Bacteria Live/Dead Staining Kit

PromoKine

Instruction Manual

Product Name	Product Description	Size	Catalog Number
Bacteria Live/Dead Staining Kit	Fluorometric detection of viable and dead bacteria.	100 - 1000 Assays	PK-CA707-30027

Introduction

This kit provides a two-color fluorescence staining on both live bacteria (green) and dead bacteria (red) using two probes DMAO and EtD-III. DMAO is a green-fluorescent nucleic acid dye which stains both live and dead bacteria with intact and damaged cell membranes. EtD-III is a red-fluorescent nucleic acid dye that only stains dead bacteria with damaged cell membranes. With an appropriate mixture of DMAO and EtD-III, bacteria with intact cell membranes stain fluorescent green, whereas bacteria with damaged cell membranes stain fluorescent red. The kit is suitable for use with fluorescence microscopes and flow cytometers. The assay principles are general and applicable to most bacteria types.

A common criterion for bacterial viability is the ability of a bacterium to reproduce in suitable nutrient media that is referred to as growth assays. This kit yields results that correlate well with growth assays in liquid or solid media. Under certain conditions, however, bacteria having damaged membranes may be able to recover and reproduce — such bacteria may be scored as "dead" in this assay. Conversely, some bacteria with intact membranes may be unable to reproduce in nutrient medium, and yet these may be scored as "alive" in this assay. These possibilities should be considered if a considerable discrepancy is observed between this assay and bacterial growth assays.

Kit Contents

Component A: DMAO, 5 mM in DMSO	2 x 100 µl
Component B: Ethidium Homodimer-III (EthD-III), 2 mM in DMSO	2 x 150 µl

At the recommended reagent concentrations and volumes, this kit contains sufficient material to perform 1000 tests using a fluorescence microscope or 100 tests using a flow cytometer.

Storage and Stability

Store at -20°C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended. DMAO and EthD-III dyes bind to nucleic acids. The mutagenicity or toxicity of these dyes is currently unknown. Both reagents should be handled using universal laboratory safety precautions.

Cell Staining Protocol

Preparation of Live and Dead Bacterial Controls

1. Grow 4 ml cultures of your bacteria to late log phase in nutrient broth.
2. Prepare two tubes of 1 ml of the bacteria culture in Eppendorf tubes and centrifuge at 5,000-10,000 ×*g* for 10–15 minutes.
3. Remove the supernatant and resuspend the pellet of one tube in 0.3 ml of 0.85% NaCl solution and another tube in 1 ml of 0.85% NaCl.
4. Add 0.7 ml isopropyl alcohol into the tube with 0.3 ml of 0.85% NaCl and mix well (final concentration of isopropyl alcohol: 70%) for preparing dead bacteria.
5. Incubate both samples at room temperature for 1 hour, mixing every 15 minutes.

6. Pellet both samples by centrifugation at 5,000-10,000 ×*g* for 10-15 minutes.
7. Resuspend the pellets in 1 ml of 0.85% NaCl and centrifuge again as in step 6.
8. Determine the optical density at 670 nm (OD_{670}) for each bacterial suspension using a spectrophotometer.
9. Adjust each cell suspension (live and killed) to 10^8 bacteria/ml (OD_{670} = ~0.3), then dilute them 1:100 in 0.85% NaCl for a final density of 10^6 bacteria/ml.
10. Mix the two cell suspensions as shown below to obtained the desired ratio of live:dead cells.

Table 1. Volumes of live and dead cell suspensions to mix to achieve desired ratio of live:dead cells in the population.

Ratio of Live:Dead Cells	Live-Cell Suspension (ml)	Dead-Cell Suspension (ml)
0:100	0	1.0
10:90	0.1	0.9
20:80	0.2	0.8
30:70	0.3	0.7
40:60	0.4	0.6
50:50	0.5	0.5
60:40	0.6	0.4
70:30	0.7	0.3
80:20	0.8	0.2
90:10	0.9	0.1
100:0	1.0	0

Staining protocol for fluorescence microscopy

Note: Care must be taken to remove traces of growth medium before staining bacteria. The nucleic acids and other media components can bind DMAO and EthD-III dyes in unpredictable ways, resulting in unacceptable variation in staining. A single wash step is usually sufficient to remove significant traces of interfering media components from the bacterial suspension. Phosphate wash buffers are not recommended because they can decrease staining efficiency.

Staining bacteria in suspension

Note: Best results are obtained by adjusting the dye concentrations to achieve distinct labeling of live cells with DMAO and of dead cells with EthD-III. The optimal concentrations may vary depending on bacteria strain. In general it is best to use the lowest dye concentration that gives sufficient signal. The following conditions are optimized for *E. coli* live/dead cell staining.

1. Combine one volume of DMAO and two volumes of EthD-III in a microcentrifuge tube, mix thoroughly and add 8 volumes of 0.85% NaCl solution to derive 100X dye solution.
2. For each 100 µl of bacterial suspension, add 1 µl of the dye mixture.
3. Mix thoroughly and incubate at room temperature in the dark for 15 minutes.
4. Mount 5 µl of the stained bacterial suspension on a slide with an 18 mm square coverslip.
5. Observe under a fluorescence microscope. The fluorescence from both live and dead bacteria may be viewed simultaneously with any standard FITC long-pass filter set. Alternatively, the live (green fluorescent) and dead (red fluorescent) cells may be imaged separately with FITC and Cy®3 or Texas Red® band-pass filter sets.

