

FilmTracer™ FM® 1-43 Green Biofilm Cell Stain

Catalog no. F10317

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
FilmTracer™ FM® 1-43 green biofilm cell stain	1 mg	<ul style="list-style-type: none"> • 2–25°C • Desiccate • Protect from light 	When stored as directed, the product is stable for at least 6 months.
Number of reactions: Sufficient material is supplied for 500 reactions, based on the protocol below.			
Approximate fluorescence excitation/emission maxima: FilmTracer™ FM® 1-43 green biofilm cell stain: 472/580 nm.			

Introduction

Bacterial 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.

FM® dyes are lipophilic styryl compounds used in a wide variety of studies involving plasma membrane and vesiculation. The water-soluble FM® dyes are virtually nonfluorescent in aqueous media, and are believed to insert into the surface membrane where they become intensely fluorescent. FM® 1-43 stain has been used successfully to stain the cell bodies specifically in a complex biofilm milieu, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus sp.*, *Acidithiobacillus caldus*, and *Vibrio cholerae*.^{1,2}

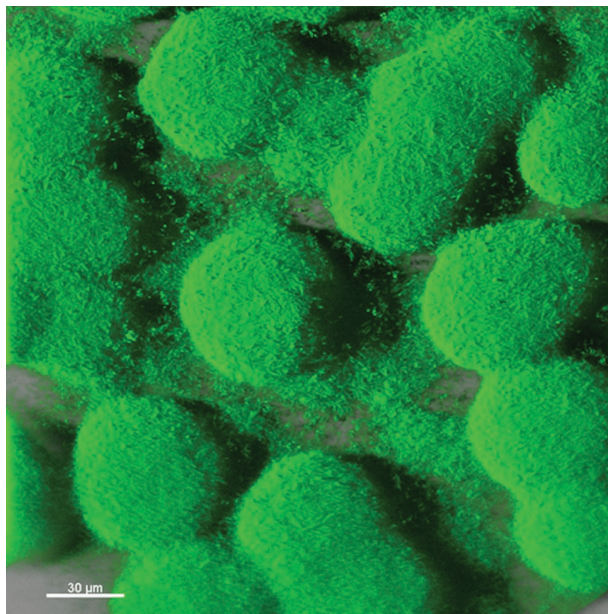


Figure 1. FM[®] 1-43 applied to a *Pseudomonas aeruginosa* biofilm. FM[®] 1-43 appears to bind to the cell membrane, and has been shown to work equally well on *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Escherichia coli*, exhibiting exceptional cell specificity in each case. The image was obtained using a Leica TCS-SP2 AOBS confocal microscope and a 63×/0.9 NA water immersion objective.

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 excitation/emission filters
- 0.2 μm filter-sterilized water
- Staining dishes (*e.g.*, 60-mm dish, 6-well plate, etc.)
- Anhydrous dimethylsulfoxide (DMSO)

Preparing Reagents

Add 100 μL DMSO to the contents of the vial. Mix thoroughly until dissolved. The stock solution in DMSO may be stored at 4°C; do not store the staining solution that is diluted in water. Dispose of dyes according to local regulations.

Caution

FilmTracer™ FM[®] 1-43 stain is potentially harmful, and may cause irritation by inhalation. Avoid prolonged or repeated exposure. Wear appropriate gloves, protective clothing and eyewear and follow safe laboratory practices. Wash thoroughly after handling. If eye or skin contact occurs, wash affected area with water for 15 minutes and seek medical advice. If inhaled, move individual to fresh air and seek medical advice. If swallowed, seek immediate medical advice.

Use appropriate protective equipment and methods to clean up spilled substances promptly. Absorb spill onto an appropriate material. Collect and dispose of all waste in accordance with applicable laws.

Experimental Protocols

Guidelines for Staining

- 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.
- If you follow the procedure below, you do not need to use fixatives on the biofilm.

Staining Procedure

The protocol below describes how to stain biofilms grown on glass CDC reactor coupons. For any other growth surface, you may need to adjust the staining volumes.

- 1.1. Prepare staining solutions by diluting 10 μL stock solution into 990 μL DMSO, followed by diluting 100 μL into 0.9 mL filter-sterilized water. We recommend a final staining concentration of 1 $\mu\text{g}/\text{mL}$.
- 1.2. 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.
- 1.3. Incubate the sample for up to 30 minutes at room temperature, **protected from light**.
- 1.4. Rinse the sample gently with filter-sterilized water. Remove all excess stain and rinse water from the base of the support material.
- 1.5. 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. Microscopy Today 16, 18 (2008); 2. J Bacteriol 190, 311 (2008).

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

Cat. no.	Product Name	Unit Size
F10317	FilmTracer™ FM® 1-43 green biofilm cell stain.....	1 mg
Related Products		
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
L10316	FilmTracer™ LIVE/DEAD™ Biofilm Viability Kit.....	1 kit
S34854	SYTO® 9 green fluorescent nucleic acid stain.....	100 µL

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