Revised: 15-July-2004

LIVE/DEAD® BacLight™ Bacterial Viability Kits

L7007 LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit *for microscopy*
L7012 LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit *for microscopy and quantitative assays*
L13152 LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit *10 applicator sets*

Quick Facts

Storage upon receipt:

Kits L7007 and L7012
- ≤–20°C
- Protect from light

Kit L13152
- Room temperature
- Protect from light

Note: Do not use Component C as immersion oil.

Introduction

Molecular Probes' LIVE/DEAD® *Bac*Light™ Bacterial Viability Kits provide a novel two-color fluorescence assay of bacterial viability that has proven useful for a diverse array of bacterial genera. Conventional direct-count assays of bacterial viability are based on metabolic characteristics or membrane integrity. However, methods relying on metabolic characteristics often only work for a limited subset of bacterial groups,[1] and methods for assessing bacterial membrane integrity commonly have high levels of background fluorescence.[2] Both types of determinations suffer from being very sensitive to growth and staining conditions.[3,4] Because of the marked differences in morphology, cytology and physiology among the many bacterial genera, a universally applicable direct-count viability assay has been very difficult to achieve. Our LIVE/DEAD *Bac*Light Bacterial Viability Kits now allow researchers to easily, reliably and quantitatively distinguish live and dead bacteria in minutes, even in a mixed population containing a range of bacterial types.

The LIVE/DEAD *Bac*Light Bacterial Viability Kits utilize mixtures of our SYTO® 9 green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, propidium iodide. These stains differ both in their spectral characteristics and in their ability to penetrate healthy bacterial cells. When used alone, the SYTO 9 stain generally labels all bacteria in a population — those with intact membranes and those with damaged membranes. In contrast, propidium iodide penetrates only bacteria with damaged membranes, causing a reduction in the SYTO 9 stain fluorescence when both dyes are present. Thus, with an appropriate mixture of the SYTO 9 and propidium iodide stains, bacteria with intact cell membranes stain fluorescent green, whereas bacteria with damaged membranes stain fluorescent red. The excitation/emission maxima for these dyes are about 480/500 nm for SYTO 9 stain and 490/635 nm for propidium iodide. The background remains virtually nonfluorescent. Furthermore, although the dye ratios suggested for the LIVE/DEAD *Bac*Light Bacterial Viability Kits have been found to work well with a broad spectrum of bacterial types, these kits also accommodate fine-tuning of the dye combinations so that optimal staining of bacteria can be achieved under a variety of environmental conditions.

A common criterion for bacterial viability is the ability of a bacterium to reproduce in suitable nutrient medium. Exponentially growing cultures of bacteria typically yield results with the LIVE/DEAD *Bac*Light bacterial viability assay that correlate well with growth assays in liquid or solid media. Under certain conditions, however, bacteria having compromised 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."[5]

The LIVE/DEAD *Bac*Light Bacterial Viability Kits have been thoroughly tested with a variety of organisms and under several different conditions (see *Bacteria That Have Been Tested*, below). The kits are well suited for use in fluorescence microscopy or for use in quantitative analysis with a fluorometer, fluorescence microplate reader, flow cytometer[6] or other instrumentation. In our original LIVE/DEAD *Bac*Light Kit (L7007), the dyes are provided mixed at different proportions in two solutions. Kit L7007 is still available for customers who have already developed protocols using that formulation. Kit L7012, however, is more flexible because it provides separate solutions of the SYTO 9 and propidium iodide stains. Having separate staining components facilitates the calibration of bacterial fluorescence for quantitative procedures. For added convenience, our LIVE/DEAD *Bac*Light kit (L13152) contains the separate dyes premeasured into pairs of polyethylene transfer pipets. Besides having the convenience of being packaged in handy applicator pipets, kit L13152 has a formulation that does not require dimethyl sulfoxide (DMSO), nor does it require refrigerated storage.

