LIFESCT
Life Scientists' Service Center

Email: support@lifesct.com
Tel: 1-240-715-2985 Fax: 240-252-7376
9610 Medical Center Drive, Rockville, MD 20850

LiFluor™ Bacteria Cell Viability Kit

Cat. #: C0028 Size: 1000 rxns

Introduction

LiFlour™ Bacterial Cell Viability Kit provides two-color fluorescence staining on both live (green) and dead (red) bacteria using two probes, SYTO 9 dye and Propidium iodide. SYTO 9 dye is a green-fluorescent nucleic acid dye that stains both live and dead bacteria with intact and damaged cell membranes. Propidium iodide is a red-fluorescent nucleic acid dye that stains only dead bacteria with damaged cell membranes. With an appropriate mixture of SYTO 9 and Propidium iodide, 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.

Package Information

Components	C0028
SYTO 9 dye (Component A)	300 µl, 3.34 mM
Propidium Iodide (Component B)	300 µl, 20 mM

Number of assays: 1,000 tests using a fluorescence microscope or 200 tests using a flow cytometer. Approximate fluorescence excitation/emission maxima, in nm: SYTO 9 dye: 480/500, bound to DNA; Propidium Iodide: 528/617, bound to DNA.

Storage

Store at -20°C and protect from light.

Experimental Protocols

The following protocols are provided as examples to guide researchers in the development of their own bacterial staining procedures. Both gram-positive and gram-negative bacteria have been validated using the following procedures.

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 (OD_{670}) 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.

Note: Care must be taken to remove traces of growth medium before staining bacteria with these kit reagents. Phosphate wash buffers are not recommended because they appear to decrease staining efficiency.

Fluorescence Microscopy Protocol

Staining Bacteria in Suspension

2.1 Combine 1.5 µl of SYTO 9 stock solution and 1.5 µl of Propidium Iodide stock solution in a microcentrifuge tube, and then add 7 µl of 0.85% NaCl solution, mix thoroughly to derive 100× dye mixture solution.

2.2 For each 100 µl of your bacteria sample and live and dead bacteria control suspensions, add 1 µl of 100× dye mixture (prepared in step 2.1).

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.

Note: Best results are obtained by adjusting the dye concentrations to achieve distinct labeling of live cells with SYTO 9 and of dead cells with Propidium Iodide. 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.

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. Typical characteristics of some appropriate filters are summarized in Table 1.

Table 1. Characteristics of common filters suitable for use with SYTO 9 and Propidium Iodide

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
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

LIFESCT
Life Scientists' Service Center

Email: support@lifesct.com
Tel: 1-240-715-2985 Fax: 240-252-7376
9610 Medical Center Drive, Rockville, MD 20850

LiFluor™ Bacteria Cell Viability Kit

Cat. #: C0028 Size: 1000 rxns

Fluorescence Microplate Protocol

3.1 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 2. Volumes of live- and dead-cell suspensions to mix to achieve various proportions of live:dead cells

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

3.2 Mix five different proportions of *E. coli* or S. aureus (Table 1) in 16× 125 mm borosilicate glass culture tubes. The total volume of each of the five samples will be 2 ml.

3.3 Mix 6 µl of Component A with 6 µl of Component B in a microfuge tube.

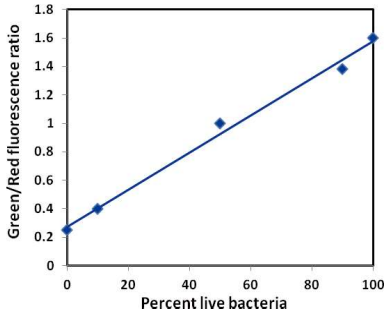
3.4 Prepare a 2× stain solution by adding the entire 12 µl of the above mixture to 2.0 ml of filter-sterilized dH_2O in a 16× 125 mm borosilicate glass culture tube and mix well.

3.5 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.

3.6 Using a new tip for each well, pipet 100 µl of the 2× staining solution (from step 3.4) to each well and mix thoroughly by pipetting up and down several times.

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

3.8 With the excitation wavelength centered at about 480 nm, measure the fluorescence intensity at a wavelength centered at about 510 nm (emission 1; green) for each well of the entire plate.



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 three measurements. The line is a least-squares fit of the relationship between % live bacteria (x) and $Ratio_{G/R}$ (y).

3.9 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.

3.10 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.

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

3.11 Plot the $Ratio_{G/R}$ versus percentage of live cells in the *E. coli* suspension.

Flow Cytometry Protocol

4.1 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.

4.2 Mix 11 different proportions of *E. coli* in 16× 125 mm borosilicate glass tubes according to Table 3. The volume of each of the 11 samples will be 1 ml.

4.3 Mix 20 µl of Component A with 20 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).

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

4.5 Analyze each bacterial sample by flow cytometry using the setting for fluorescein for nucView green positive cells and propidium iodide for propidium iodide positive cells.

Table 3. 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