FilmTracer™ LIVE/DEAD® Biofilm Viability Kit

Catalog no. L10316

Table 1. Contents and storage information.

Material	Amount	Concentratiom	Storage	Stability
SYTO® 9 green fluorescent nucleic acid stain (Component A)	300 µL	3.34 mM solution in dimethylsulfoxide (DMSO)	• ≤–20°C • Protect from light	When stored as directed, the product is stable for at least 1 year.
Propidium iodide (Component B)		20 mM in DMSO		
Number of reactions: Sufficient material is supplied for 100 reactions, based on the protocol below.				
Approximate fluorescence excitation/emission maxima: SYTO® 9 green fluorescent nucleic acid stain: 482/500 nm; propidium iodide: 490/635 nm.				

Introduction

Biofilms present a unique set of challenges for fluorescent staining and subsequent imaging. A typical biofilm not only exhibits heterogeneous thickness throughout the surface, placing stringent restrictions on stain penetration, but also contains regions of widely varying environmental conditions. Evidence suggests that bacterial cells exist in various physiological states within these biofilm microenvironments. Furthermore, biofilms contain many undefined components (*e.g.*, the extracellular polymeric matrix) that differ with species and conditions.

The FilmTracer™ LIVE/DEAD® Biofilm Viability kit provides a two-color fluorescence assay of bacterial viability, based on membrane integrity, that has proven useful for a diverse array of bacterial genera including those growing in biofilm communities. The scientific literature contains references demonstrating the use of the LIVE/DEAD™ staining combination on a wide variety of biofilm organisms including *Acinetobacter baumanni*,[1] *Acinetobacter* sp. BD413,[2] *Actinobacillus actinomycetemcomitans*,[3] *Bdellovibrio bacteriovorus*,[4] *Desulfovibrio vulgaris*,[5] *Enterococcus faecalis*,[6] *Escherichia coli*,[7] *Fusobacterium nucleatum*,[3] *Haemophilus influenzae*,[8] *Helicobacter pylori*,[9] *Legionella pneumophilia*,[10] *Listeria monocytogenes*,[11] *Mycoplasma bovis*,[12] *Mycoplasma putrefaciens*,[12] *Neisseria gonorrhoeae*,[13] non-pigmented mycobacteria,[14] *Novosphingobium stygiae*,[15] *Porphyromonas gingivalis*,[16] *Pseudoalteromonas ruthenica*,[17] *Pseudomonas aeruginosa*,[18] *Pseudomonas veroni*,[15] *Rhodococcus ruber*,[19] *Salmonella enteritidis*,[20,21] *Shewanella putrefaciens*,[5] *Sphingopyxis alaskensis*,[15] *Staphylococcus aureus*,[22] *Staphylococcus aureus* (MRSA),[23] *Staphylococcus epidermidis*,[24] *Streptococcus gordonii*,[3] *Streptococcus mutans*,[25] *Streptococcus pneumoniae*,[26] *Streptococcus pyogenes*,[27] and *Vibrio vulnificus*.[28]

The LIVE/DEAD® Biofilm Viability kit allows researchers to distinguish live and dead bacteria quickly, without waiting for growth plate results. The LIVE/DEAD® Biofilm Viability kit utilizes mixtures of the 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 (*i.e.,* live) stain fluorescent green, whereas bacteria with damaged membranes (*i.e.,* dead) stain fluorescent red. The excitation/emission maxima for these dyes are approximately 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® Biofilm Viability kit 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.

Before You Begin

Materials Required but Not Provided

- Biofilm samples: Biofilms may be grown on coupons in a biofilm reactor, as colony biofilms, in flow-cell system, or in drip-flow reactors. This protocol describes staining biofilms grown on glass coupons in a CDC reactor. For more information on the CDC reactor or other reactor types, refer to the BioSurface Technologies website (www.imt.net/~mitbst/Products.html), contact Center for Biofilm Engineering, Montana State University, Bozeman, Montana, or refer to the standard protocols outlined in the following ASTM methods: ASTM E2647, ASTM E2562, ASTM E2196.
- Fluorescence microscope with appropriate FITC longpass excitation/emission filters. Alternatively, you may view the live (green fluorescent) and dead (red fluorescent) cells separately with fluorescein and Texas Red® bandpass filter sets.
- 0.2 µm filter-sterilized water
- Staining dishes (*e.g.*, 60-mm dish, 6-well plate, etc.)

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. Use both reagents with appropriate care. Handle the DMSO stock solutions 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, pour solutions containing these reagents through activated charcoal before disposal. Afterwards, incinerate the charcoal to destroy the dyes.