The LIVE/DEAD *Bac*Light Bacterial Viability Kits are intended as research tools and our Technical Assistance Department welcomes any feedback on the performance of these kits with bacterial strains and environmental conditions not described in this enclosure.

Materials

Kit Contents for Viability Kit, L7007

- **SYTO 9 dye, 1.67 mM / Propidium iodide, 1.67 mM** (Component A), 300 µL solution in DMSO
- **SYTO 9 dye, 1.67 mM / Propidium iodide, 18.3 mM** (Component B), 300 µL solution in DMSO
- ***Bac*Light mounting oil** (Component C), 10 mL, for bacteria immobilized on membranes. The refractive index at 25°C is 1.517 ± 0.003. DO NOT USE FOR IMMERSION OIL.

Kit Contents for Viability Kit, L7012

- **SYTO 9 dye, 3.34 mM** (Component A), 300 µL solution in DMSO
- **Propidium iodide, 20 mM** (Component B), 300 µL solution in DMSO
- ***Bac*Light mounting oil** (Component C), 10 mL, for bacteria immobilized on membranes. The refractive index at 25°C is 1.517 ± 0.003. DO NOT USE FOR IMMERSION OIL.

Note that a 1:1 mixture of Components A and B of kit L7012 is exactly equivalent to a 1:1 mixture of Components A and B of kit L7007.

Kit Contents for Viability Kit, L13152

- **SYTO 9 dye** (Component A), stabilized as a solid in 10 sealed applicator pipets
- **Propidium iodide** (Component B), as a solid in 10 sealed applicator pipets
- ***Bac*Light mounting oil** (Component C), 10 mL, for bacteria immobilized on membranes. The refractive index at 25°C is 1.517 ± 0.003. DO NOT USE FOR IMMERSION OIL.

For use of the applicator pipets provided in kit L13152, snip off the sealed ends and dissolve the contents in deionized water, as described in the protocols below.

Number of Tests Possible

At the recommended reagent dilutions and volumes, kits L7007 and L7012 contain sufficient material to perform ≥1000 individual tests in 96-well assay plates, many more tests by fluorescence microscopy or ~200 tests by flow cytometry. In kit L13152, each applicator pair contains sufficient dye to perform 50 individual tests in a 96-well assay plate, ~1000 assays by fluorescence microscopy or 10 tests by flow cytometry.

Storage and Handling

For either kit L7007 or L7012, the DMSO stock solutions should be stored frozen at ≤–20°C and protected from light. Allow reagents to warm to room temperature and centrifuge briefly before opening the vials. Before refreezing, seal all vials tightly. When stored properly, these stock solutions are stable for at least one year.

For kit L13152, store at room temperature, protected from light. The new stain formulation is solid phase and is chemically stable when stored at 37°C for more than six months, protected from light. The dissolved dye solutions are stable for up to a year, when stored frozen at ≤–20°C and protected from light.

The *Bac*Light mounting oil may be stored at room temperature, and is stable indefinitely.

Caution: Propidium iodide and SYTO 9 stain bind to nucleic acids. Propidium iodide is a potential mutagen, and we have no data addressing the mutagenicity or toxicity of the SYTO 9 stain. Both reagents should be used with appropriate care. The DMSO stock solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solutions. As with all nucleic acid stains, solutions containing these reagents should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dyes.

Experimental Protocols, General Considerations

The following protocols are provided as examples to guide researchers in the development of their own bacterial staining procedures. Researchers at Molecular Probes have used these procedures and found them to be simple and reliable for both gram-positive and gram-negative bacteria.

Culture Conditions and Preparation of Bacterial Suspensions

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 the SYTO 9 and propidium iodide 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.

1.1 Grow 30 mL cultures of either *Escherichia coli* or *Staphylococcus aureus* to late log phase in nutrient broth (e.g., DIFCO catalog number 0003-01-6).

