Cat. No. 303

**BacSee®**

# Total/Dead dual-staining kit for bacterial viability kit

## Product Information

## Quick Facts

1. **Storage conditions before opening:<-20 ℃/ Protect from light.**
2. **Ex/Em (nm)：Total bacteria (470/520 )、Dead bacteria (530/640 ).**
3. **Recommended dye concentration for staining working solution：**
   **12 μM GA & 20 μM RD (Dilution ratio：1000X).**

## Introduction

This product provides two fluorescent reagents with different colors to quickly assess the percentage of live and dead bacterial cells. The **TOTAL** bacterial stain reagent is permeant to all cells and thus all bacteria cell will be stained allowing the total number to be calculated; The **DEAD** bacterial stain reagent is impermeant to living cells, thus just dead bacteria cell can be stained. With both of two fluorescent reagents, the ratio of dead (and/or live) to total bacterial cell can be calculated. This kit is suitable for use with fluorescent microscopy, fluorescent microplate reader, flow cytometers and other fluorescent devices. This product can be used in both of Gram Positive and Negative bacterial cells. We have tested this product on the following bacterial species：Staphylococcus aureus, Streptococcus oralis, Escherichia coli, Klebsiella pneumoniae respectively. All of these bacterial types shown favorable results.

## Shipping and Storage Information

| Product Name | Shipping | Storage |
|---|---|---|
| BacSee (Cat. No.：303) | under-20 °C with protection from light. | 12 months under-20 °C with protection from light. |

## Handling of Reagents

1. Allow the reagents to warm to room temperature (about 30 mins) and centrifuge briefly before opening. Before refreezing again, seal all stock solutions tightly.
2. GA and RD fluorescent reagents are sensitive to light. Prepare aqueous working solution immediately prior to use, and use it right away.

## Kit Contents (200 tests)

1. BacSee GA stock solution for **TOTAL** bacteria cell (Green label, brown vials)：
   Two vials, 12 mM, 100 μL each.
2. BacSee RD stock solution for **DEAD** bacteria cell (Red label, brown vials)：
   Two vials, 20 mM, 100 μL each.

## Protocol

### 1. Preparation of Bacterial Suspensions：

**1.1** Grow bacterial cells overnight in 5 mL appropriate media.

**1.2** Concentrate the bacterial culture by centrifugation at 10,000 g for 10–15 minutes.

**1.3** Remove the supernatant and resuspend the pellet in 2 mL of 0.85% NaCl.

**1.4** Add 1 mL of this suspension to each of two 10 mL centrifuge tubes containing either 5 mL of 0.85% NaCl (for **LIVE** bacteria) or 5 mL of 70% isopropyl alcohol (for

**DEAD** be killed bacteria).

**1.5** Incubate both samples at room temperature for 1 hour, mixing every 15 minutes.

**1.6** Pellet both samples by centrifugation at 10,000 g for 10–15 minutes.

**1.7** Resuspend both pellets in 5 mL of 0.85% NaCl.

**1.8** Pellet both samples by centrifugation at 10,000 g for 10–15 minutes.

**1.9** Resuspend both pellets in 1 mL of 0.85% NaCl.

**1.10** If necessary, determine the optical density at 670 nm (OD670) in glass or acrylic absorption cuvettes.

**Note :** Although a single wash step is usually sufficient to remove significant traces of interfering media components from the bacterial suspension, two times of washing steps were recommended. Phosphate wash buffers are not recommended because they can decrease staining efficient.

**Note :** If necessary, before the bacterial staining step, prepare a working solution by diluting with 0.85% NaCl and/or mixing the two provided fluorescent reagents.

## 2. Fluorescent microscopy :

### 2.1 Select the Optical Filters :

The stained **TOTAL** and **DEAD** bacteria can be viewed simultaneously or separately. Typical characteristics of some appropriate filters are summarized in the following table :

| | **Omega Filters** | **Chroma Filters** |
|---|---|---|
| Simultaneous viewing of **TOTAL** and **DEAD** cells | XF25, XF26, XF115 | 11001, 41012, 71010 |
| Viewing **TOTAL** cell only | XF22, XF23 | 31001, 41001 |
| Viewing **DEAD** cell only | XF32, XF43, XF102, XF108 | 31002, 31004, 41002, 41004 |

### 2.2 Staining the bacteria :

a. To each 1 mL sample tube prepared in step **1.9**, add 1 µL **GA** Stain into **LIVE** tube and 1 µL **RD** stain into **DEAD** tube.
b. Incubate at room temperature in the dark for 15-60 minutes.
c. Wet mount a small volume of each sample on a microscope slide or appropriate well of a culture dish.
d. Observe in a fluorescent microscope equipped with the filter suggested in the table (step **2.1**).
e. 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 the following equation:

$$\text{Percentage of Viability } (\%) = 100 - \frac{\text{No. of red cells}}{\text{No. of green cells}} X\ 100\%$$

## 3. fluorescent microplate reader :

### 3.1 Select the Optical Filters for the Microplate Reader :

a. **GA** fluorescent reagent for **TOTAL** cell :
Exciting at 470 ± 10 nm ; emission at 520 ± 10 nm.
b. **RD** fluorescent reagent for **DEAD** cell :

Exciting at 530 ± 10 nm；emission at 640 ± 20 nm.

**3.2 Staining the bacteria：**

a. Adjust the concentrations of two bacterial suspensions in step **1.9**. The concentration should over 2 X $10^7$(~0.30 $OD_{670}$).
b. Mix 5 different proportions of two suspensions (**LIVE** & **DEAD**), for example：1.0/0.0; 0.8/0.2; 0.5/0.5; 0.2/0.8; 0.0/1.0 for 5 standard solutions of different percentages of bacteria viability as 100%, 80%, 50%, 20%, 0%, respectively .
c. To each sample tube (1 mL) add 1 µL Total **GA** stain and 1 µL Dead **RD** stain.
d. Prepare a blank solution containing 1 mL of 0.85% NaCl, 1 µL Total **GA** stain and 1 µL Dead **RD** stain (without bacteria).
e. Incubate at room temperature in the dark for 15-60 minutes.
f. Pipette 200 µL of each sample and the blank solutions into individual wells of a 96-well plate in triplicate. The outside wells (rows A and H and columns 1 and 12) are usually kept empty to avoid spurious readings.
g. Measure the fluorescence intensity at Ex 470nm/EM 520nm (Reading 1) and Ex 530 nm/EM 640 nm (Reading 2) for all wells.

**3.3 Calculation：**

a. After blank correction, analyze the data by dividing the Reading 1 with Reading 2 as following equation：

$$\text{Ratio (G/R)} = \frac{\text{Fluorescent intensity of Total cell (Reading 1)}}{\text{Fluorescent intensity of Dead cell (Reading 2)}}$$

b. Plot the Ratio (G/R) (Y axis) versus percentages of live bacteria (X axis, step **3.2c**).
c. The percentage of live bacteria in the unknow sample can be calculated.

## 4. Troubleshooting

**4.1** Dye deterioration：

Total **GA** stain (brown vial, green label) and DEAD **RD** (brown vials, Red label) will not deteriorate under normal storage conditions. If it is deteriorated, it will produce strong fluorescence, resulting in a decrease in sensitivity. Please wash the bacterial pellet with 0.85% NaCl after staining the bacteria. If this problem is still existed, please contact us.

**4.2** If you have other questions, please contact our technical staff.

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