Live-Dead Bacterial Staining Kit (DMAO/PI)

Introduction

This kit adopts a dual-fluorescence staining system of DMAO and PI for bacterial viability detection of both Gram-positive and Gram-negative bacteria. DMAO is a membrane-permeable nucleic acid dye that crosses intact bacterial cell membranes and binds double-stranded DNA; it labels both live and dead bacteria and produces green fluorescence. PI is a membrane-impermeable nucleic-acid dye that only enters dead bacteria with compromised membrane integrity and generates red fluorescence upon binding to DNA. Under dual-staining conditions, live bacteria exhibit green fluorescence only, whereas dead bacteria display both green and red fluorescence. Assays can be performed using fluorescence microscopes, fluorescence microplate readers or flow cytometers.

Components and Storage

Components \ Size	200 Assays	1000 Assays	Storage
Assay Buffer	1 mL	5 mL	-20°C
DMAO (1000X)	20 μL	100 μL	-20°C away from light
PI (1000X)	20 μL	100 μL	-20°C away from light
Shipping: Blue ice	**Shelf life: 1 year**		

Protocol

1. Preparation of 100X Dual Staining Working Solution: Dilute an appropriate amount of DMAO (1000X) and PI (1000X) with Assay Buffer to prepare a 100X Dual Staining Working Solution. For instance, mix 10 μL DMAO (1000X), 10 μL PI (1000X) and 80 μL Assay Buffer for 100 μL of 100X Dual Staining Working Solution.

***Note:**

a) Prepare the 100X Dual Staining Working Solution fresh and protect it from light.

b) Final concentrations of DMAO and PI in the working solution may be optimized for individual experiments.

c) Upon first use, aliquot DMAO (1000X) and PI (1000X) to avoid repeated freeze-thaw cycles.

2. Detection via Fluorescence Microscope

1) Grow bacteria in suitable liquid medium to logarithmic-growth phase.

2) Collect appropriate bacterial suspension and centrifuge at 10000 × *g* for 5 min at room temperature.

3) Discard supernatant, wash bacteria once with normal saline. Resuspend bacteria in normal saline and adjust cell density to 10^8 bacteria/mL (OD_{670}≈0.3).

4) Mix 100 µL bacterial suspension with 1 µL of 100X Dual Staining Working Solution. Incubate at 37°C in the dark for 15 min.

***Note:** Optimal incubation time varies among bacterial strains; use 15 min as a starting point and adjust according to actual staining performance.

5) After incubation, transfer 10 µL stained bacterial suspension onto a clean glass slide, mount with a coverslip and observe immediately.

DMAO: green fluorescence, Ex/Em = 503/530 nm

PI: red fluorescence, Ex/Em = 535/617 nm

***Note:** Bacteria are small and fluorescence is prone to photobleaching. Perform pilot experiments to determine optimal detection parameters.

3. Detection via Flow Cytometer

1) Grow bacteria in suitable liquid medium to logarithmic-growth phase.

***Note:** Debris in bacterial culture medium may interfere with detection; filter bacterial medium through a 0.2 µm filter before use.

2) Collect appropriate bacterial suspension and centrifuge at 10000 × *g* for 5 min at room temperature.

3) Discard supernatant, wash bacteria once with normal saline. Resuspend bacteria in normal saline and adjust density to 10^8 bacteria/mL (OD_{670}≈0.3). Further dilute 1:100 with normal saline to a final density of 10^6 bacteria/mL.

4) Prepare 100X Single Staining Working Solution for compensation. For instance, dilute 1 µL DMAO (1000X) in 9 µL Assay Buffer to prepare 10 µL 100X DMAO working solution. Dilute 1 µL PI (1000X) in 9 µL Assay Buffer to prepare 10 µL 100X PI working solution.

5) Mix 1 mL bacterial suspension with 10 µL of 100X Dual Staining Working Solution. Meanwhile, mix 1 mL bacterial suspension with 10 µL Assay Buffer to serve as the negative control. Prepare two additional tubes of 1 mL bacterial suspension, add 10 µL 100X DMAO working solution and 10 µL 100X PI working solution respectively for compensation setup. Incubate all tubes at 37°C in the dark for 15 min.

***Note:** Optimal incubation time varies among bacterial strains; use 15 min as a starting point and adjust according to actual staining performance.