1.2 Concentrate 25 mL of the bacterial culture by centrifugation at 10,000 × g for 10–15 minutes.

1.3 Remove the supernatant and resuspend the pellet in 2 mL of 0.85% NaCl or appropriate buffer.

1.4 Add 1 mL of this suspension to each of two 30–40 mL centrifuge tubes containing either 20 mL of 0.85% NaCl or appropriate buffer (for live bacteria) or 20 mL of 70% isopropyl alcohol (for killed 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 20 mL of 0.85% NaCl or appropriate buffer and centrifuge again as in step 1.6.

1.8 Resuspend both pellets in separate tubes with 10 mL of 0.85% NaCl or appropriate buffer each.

1.9 Determine the optical density at 670 nm (OD_{670}) of a 3 mL aliquot of the bacterial suspensions in glass or acrylic absorption cuvettes (1 cm pathlength).

1.10 For suggested concentrations of *E. coli* or *S. aureus* suspensions, please refer to the section appropriate for your

instrumentation: fluorescence microscope, fluorometer, fluorescence microplate reader or flow cytometer.

Bacteria That Have Been Tested

The LIVE/DEAD *Bac*Light Bacterial Viability Kits have been tested at Molecular Probes on the following bacterial species: *Bacillus cereus*, *B. subtilis*, *Clostridium perfringens*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Mycobacterium phlei*, *Pseudomonas aeruginosa*, *P. syringae*, *Salmonella oranienburg*, *Serratia marcescens*, *Shigella sonnei*, *Staphylococcus aureus* and *Streptococcus pyogenes*. All of these bacterial types have shown a good correlation between the results obtained with the LIVE/DEAD *BacLight* Bacterial Viability Kits and those obtained with standard plate counts. These tests were performed on logarithmically growing cultures of organisms. In addition, we have received favorable reports from researchers who have used these kits with: *Agrobacterium tumefaciens*, *Edwardsiella ictaluri*, *Eurioplasma eurilytica*, *Lactobacillus* sp., *Mycoplasma hominus*, *Propionibacterium* sp., *Proteus mirabilis* and *Zymomonas* sp.

Optimization of Staining

The two dye components provided with the LIVE/DEAD *Bac*Light Bacterial Viability Kits have been balanced so that a 1:1 mixture provides good live/dead discrimination in most applications. Occasionally, however, the proportions of the two dyes must be adjusted for optimal discrimination. For example, if green fluorescence is too prominent in the preparation, we suggest that you try either lowering the concentration of SYTO 9 stain (by using less of Component A) or by raising the concentration of propidium iodide (by using more of Component B).

To thoroughly optimize the staining, we recommend experimenting with a range of concentrations of SYTO 9 dye, each in combination with a range of propidium iodide concentrations. In the case of Kits L7007 and L7012, you may wish to try staining 1.0 mL of the bacterial suspension with 3 µL of dye pre-mixed at different Component A:Component B ratios. In the case of kit L13152, separate dye solutions can be made by dissolving the contents of one Component A pipet in 2.5 mL filter-sterilized dH_2O and the contents of one Component B pipet in 2.5 mL filter-sterilized dH_2O. These separate solutions can be blended at different ratios, and then the mixtures applied 1:1 with the bacterial suspension.

Fluorescence Microscopy Protocols

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. A summary of the fluorescence microscope filter sets recommended for use with the LIVE/DEAD *Bac*Light Bacterial Viability Kits shown in Table 1.

Staining Bacteria in Suspension with either Kit L7007 or L7012

2.1 Combine equal volumes of Component A and Component B in a microfuge tube, mix thoroughly.

2.2 Add 3 µL of the dye mixture for each mL of the bacterial suspension. When used at the recommended dilutions, the reagent mixture will contribute 0.3% DMSO to the staining solution. Higher DMSO concentrations may adversely affect staining.

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 in a fluorescence microscope equipped with any of the filter sets listed in Table 1.

