DATA SHEET

Viability/Cytotoxicity Assay Kit
For
Bacteria Live & Dead Cells

Catalog Number: 30027 (100-1000 assays)

Introduction

Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cell Staining Kit (30027) provides two-color fluorescence staining on both live (green) and dead (red) bacteria using two probes, DMAO and EtD-III. DMAO is a green-fluorescent nucleic acid dye that stains both live and dead bacteria with intact and damaged cell membranes. EtD-III is a red-fluorescent nucleic acid dye that stains only dead bacteria with damaged cell membranes. With an appropriate mixture of DMAO and EtD-III, bacteria with intact cell membranes is stained fluorescent green, whereas bacteria with damaged cell membranes is stained 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". Therefore, these situations need to be considered if a vast difference of live and dead bacteria counts is observed between this assay and growth assays.

Kit Components

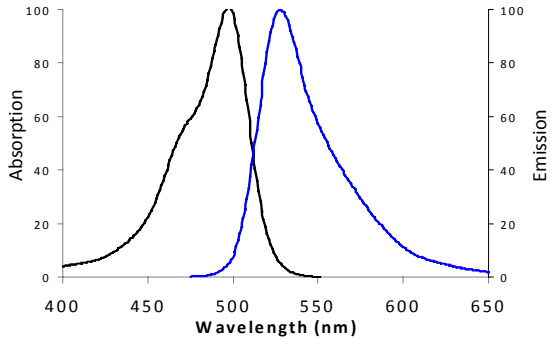
Component A, **DMAO**: 2x100 µL, 5 mM in DMSO
Component B, **EthD-III**: 2x150 µL, 2 mM in DMSO
At the recommended reagent concentrations and volumes, this kit contains sufficient material to perform 1000 tests using a fluorescence microscope or 100 tests using a flow cytometer.

Storage and Handling of Reagents

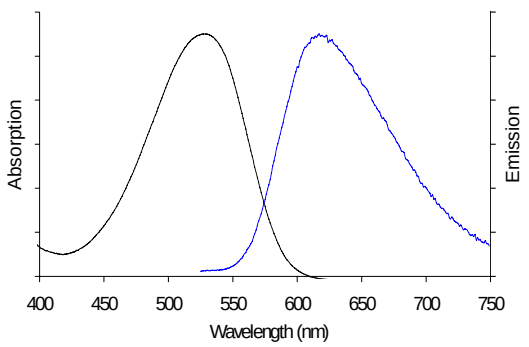
Reagents in this kit should be stored sealed and protected from light and frozen at -20°C. Allow the reagents to warm up to room temperature and centrifuge briefly before opening. Before refreezing, seal all stock solutions tightly. When stored properly, these stock solutions are stable for at least six months.
Caution: DMAO and EtD-III dyes bind to nucleic acids. Currently we have no data addressing the mutagenicity or toxicity of these dyes. Both reagents should be used with appropriate caution.

Absorption and emission of DMAO and EtHD-III bound DNA

DMAO



EtD-III



Preparation of Live and Dead Bacterial Suspensions as Controls

1.1 Grow 4 mL cultures of your bacteria to late log phase in nutrient broth.

1.2 Prepare two tubes of 1 mL of the bacteria culture in Eppendorf tubes and centrifuge at 10,000 × g for 10–15 minutes.

1.3 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.

1.4 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.

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 the pellets in 1 mL of 0.85% NaCl and centrifuge again as in step 1.6.

1.8 Determine the optical density at 670 nm (OD670) for a 3 mL aliquot of the bacterial suspensions in glass or acrylic absorption cuvettes (1 cm pathlength).

1.9 Use live and dead bacteria at your desired concentration for staining experiments shown below.

Fluorescence Microscopy Protocol

Note: Care must be taken to remove traces of growth medium before staining bacteria with these kit reagents. The nucleic acids and other media components can bind DMAO and EthD-III dyes in unpredictable ways, resulting in unacceptable variations 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 appear to 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 are likely to vary depending on the bacteria types. In general it is best to use the lowest dye concentration that gives sufficient signal. The following condition is optimal for *E. coli* live and dead cell staining.

2.1 Combine one volume of Component A and two volumes of Component B in a microcentrifuge tube, mix thoroughly and add 8 volumes of 0.85% NaCl solution to derive 100X dye solution.