Experimental Protocols

Guidelines for Staining

- The following protocol was developed by the Center for Biofilm Engineering Microscopy Facility researchers. Researchers have used this procedure and found it to be simple and reliable for gram-positive and gram-negative bacteria. The protocol describes how to stain biofilms grown on glass CDC reactor coupons. For any other growth surface, you may need to adjust the staining volumes. For additional protocols, refer to the Materials and Methods sections in the published literature (see **References**).
- Unless otherwise noted, keep all fluorescent stains frozen, diluted only in DMSO, until ready for use. Thaw frozen stains quickly, remove desired small volumes, and immediately return vials to the freezer.

- We recommend performing staining in water as the phosphates in buffers may interfere with fluorescent staining.
- If you need to stain and image multiple samples, do not stain more than two samples at a time. Evidence suggests that, in many cases, stain might be drawn from cells over time as they sit in water. Stagger staining, so that samples are stained, rinsed, and imaged following the same schedule. Image immediately following rinsing.
- For imaging biofilm on CDC reactor coupons, use glass coupons only. In particular, avoid polycarbonate coupons for imaging purposes as polycarbonate is autofluorescent, and the rough surface interferes with imaging. After imaging, soak the coupons in ethanol to remove any stain.
- If you follow the protocol below, you do not need to use fixatives on the biofilm.

Staining Procedure

1.1. Prepare working solution of fluorescent stains by adding 3 µL of SYTO® 9 stain and 3 µL of propidium iodide stain to 1 mL of filter-sterilized water. Note that one coupon requires only 200 µL of stain, and once diluted in water, use the staining solution that day or discard it as the dyes are not stable in water.

1.2. Place sample coupon or other sample support material in a staining dish.

1.3. Add 200 µL (or appropriate volume) of staining solution onto the biofilm sample. Add the stain very gently so as not to disturb the biofilm. It is important to immediately add the stain before the biofilm dries.

1.4. Cover the staining dish, and incubate the sample for 20–30 minutes at room temperature, **protected from light**.

1.5. Rinse the sample gently with filter-sterilized water. Remove all excess stain and rinse water from the base of the support material.

1.6. For best results with reactor coupons, place coupon in a 60-mm dish, fill the dish with filter-sterilized water to cover the coupon surface by 1–3 mm, and observe on the microscope using a 40X 0.7NA 3.3 mm WD water objective or a 63X 0.9NA 2.2 mm WD water immersion objective.

References

1. J Bacteriol 190, 1036 (2008); **2.** Water Science & Technology 52, 195 (2005); **3.** J Dent Research 83, 802 (2004); **4.** Appl Environ Microbiol 71, 4044 (2005); **5.** Water SA 28, 123 (2002); **6.** Appl Environ Microbiol 73, 7023 (2007); **7.** Appl Environ Microbiol 72, 7701 (2006); **8.** JAMA 296, 202 (2006); **9.** J Bacteriol 186, 3124 (2004); **10.** Curr Microbiol 57, 497 (2008); **11.** J Food Protection 71, 1379 (2008); **12.** Microbiology 152, 913 (2006); **13.** Infection and Immunity 73, 1964 (2005); **14.** BMC Microbiology 8, 184 (2008); **15.** Lett Appl Microbiol 43, 417 (2006); **16.** J Microbiol Methods 40, 233 (2000); **17.** Biofouling 22, 371 (2006); **18.** Microbiology 151, 2452 (2005); **19.** Appl Microbiol Biotech 72, 346 (2006); **20.** Appl Environ Microbiol 70, 4326 (1997); **21.** Antimicrobial Agents and Chemotherapy 50, 3588 (2006); **22.** Biomaterials 27, 1129 (2006); **23.** J Med Microbiol 57, 966 (2008); **24.** Lett Appl Microbiol 44, 454 (2007); **25.** Appl Environ Microbiol 73, 5633 (2007); **26.** BMC Microbiology 8, 173 (2008); **27.** J Antimicrobial Chemotherapy 57, 1227 (2006); **28.** J Bacteriol 186, 889 (2004).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
L10316	FilmTracer™ LIVE/DEAD® Biofilm Viability Kit	1 kit
Related Products		
F10317	FilmTracer™ FM® 1-43 green biofilm cell stain	1 mg
F10318	FilmTracer™ SYPRO® Ruby biofilm matrix stain	200 mL
F10319	FilmTracer™ calcein red-orange biofilm stain	20 × 50 µg
F10320	FilmTracer™ calcein violet biofilm stain	20 × 25 µg
F10322	FilmTracer™ calcein green biofilm stain	20 × 50 µg
F35355	FM® 1-43FX *fixable analog of FM® 1-43 membrane stain*	10 × 100 µg
L34952	LIVE/DEAD® *Funga*Light™ Yeast Viability Kit *for flow cytometry*	1 kit
S34854	SYTO® 9 green fluorescent nucleic acid stain *5 mM solution in DMSO*	100 µL

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