Staining Bacteria in Suspension with Kit L13152

3.1 Prepare a 2X stock solution of the LIVE/DEAD *Bac*Light staining reagent mixture by dissolving the contents of one Component A pipet (containing yellow-orange solids) and one Component B pipet (containing red solids) in a common 5 mL–volume of filter-sterilized dH_2O.

3.2 Combine a sample of the 2X stock solution with an equal volume of the bacterial suspension. The final concentration of each dye will be 6 µM SYTO 9 stain and 30 µM propidium iodide.

Table 1. *Characteristics of common filters suitable for use with the LIVE/DEAD BacLight Bacterial Viability Kits.*

Omega Filters*	Chroma Filters*	Notes
XF25, XF26, XF115	11001, 41012, 71010	Longpass and dual emission filters useful for simultaneous viewing of SYTO 9 and propidium iodide stains
XF22, XF23	31001, 41001	Bandpass filters for viewing SYTO 9 alone
XF32, XF43 XF102, XF108	31002, 31004 41002, 41004	Bandpass filters for viewing propidium iodide alone
***** Catalog numbers for recommended bandpass filter sets for fluorescence microscopy. Omega® filters are supplied by Omega Optical Inc. (www.omegafilters.com). Chroma filters are supplied by Chroma Technology Corp. (www.chroma.com).		

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

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

3.5 Observe in a fluorescence microscope equipped with any of the filter sets listed in Table 1.

Fluorescence Spectroscopy Protocols

Staining Bacteria with either Kit L7007 or L7012

4.1 Adjust the *E. coli* suspensions (live and killed) to 1×10^8 bacteria/mL (~0.03 OD_{670}) or the *S. aureus* suspensions (live and killed) to 1×10^7 bacteria/mL (~0.15 OD_{670}). *S. aureus* suspensions typically should be 10-fold less concentrated than *E. coli* for fluorescence spectroscopy.

4.2 Mix five different proportions of the bacterial suspensions in 1 cm acrylic, glass or quartz fluorescence cuvettes (Table 2). The total volume of each of the five samples will be 3 mL.

4.3 Prepare a combined reagent mixture in a microfuge tube by adding 30 µL of Component A to 30 µL of Component B.

4.4 Add 9 µL of the combined reagent mixture to each of the five samples (5 samples × 9 µL = 45 µL total) and mix thoroughly by pipetting up and down several times.

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

Staining Bacteria with Kit L13152

5.1 Adjust the *E. coli* suspensions (live and killed) to 2×10^8 bacteria/mL (~0.06 OD_{670}) or the *S. aureus* suspensions (live and killed) to 2×10^7 bacteria/mL (~0.30 OD_{670}). *S. aureus* suspensions typically should be 10-fold less concentrated than *E. coli* for fluorescence spectroscopy.

Table 2*. Volumes of live- and dead-cell suspensions to mix to achieve various proportions of live:dead cells for fluorescence spectroscopy.*

Ratio of Live:Dead Cells	mL Live-Cell Suspension	mL Dead-Cell Suspension
0:100	0	3.0
10:90	0.3	2.7
50:50	1.5	1.5
90:10	2.7	0.3
100:0	3.0	0

5.2 Mix five different proportions of the bacterial suspensions in 1 cm acrylic, glass or quartz fluorescence cuvettes (Table 2). Note that when using kit L13152, only one-half of the cell suspension volume (1.5 mL) listed in Table 2 will be used.

5.3 Prepare a 2X working solution of the LIVE/DEAD *Bac*Light staining reagent mixture by dissolving the contents of one Component A pipet (containing yellow-orange solids) and one Component B pipet (containing red solids) in a common 5 mL–volume of filter-sterilized dH_2O.

5.4 Mix 1.5 mL of the 2X staining reagent mixture with an equal volume (1.5 mL) of each bacterial suspension. Note that, as described above, two applicator sets will be needed (5 samples × 1.5 mL = 7.5 mL total); however, it may be possible to use smaller volumes.

