Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

# Live/Dead Bacterial Viability Kit (SYTO 9/PI)

Cat #: KTA1003 Size: 200 T/1000 T

| | | | |
|---|---|---|---|
| | **Live/Dead Bacterial Viability Kit (SYTO 9/PI)** | | |
| REF | **Cat #:** KTA1003 | LOT | **Lot #:** Refer to product label |
| | **Applicable samples:** Bacteria | | |
| | **Instrument:**Fluorescence microscope / Flow cytometer / Fluorescence microplate reader | | |
| | **Storage:** Stored at -20℃ for 12 months, protected from light | | |

## Assay Principle

The viable bacteria viability kit contains SYTO 9 (Ex/Em = 470/540 nm) a green fluorescent nucleic acid dye and PI (Ex/Em = 470/617 nm) a red fluorescent nucleic acid dye. When used alone, SYTO 9 can label all bacteria including those with intact membranes and those with damaged membranes, while PI can only label bacteria with damaged membranes; when SYTO 9 and PI are used together, live bacteria with intact cell membranes show green fluorescence, while dead bacteria with damaged cell membranes show red fluorescence.

## Materials Supplied and Storage Conditions

| Kit components | Size | | Storage conditions |
|---|---|---|---|
| | 200 T | 1000 T | |
| SYTO 9 | 20 μL | 100 μL | -20℃, protected from light |
| PI | 20 μL | 100 μL | -20℃, protected from light |

**Note: For the 200 T and 1000 T of this kit: 1000 and 5000 assays respectively for fluorescence microscope; 60 and 300 assays respectively for flow cytometer; 200 and 1000 assays respectively for fluorescence microplate reader.**

## Materials Required but Not Supplied

· Fluorescence microscope, Flow cytometer, Fluorescence microplate reader, Spectrophotometer
· Centrifuge, incubator, precision pipettes, disposable pipette tips, slides, cover slips, flow tubes, 96-well all-black microplates
· Normal saline (0.85% NaCl), anhydrous ethanol or isopropanol

## Reagent Preparation

**SYTO 9:** Ready to use as supplied. Allow it to reach room temperature before use. Stored at -20℃ and protected from light.
**PI:** Ready to use as supplied. Allow it to reach room temperature before use. Stored at -20℃ and protected from light.

## Assay Protocol

**A. Fluorescence microscope**

1. Culture bacteria in an appropriate liquid medium to log phase.

2. Transfer an appropriate volume of bacterial suspension, centrifuge at 10000 g for 5 min, discard the supernatant, and wash the pellet once with normal saline. Measure the absorbance of the bacterial suspension at 670 nm (1 cm path length) using a spectrophotometer.

3. Adjust the bacterial concentration to about $2\times10^8$ bacteria/mL with normal saline. (*E. coli* reaches ~$4\times10^8$ bacteria/mL at $OD_{670}$ = 0.3; *Staphylococcus epidermidis* reaches ~$2\times10^8$ bacteria/mL at $OD_{670}$ = 0.4.)

4. Preparation of Staining Working Solution: Add 1 μL SYTO 9 and 1 μL PI into 500 μL normal saline and mix well. Prepare fresh immediately before use. Each sample requires 10 μL Staining Working Solution for fluorescence microscopy; adjust the actual preparation volume according to the number of samples.

5. Add 10 μL bacterial suspension to 10 μL Staining Working solution, mix well, incubate at 37 ℃ for 15 min in the dark.

6. Pipette 10 μL of the stained sample onto a glass slide and cover with a coverslip (allow 10 min standing for bacterial sedimentation if needed). Examine staining results under a fluorescence microscope. SYTO 9: Ex/Em = 470/540 nm; PI: Ex/Em = 470/617 nm.

**Note: For fluorescence microscopy detection, green fluorescence is more susceptible to quenching than red fluorescence. Adjust the field-of-view under bright-field prior to capturing fluorescence images. Upon merging green and red fluorescence images, green signal indicates live bacteria and red signal indicates dead bacteria; the two signals are independent with no spectral overlap.**

**B. Flow cytometer**

1. Culture bacteria in an appropriate liquid medium to log phase.

2. Take appropriate bacterial suspension, centrifuge at 10000 g for 5 min, discard the supernatant, and wash the pellet once with normal saline. Measure the absorbance of the bacterial suspension at 670 nm (1 cm path length) using a spectrophotometer.

3. Adjust the bacterial concentration to about $2\times10^8$ bacteria/mL with normal saline. (*E. coli* reaches ~$4\times10^8$ bacteria/mL at $OD_{670}$ = 0.3; *Staphylococcus epidermidis* reaches ~$2\times10^8$ bacteria/mL at $OD_{670}$ = 0.4.) Dilute the suspension 200 times with normal saline to make the bacterial solution about $1\times10^6$ bacteria/mL.

4. Preparation of Dual-Staining Working Solution: Add 1 μL SYTO 9 and 1 μL PI into 28 μL normal saline, and mix well. Prepare freshly before use. 10 μL of Dual-Staining Working Solution is required for each sample in flow cytometric detection; the actual preparation volume can be adjusted according to the number of samples.