Staining protocol for flow cytometry

1. Before beginning, see the notes under the staining protocol for fluorescence microscopy above.
2. Mix 11 different ratios of live and dead cells in 16 × 125 mm borosilicate glass tubes according to Table 1. The volume of each of the 11 samples will be 1 ml.
3. Mix 12 µl of DMAO stock solution with 24 µl of EthD-III stock solution in a microcentrifuge tube. Add 3 µl of the combined reagent mixture to each of the 11 samples, plus your experimental samples, and mix thoroughly by pipetting up and down several times.

   Note: It may be desirable to prepare additional control bacterial samples for staining with DMAO alone and with EthD-III alone.

4. Incubate at room temperature in the dark for 15 minutes.
5. Analyze each sample by flow cytometry using the FITC channel for DMAO positive cells and the propidium iodide or PE channel for EthD-III positive cells.

Figure 1: Live and dead E. coli analyzed by flow cytometry. Live bacteria (left panel) and dead bacteria (right panel) were stained with DMAO and EtD-III according to the protocol and analyzed by Coulter XL-MCL flow cytometer equipped with an argon-ion laser at 488 nm and 15 mW output. The emission light path contained a 525 nm and a 575 nm blocking filters.

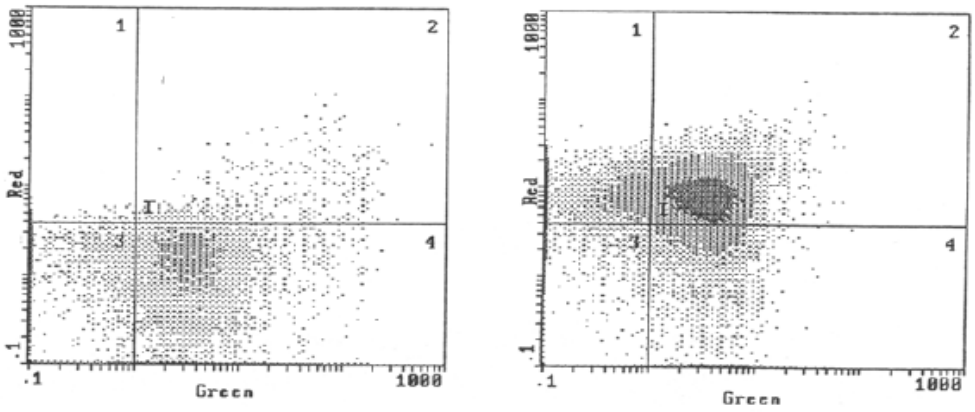
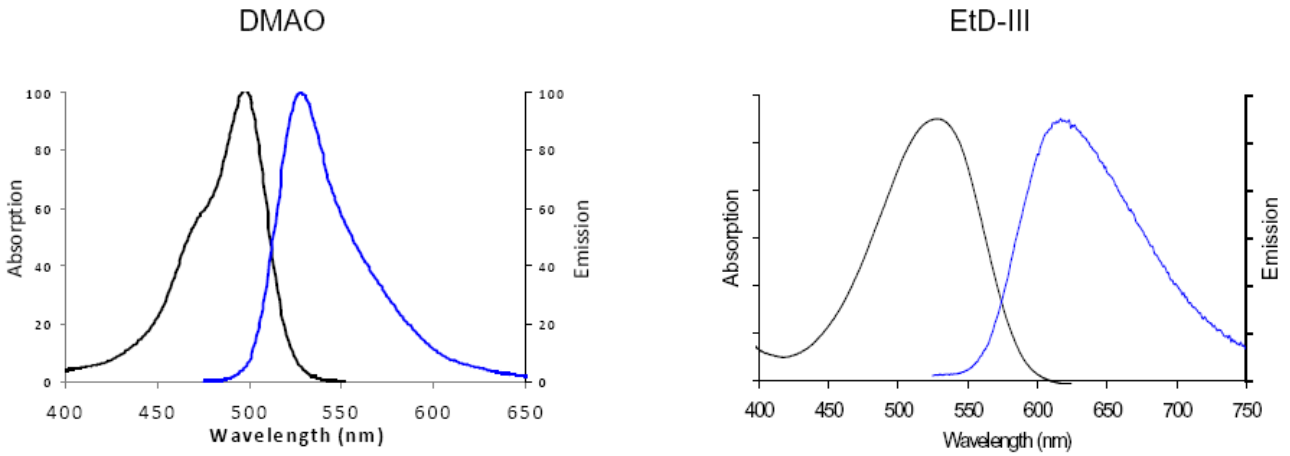


Figure 2: Absorption and emission of DMAO and EtHD-III bound DNA



Intended Use

For *in vitro* research use only. Not for diagnostic or therapeutic procedures.

References

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PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promokine.info
www.promokine.info

North America
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

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