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

Fluorescence Spectroscopy and Data Analysis

6.1 Measure the fluorescence emission spectrum (excitation 470 nm, emission 490–700 nm) of each cell suspension (F_{cell}) in a fluorescence spectrophotometer (Figure 1a).

6.2 Calculate the ratio of the integrated intensity of the portion of each spectrum between 510–540 nm (em1; green) to that

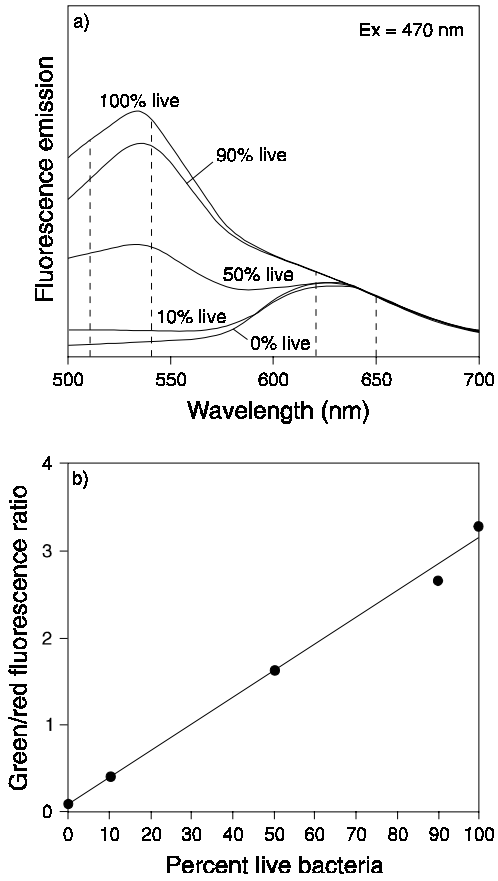

Figure 1. *Analysis of relative viability of E. coli suspensions by fluorescence spectroscopy. a) Emission spectra of suspensions of various proportions of live and isopropyl alcohol–killed E. coli were obtained from samples prepared and stained as outlined in the text. Integrated fluorescence emission intensities were determined from the spectral regions indicated by dashed vertical lines. b) Integrated intensities of the green (510–540 nm) and red (620–650 nm) emission were acquired, and the green/red fluorescence ratios ($Ratio_{G/R}$) were calculated for each proportion of live/dead E. coli. The line is a least-squares fit of the relationship between % live bacteria (x) and $Ratio_{G/R}$ (y).*

between 620–650 (em2; red) for each bacterial suspension.

$$\text{Ratio}_{G/R} = \frac{F_{cell,em1}}{F_{cell,em2}}$$

6.3 Plot the ratio of integrated green fluorescence to integrated red fluorescence ($R_{G/R}$) versus percentage of live cells in the *E. coli* suspension (Figure 1b).

Fluorescence Microplate Readers

Conditions required for measurement of fluorescence in microplate readers are very similar to those required for fluorescence spectroscopy of bacterial cell suspensions. As in fluorescence spectroscopy experimental protocols, reagent concentrations are the same as those recommended for fluorescence microscopy, and the ratio of green to red fluorescence emission is proportional to the relative numbers of live bacteria.

Staining Bacterial Suspensions with either Kit L7007 or L7012

7.1 Adjust the *E. coli* suspensions (live and killed) to 2×10^8 bacteria/mL (~0.06 OD_{670}) or the *S. aureus* suspensions (live and killed) to 2×10^7 bacteria/mL (~0.30 OD_{670}). *S. aureus* suspensions typically should be 10-fold less concentrated than *E. coli* when using a fluorescence microplate reader.