5. Preparation of Single-Staining Working Solution (optional): Add 1 μL SYTO 9 into 29 μL normal saline and mix well to obtain SYTO 9 Single-Staining Working Solution. Add 1 μL PI into 29 μL normal saline and mix well to obtain PI single-staining working solution. 10 μL of Single-Staining Working Solution is required per sample for flow cytometry; the actual preparation volume can be adjusted based on sample quantity. (Generally, single-stained tubes and compensation adjustment are not required for live/dead bacterial staining.)

6. Add 10 μL of Dual- or Single-Staining Working Solution to 1 mL bacterial suspension, mix thoroughly, and incubate at 37℃ for 15 min in the dark.

7. Perform detection by flow cytometry. FITC (FL1) and PerCP (FL3) channels are recommended.

**Note: For flow cytometry detection, the threshold values vary greatly among different bacterial species. It is recommended to optimize parameters using unstained bacterial suspension prior to the formal experiment. Gating strategies can be set according to your experimental requirements**

**C. Fluorescence microplate reader**

1. Culture bacteria in an appropriate liquid medium to log phase.

2. Take two equal aliquots of bacterial suspension, centrifuge at 10000 g for 5 min, discard the supernatant, and wash once with normal saline.

3. Resuspend one pellet in 1 mL normal saline to prepare the live bacterial sample. Resuspend the other pellet in 0.3 mL normal saline, add 0.7 mL absolute ethanol or isopropanol, and mix well to prepare the dead bacterial sample.

4. Incubate both samples at room temperature for 1 h. Mix by inversion every 15 min (a mixer or shaker may also be used).

5. Centrifuge at 10000 g for 5 min, discard the supernatant, and wash once with normal saline. Measure the absorbance of the bacterial suspension at 670 nm (1 cm path length) using a spectrophotometer.

6. Adjust the concentrations of live and dead bacterial suspensions separately with normal saline to approximately $2\times10^8$ bacteria/mL. (*E. coli* reaches ~$4\times10^8$ bacteria/mL at $OD_{670}$ = 0.3; *Staphylococcus epidermidis* reaches ~$2\times10^8$ bacteria/mL at $OD_{670}$ = 0.4.)

7. Preparation of live/dead bacterial standards:

| No. | Live bacterial percentage (%) | Live bacterial sample (μL) | Dead bacterial sample (μL) |
|---|---|---|---|
| **1** | 0 | 0 | 100 |
| **2** | 10 | 10 | 90 |
| **3** | 20 | 20 | 80 |
| **4** | 30 | 30 | 70 |
| **5** | 40 | 40 | 60 |
| **6** | 50 | 50 | 50 |
| **7** | 60 | 60 | 40 |
| **8** | 70 | 70 | 30 |
| **9** | 80 | 80 | 20 |
| **10** | 90 | 90 | 10 |
| **11** | 100 | 100 | 0 |

8. Preparation of Staining Working Solution: Add 2 μL SYTO 9 and 2 μL PI into 1 mL normal saline and mix thoroughly. Prepare freshly before use. The volume of staining working solution required for each sample in fluorescence microplate reader detection is 50 μL; the actual preparation volume can be adjusted according to the number of samples.

9. Pipette 50 μL bacterial suspension (standards and test samples) into wells of a microplate, add 50 μL staining working solution, mix well, and incubate at 37℃ for 15 min in the dark.

10. Detect with a fluorescence microplate reader. SYTO 9: Ex/Em = 470/540 nm; PI: Ex/Em = 470/617 nm.

11. Plot the bacteria live/dead standard curve using X = Live bacterial Percentage (%), Y = $\frac{100\times\text{SYTO 9/PI}}{1+\text{SYTO 9/PI}}$. The percentage of live bacteria in unknown test samples can be calculated using this standard curve.

**Note: For fluorescence microplate reader detection, since SYTO 9 in this kit stains only live bacteria and PI stains only dead bacteria, the relationship between the percentage of live bacteria and dye-derived signals is as follows:**

$$\frac{\textbf{Live bacterial percentage}\ (\%)}{\textbf{100}\ -\ \textbf{Live bacterial percentage}\ (\%)} \propto \frac{\textbf{SYTO 9}}{\textbf{PI}}$$

**This formula can be further arranged as:**

$$\textbf{Live bacterial percentage}\ (\%) \propto \frac{\textbf{100}\ \times\ \textbf{SYTO 9/PI}}{\textbf{1}\ +\ \textbf{SYTO 9/PI}}$$

