# Product Information

## Viability/Cytotoxicity Assay for Bacteria Live & Dead Cells

**Catalog Number:** 30027

**Unit Size:** 100 flow cytometry assays or 1000 fluorescence microscopy assays

### Kit Contents

| Component | Size |
|---|---|
| 30027A: DMAO | 2 vials (100 uL each) |
| 99905: EthD-III | 2 vials (150 uL each) |

### Storage and Handling

Store at 4°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.

### Spectral Properties

DMAO: Ex/Em: 503/530 nm (with DNA)
EthD-III: Ex/Em: 530/620 nm (with DNA)

### Product Description

The Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cells provides green/red fluorescence staining of live and dead bacteria, respectively. The assay employs two probes, DMAO and EthD-III. DMAO is a green-fluorescent nucleic acid dye that stains both live and dead bacteria. EthD-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 fluoresce green, while bacteria with damaged cell membranes fluoresce green and red. Staining can be analyzed by fluorescence microscopy or flow cytometry. 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, referred to as a growth assay. This kit generally 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 in nutrient medium, even though such bacteria could be scored as dead in this assay. Conversely, some bacteria with intact membranes may be unable to reproduce in nutrient medium, even though these bacteria would 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.

### 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).

### Assay Protocols

**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.

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 uL of bacterial suspension, add 1 uL of the dye mixture.
3. Mix thoroughly and incubate at room temperature in the dark for 15 minutes.
4. Mount 5 uL 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**

Before beginning, see the notes under the staining protocol for fluorescence microscopy above.

1. 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.
2. Mix 12 uL of DMAO stock solution with 24 uL of EthD-III stock solution in a microcentrifuge tube. Add 3 uL 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.
3. Incubate at room temperature in the dark for 15 minutes.
4. 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.

**Related Products**

| Catalog number | Product |
|---|---|
| 32001 | Bacterial Viability and Gram Stain Kit |
| 32000 | Live Bacteria Gram Stain Kit |
| 40069 | PMAxx™ Dye for viability PCR, 20 mM in water |
| 40013 | PMA Dye for viability PCR |
| 40019 | PMA Dye for viability PCR, 20 mM in water |
| E90002 | PMA-Lite™ LED Photolysis Device |
| 31033-31037; 31050, 31051, 31053 | Real-Time PCR Bacterial Viability Kits (choose from kits for 8 bacterial strains) |
| 32002-32009 | Live-or-Dye™ Fixable Viability Staining Kits |
| 29021-29029; 29059; 29064 | CF™ Dye Wheat Germ Agglutinin (WGA) |
| 10063 | CTC, bacterial respiration dye |
| 31062 | Yeast Vitality Staining Kit |
| 31063 | Yeast Viability Staining Kit |
| 31064 | Yeast Fixable Live/Dead Staining Kit |
| 30002 | Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells |

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