Table 3. *Volumes of live- and dead-cell suspensions to mix to achieve various proportions of live:dead cells for fluorescence microplate readers.*

Ratio of Live:Dead Cells	mL Live-Cell Suspension	mL Dead-Cell Suspension
0:100	0	2.0
10:90	0.2	1.8
50:50	1.0	1.0
90:10	1.8	0.2
100:0	2.0	0

7.2 Mix five different proportions of *E. coli* or *S. aureus* (Table 3) in 16 × 125 mm borosilicate glass culture tubes. The total volume of each of the five samples will be 2 mL.

7.3 Mix 6 µL of Component A with 6 µL of Component B in a microfuge tube.

7.4 Prepare a 2X stain solution by adding the entire 12 µL of the above mixture to 2.0 mL of filter-sterilized dH_2O in a 16 × 125 mm borosilicate glass culture tube and mix well.

7.5 Pipet 100 µL of each of the bacterial cell suspension mixtures into separate wells of a 96-well flat-bottom microplate. We recommend that you prepare samples in triplicate. The outside wells (rows A and H and columns 1 and 12) are usually kept empty to avoid spurious readings.

7.6 Using a new tip for each well, pipet 100 µL of the 2X staining solution (from step 7.4) to each well and mix thoroughly by pipetting up and down several times.

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

Staining Bacterial Suspensions with Kit L13152

8.1 Adjust the *E. coli* suspensions (live and killed) to 4×10^8 bacteria/mL (~0.12 OD_{670}) or the *S. aureus* suspensions (live and killed) to 4×10^7 bacteria/mL (~0.60 OD_{670}). *S. aureus* suspensions typically should be 10-fold less concentrated than *E. coli* when using a fluorescence microplate reader.

8.2 Mix five different proportions of *E. coli* or *S. aureus* (Table 3) in 16 × 125 mm borosilicate glass culture tubes.

8.3 Prepare a 2X working solution of the LIVE/DEAD *Bac*Light staining reagent mixture by dissolving the contents of one Component A pipet (containing yellow-orange solids) and one Component B pipet (containing red solids) in a common 5 mL–volume of filter-sterilized dH_2O.

8.4 Pipet 100 µL of each of the bacterial cell suspension mixtures into separate wells of a 96-well flat-bottom microplate. We recommend that you prepare samples in triplicate. The outside wells (rows A and H and columns 1 and 12) are usually kept empty to avoid spurious readings.

8.5 Using a new tip for each well, pipet 100 µL of the 2X working stain solution (from step 8.3) to each well and mix thoroughly by pipetting up and down several times.

8.6 Incubate the sample at room temperature in the dark for 15 minutes.

Fluorescence Measurement and Data Analysis

9.1 With the excitation wavelength centered at about 485 nm, measure the fluorescence intensity at a wavelength centered at about 530 nm (emission 1; green) for each well of the entire plate.

9.2 With the excitation wavelength still centered at about 485 nm, measure the fluorescence intensity at a wavelength centered about 630 nm (emission 2; red) for each well of the entire plate.

9.3 Analyze the data by dividing the fluorescence intensity of the stained bacterial suspensions (F_{cell}) at emission 1 by the fluorescence intensity at emission 2.