6) After incubation, perform detection directly. Alternatively, centrifuge samples at 10000 × *g* for 5 min at room temperature, discard supernatant and resuspend bacterial pellet in 500 µL normal saline before acquisition. Keep stained samples on ice and complete data acquisition within 1 h.

DMAO: green fluorescence, Ex/Em = 503/530 nm

PI: red fluorescence, Ex/Em = 535/617 nm

***Note:**

a) Use a flow cytometer configured for bacterial detection.

b) Unstained bacterial suspension containing only Assay Buffer serves as the negative control.

c) Exclude bacterial debris during gating. Perform compensation adjustment using bacteria stained with DMAO alone and PI alone, respectively. Dual-stained samples produce two distinct bacterial populations: live bacteria with green-only fluorescence and dead bacteria with both green and red fluorescence.

d) Flow cytometry is highly sensitive; final probe concentrations may need to be lower than those used for fluorescence-microscopy detection. Adjust DMAO and PI final concentrations experimentally.

e) Instrument settings are critical for reliable detection. Gate the bacterial population on the Green Fluorescence vs. Side-Scatter (SSC) plot; display population separation on the Green-Fluorescence vs. Red-Fluorescence plot.

f) Distribution of live and dead bacterial populations varies with bacterial species and instrument settings. Some events may fall outside defined gates; adjust gating boundaries for individual experiments.

4. Detection via Fluorescence Microplate Reader

1) Grow bacteria in suitable liquid medium to logarithmic-growth phase.

2) Collect appropriate bacterial suspension and centrifuge at 10000 × *g* for 5 min at room temperature.

3) Discard supernatant, wash bacteria once with normal saline. Resuspend bacteria in normal saline and adjust density to 10^8 bacteria/mL (OD_{670}≈0.3). Further dilute 1:100 with normal saline to a final density of 10^6 bacteria/mL.

4) Add 100 µL bacterial suspension per well into a 96-well plate. Add 1 µL of 100X Dual Staining Working Solution to each well and mix thoroughly. Incubate at 37°C in the dark for 15 min.

***Note:**

a) Use fluorescence-compatible black or white 96-well plates, e.g., fully black 96-well cell-culture plates or black 96-well plates with transparent bottoms.

b) Optimal incubation time varies among bacterial strains; use 15 min as a starting point and adjust according to actual staining performance.

5) Upon incubation completion, measure fluorescence signals using a fluorescence microplate reader.

DMAO: green fluorescence, Ex/Em = 503/530 nm

PI: red fluorescence, Ex/Em = 535/617 nm

5. Determination of Live-to-Dead Bacterial Ratio via Standard-Curve Calibration

1) Grow bacteria in suitable liquid medium to logarithmic-growth phase.

2) Split equal volumes of bacterial suspension into two 1.5 mL microcentrifuge tubes. Centrifuge at 10000 × *g* for 5 min at room temperature and discard supernatant. Wash once with normal saline.

3) Resuspend one aliquot in normal saline (live-bacterial sample). Resuspend the second aliquot in ethanol/normal‑saline mixture (ethanol or isopropanol : normal saline = 7:3) to prepare dead-bacterial

sample. Adjust bacterial density of both samples to 10^8 bacteria/mL (OD_{670}≈ 0.3) using their respective solutions.

4) Incubate at room temperature for 1 h; mix thoroughly every 15 min.

5) Centrifuge at 10000 × *g* for 5 min at room temperature. Discard supernatant and wash once with normal saline. Resuspend pellets in normal saline to 10^8 bacteria/mL, then dilute 1:100 with normal saline to a final density of 10^6 bacteria/mL.

6) Prepare live-dead standard samples in a 96-well plate according to the table below:

#	Percentage of live bacteria (%)	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

7) Perform staining and detection as described in section 4, steps 4-5.

8) Fit a standard curve using acquired data.

Note

1. Remove culture medium prior to staining. Medium components may bind fluorescent dyes and reduce staining efficiency.
2. Fluorescent dyes are susceptible to photobleaching. Protect reagents and samples from light during handling.
3. Avoid microbial contamination of Assay Buffer, which compromises staining performance. Discard Assay Buffer if turbidity or other signs of contamination appear.
4. For your safety and health, please wear lab coats and gloves during the experiment.

5. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com