2.2 For each 100 uL of your bacteria sample and live and dead bacteria control suspensions, add 1 uL of the dye mixture.

2.3 Mix thoroughly and incubate at room temperature in the dark for 15 minutes.

2.4 Trap 5 µL of the stained bacterial suspension between a slide and an 18 mm square coverslip.

2.5 Observe under a fluorescence microscope equipped with any of the filter sets as below.

Selection of Optical Filters

The fluorescence from both live and dead bacteria may be viewed simultaneously with any standard fluorescein longpass filter set. Alternatively, the live (green fluorescent) and dead (red fluorescent) cells may be viewed separately with fluorescein and Texas Red bandpass filter sets.

The following is the summary of the fluorescence microscope filter sets recommended for use with this kit.

Longpass and dual emission filters useful for simultaneous viewing of DMAO and EthD-III stains
Omega Filters: XF25, XF26, XF115
Chroma Filters: 11001, 41012, 71010
Bandpass filters for viewing DMAO alone
Omega Filters: XF22, XF23
Chroma Filters: 31001, 41001
Bandpass filters for viewing EthD-III alone
Omega Filters: XF32, XF43, XF102, XF108
Chroma Filters: 31002, 31004, 41002, 41004

Flow Cytometry

3.1 Adjust the *E. coli* suspensions (live and killed) to 1×10^8 bacteria/mL (~0.03 OD_{670}), then dilute them 1:100 in filter-sterilized dH_2O to reach a final density of 1×10^6 bacteria/mL if needed.

3.2 Mix 11 different proportions of *E. coli* in 16 × 125 mm borosilicate glass tubes according to Table 1. The volume of each of the 11 samples will be 1 mL.

3.3 Mix 12 µL of Component A with 24 µL of Component B in a microcentrifuge tube. Add 3 µL of the combined reagent mixture to each of the 11 samples and mix thoroughly by pipetting up and down several times.
Note: It may be desirable to prepare additional bacterial samples for staining with component A alone (stain both live and dead bacteria) and with Component B alone (stain dead bacteria only).

3.4 Incubate at room temperature in the dark for 15 minutes.

3.5 Analyze each bacterial sample by flow cytometry using the setting for fluorescein for DMAO positive cells and propidium iodide for EtD-III positive cells.

Table 1: *Volume of Live- and dead-cell suspension to mix to achieve desired ratio of live:dead cell population*

Ratio of Live:Dead Cells	mL Live-Cell Suspension	mL Dead-Cell Suspension
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

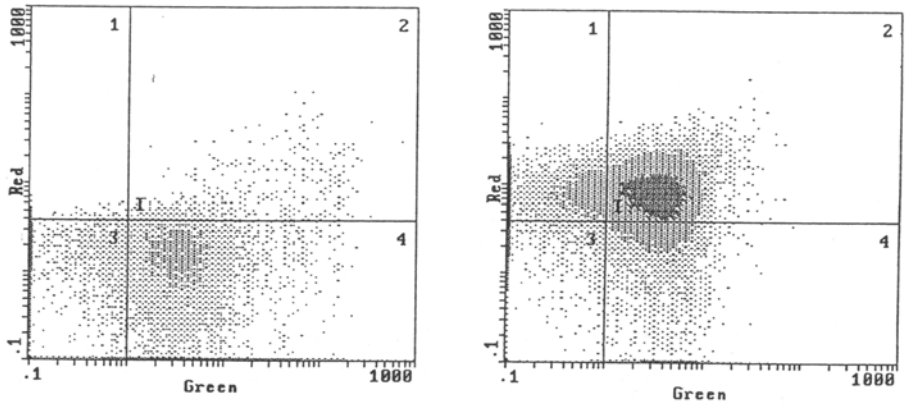


Figure 1: Live and dead E. coli analyzed by flow cytometry. Live bacteria (left panel) and dead bacteria(right panel) were stained with DMAO and EtD-III according to the protocol and analyzed by Coulter XL-MCL flow cytometer equipped with an argon-ion laser at 488 nm and 15 mW output. The emission light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

References

1. J Appl Bacteriol 72, 410 (1992); **2.** Lett Appl Microbiol 13, 58 (1991); **3.** Curr Microbiol 4, 321 (1980); **4.** J Microbiol Methods 13, 87 (1991); **5.** Microbiol Rev 51, 365 (1987); **6.** J Med Microbiol 39, 147 (1993).

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