$$\text{Ratio}_{G/R} = \frac{F_{cell,em1}}{F_{cell,em2}}$$

9.4 Plot the $\text{Ratio}_{G/R}$ versus percentage of live cells in the *E. coli* suspension (Figure 2).

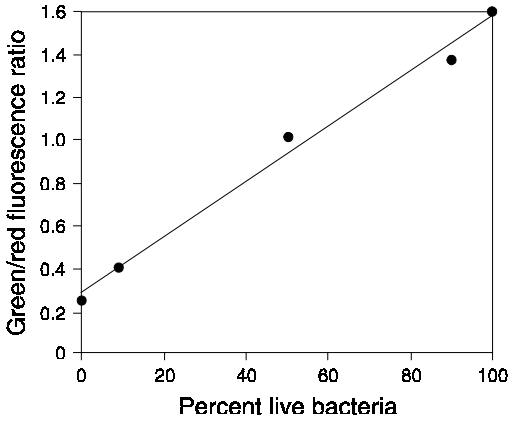

Figure 2. *Analysis of relative viability of E. coli suspensions in a fluorescence microplate reader. Samples of E. coli were prepared and stained as outlined in the text. The integrated intensities of the green (530 ± 12.5 nm) and red (620 ± 20 nm) emission of suspensions excited at 485 ± 10 nm were acquired, and the green/red fluorescence ratios ($Ratio_{G/R}$) were calculated for each proportion of live/dead E. coli. Each point represents the mean of ten measurements. The line is a least-squares fit of the relationship between % live bacteria (x) and $Ratio_{G/R}$ (y).*

Flow Cytometry

Instrument capabilities may vary considerably but the techniques and parameters established here should aid considerably in setting up similar analyses in the majority of flow cytometers now in use, both in research and clinical environments.

Table 4. *Volumes of live- and dead-cell suspensions to mix to achieve various proportions of live:dead cells for flow cytometry.*

Ratio of Live:Dead Cells	mL Live-Cell Suspension	mL Dead-Cell Suspension
0:100	0	2.0
10:90	0.2	1.8
20:80	0.4	1.6
30:70	0.6	1.4
40:60	0.8	1.2
50:50	1.0	1.0
60:40	1.2	0.8
70:30	1.4	0.6
80:20	1.6	0.4
90:10	1.8	0.2
100:0	2.0	0

Staining Bacterial Suspensions with either Kit L7007 or L7012

10.1 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.

10.2 Mix 11 different proportions of *E. coli* in 16 × 125 mm borosilicate glass tubes according to Table 4. The volume of each of the 11 samples will be 2 mL.

10.3 Mix 35 µL of Component A with 35 µL of Component B in a microfuge tube. If Kit L7012 is used, it may be desirable to prepare additional bacterial samples for staining with Component A alone and with Component B alone.

10.4 Add 6 µL of the combined reagent mixture to each of the 11 samples (11 samples x 6 µL = 66 µL total) and mix thoroughly by pipetting up and down several times.

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

Staining Bacterial Suspensions with Kit L13152

11.1 Adjust the *E. coli* suspensions (live and killed) to 1×10^8 bacteria/mL (~0.03 OD_{670}), then dilute them 1:50 in filter-sterilized dH_20 to reach a final density of 2×10^6 bacteria/mL.

11.2 Mix 11 different proportions of *E. coli* in 16 × 125 mm borosilicate glass tubes according to Table 4. Note that when using kit L13152, only one-half of the cell suspension volume (1.0 mL) listed in Table 3 will be used.

11.3 Prepare a 2X working solution of the LIVE/DEAD *Bac*Light staining reagent mixture by dissolving the contents of one Component A pipet (containing yellow-orange solids) and one Component B pipet (containing red solids) in a common 5 mL–volume of filter-sterilized dH_2O. It may be desirable to prepare additional bacterial samples for staining with Component A alone (dissolved in 5 mL of filter-sterilized dH_2O) and with Component B alone (dissolved in 5 mL of filter-sterilized dH_2O).

11.4 Mix 1 mL of the 2X working solution of the LIVE/DEAD *Bac*Light staining reagent mixture with an equal volume (1 mL) of the bacterial suspension. Note that, as described above, three applicator sets will be needed (11 samples × 1 mL = 11 mL total); however it may be possible to use smaller volumes.

11.5 Incubate the sample at room temperature in the dark for 15 minutes.

Instrument Parameters

The data shown in this example were acquired with a Coulter EPICS V™ flow cytometer equipped with an argon-ion laser at 488 nm and 400 mW output. Data acquisition and analysis were controlled using Cytomation CICERO software and a hardware interface. The emission light path contained a 515 nm blocking filter, 590 nm dichroic filter before the Green PMT and a 610 nm absorbance filter before the Red PMT. The density of the *E. coli* bacterial suspension was 1×10^6 cells/mL and the sampling rate was ~300 particles/sec. The sheath fluid was distilled water and the flow tip was a 76 µm air tip.

