visualize using an epi-fluorescence microscope fitted with appropriate filters for Ex 490nm/Em 525nm and Ex 536nm/Em 617nm (FITC/TAMRA filter sets, respectively).
- The percentage of dead cells can be calculated by dividing the number of red (dead) cells by the number of green (total) cells and multiplying by 100. Viability is calculated according to the formula:

Percentage Viability = 100 – ([# red cells / # green cells] x 100)

C. Protocol for Measurement in a Plate Reader

- To each sample tube (1 mL) add 1 µL Total Cell Stain and 1 µL Dead Cell stain. Prepare a blank solution containing 1 mL 1X Wash Buffer and 1 µL each staining solution.
- Incubate at room temperature in the dark for 1 hour.
- Pipette 200 µL of each sample and the blank solution into individual wells of a 96-well plate in triplicate.
- Measure fluorescence at Ex 490nm/EM 525nm (Reading 1) and Ex 536nm/EM 617nm (Reading 2) for all wells.

  NOTE: Adjust the gain of one reading to give a ratio of Reading 2/Reading 1 of approximately = 1 after blank correction, in the dead sample.
- The percentage of dead cells in the live sample can be calculated by dividing Reading 2 by Reading 1 and multiplying by 100. Representative data is shown in Figure 1 below.

7. Data Analysis

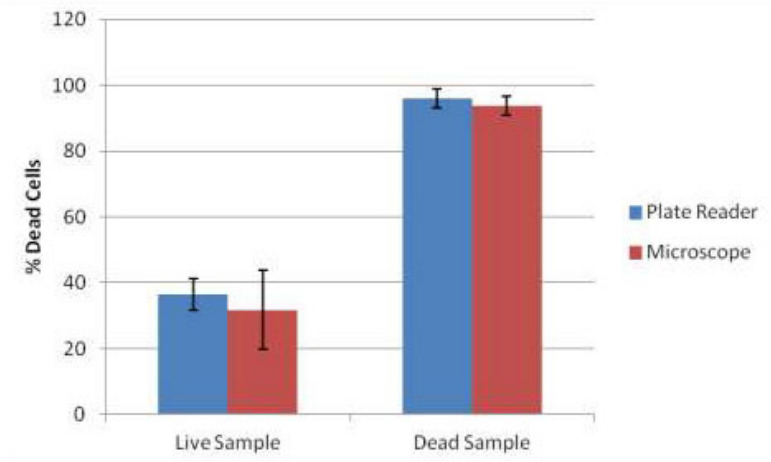


Figure 1. E. coli JM109 were stained as described using the protocols, and fluorescence was measured using a plate reader and a microscope. Number of stained cells were quantified.

Technical Support

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