## Data Analysis

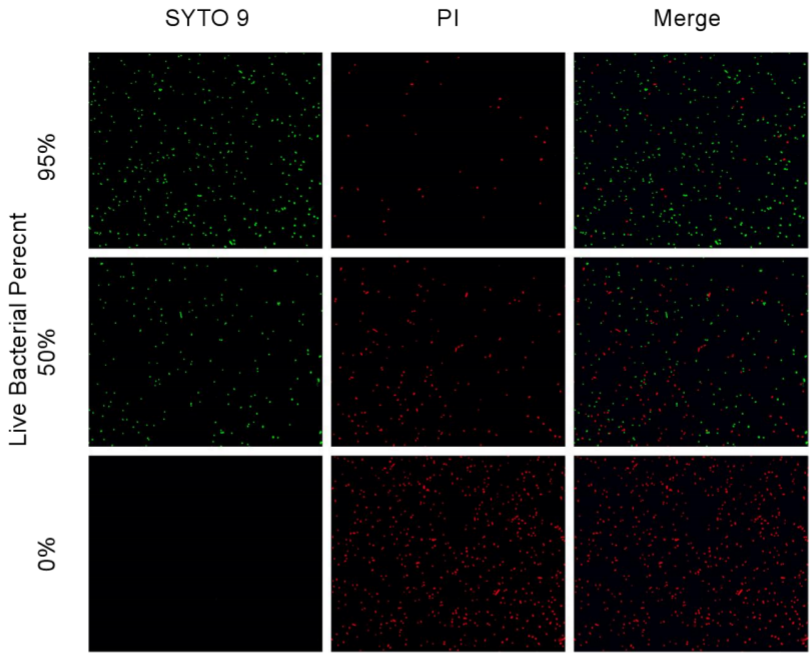


Figure 1. Staining effect diagram of Escherichia coli for detecting the proportion of different live bacteria by fluorescence microscopy.

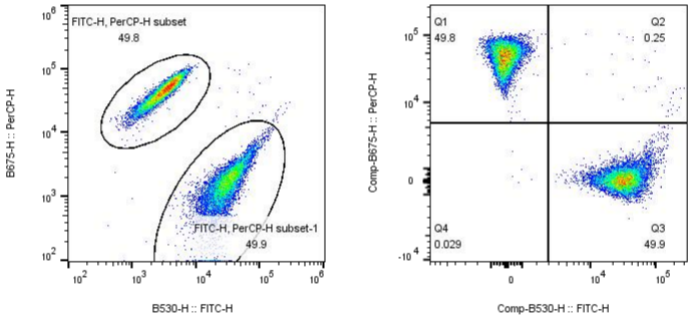


Figure 2. Staining effect of Escherichia coli with a live-to-dead ratio of 1:1 detected by flow cytometry, before compensation adjustment (left) and after compensation adjustment (right).

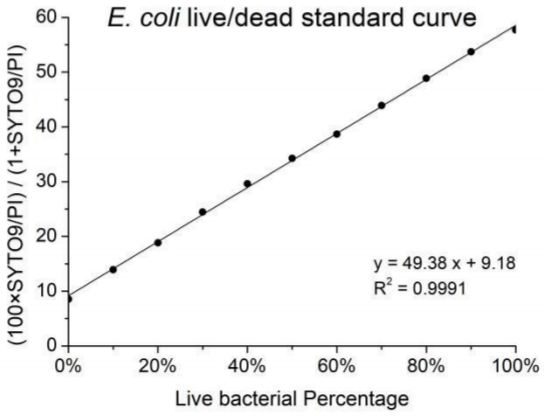


Figure 3. Standard curve for live/dead Escherichia coli detected by fluorescence microplate reader

## FAQ

1.

Q: Can different types of bacteria be detected using the protocols provided in the instructions?

A: Yes. The protocol supplied with this kit have been optimized for most bacterial species. You may also empirically adjust parameters such as dye concentration, sample concentration, and incubation time to better fit your experimental requirements.

2.

Q: Why do the bacterial live/dead assay results not match bacterial proliferation rates?

A: Assay results from this kit generally show good correlation with the proliferative capacity of bacteria in culture media. Under certain conditions, however, some bacteria with damaged cell membranes may recover and proliferate, yet are scored as dead in this assay. Conversely, some bacteria with intact cell membranes may fail to proliferate in culture media, but are scored as live in this assay.

3.

Q: How to measure $OD_{670}$ using a microplate reader?

A: Enable the “path-length correction” function on the microplate reader. If this function is unavailable, follow the steps below: Pipette ultrapure water into microplate wells and measure the difference between $OD_{977}$ and $OD_{900}$. Divide this difference by 0.18 to obtain the path-length in centimeters. Next, measure the $OD_{670}$ of bacterial suspension of the same volume, and convert the value to that corresponding to a 1 cm path-length.

Example: Add 200 µL ultrapure water into a microplate well. Measured $OD_{977}$ = 0.17, $OD_{900}$ = 0.055. The path-length = (0.17 - 0.055) ÷ 0.18 = 0.64 cm. Add 200 µL bacterial suspension into a well; measured $OD_{670}$ = 0.2. The $OD_{670}$ at 1 cm path length = 0.2 ÷ 0.64 × 1 = 0.31.

## Recommended Products

| Catalog No. | Product Name |
|---|---|
| KTA1001 | Live and Dead Cell Double Staining Kit |
| KTA1002 | Live Cell Tracking Kit (Green Fluorescence) |

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.