Fluorescence Measurements and Data Analysis

Because both live and dead cells exhibit green fluorescence, the signal discriminator was set at 15% of the log-integrated green fluorescence (LIGFL) to eliminate debris. Populations of bacteria were discriminated as three regions of the log-integrated red fluorescence (LIRFL) versus LIGFL plot (Figure 3a), and the numbers of bacteria found within these regions were used to estimate the percentage of viable organisms in the population (Figure 3b).

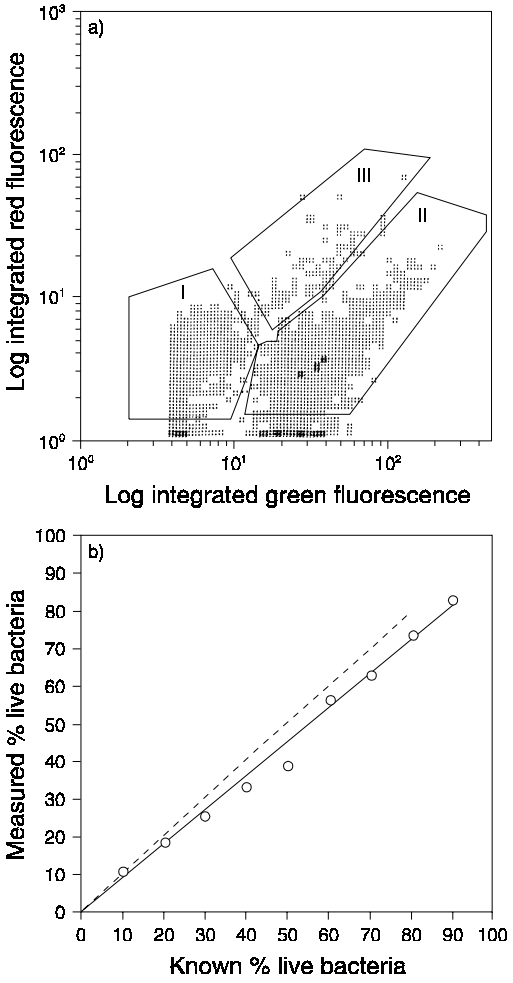

Figure 3. *Analysis of relative viability of E. coli suspensions by flow cytometry. Samples of E. coli were prepared, stained and analyzed as outlined in the text. a) A two-parameter comparison of the green and red components of fluorescence emission of individual bacteria from a population containing 70% "killed" organisms indicates two major regions (I & II) and one minor region (III). The majority of the bacteria are represented by region I (dead cells) and region II (live cells), which have similar red fluorescence intensity and different proportions of green fluorescence. E. coli organisms appearing in region III generally represent less than 5% of the population and are as yet uncharacterized in terms of viability. b) Known viability is defined as the proportion of "live" to "killed" bacteria. Measured viability is defined by the following equation: Measured % live bacteria = (# of bacteria in region II/# of bacteria in regions I+II) × 100. A least-squares fit extrapolated to the 100% "live" point suggested a 13% component of dead bacteria in the "live" population. The upper (dashed) line is corrected for a 13% dead component in the "live" cell suspension.*

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).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
L7007	LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit *for microscopy* *1000 assays*	1 kit
L7012	LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit *for microscopy and quantitative assays* *1000 assays*	1 kit
L13152	LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit *10 applicator sets*	1 kit

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

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Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 ● Fax: (541) 335-0305 ● order@probes.com

Toll-Free Ordering for USA and Canada:
Order Phone: (800) 438-2209 ● Order Fax: (800) 438-0228

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
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