FilmTracer™ SYPRO® Ruby Biofilm Matrix Stain

Catalog no. F10318

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
FilmTracer™ SYPRO® Ruby biofilm matrix stain	200 mL	• 2–25°C • Desiccate • Protect from light • DO NOT FREEZE	When stored as directed, the product is stable for at least 9 months.
Number of reactions: Sufficient material is supplied for 1000 reactions, based on the protocol below.			
Approximate fluorescence excitation/emission maxima: FilmTracer™ SYPRO® Ruby biofilm matrix stain: 280, 450/610 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.

SYPRO® Ruby stain labels most classes of proteins, including glycoproteins, phosphoproteins, lipoproteins, calcium binding proteins, fibrillar proteins, and other proteins that are difficult to stain.[1] SYPRO® Ruby stain has been tested to stain the matrix of *Pseudomonas aeruginosa* (ATCC 15442) and some strains of *E. coli* (not *E. coli* K-12, which does not produce cellulose). Staining patterns may vary depending upon the organism and the matrix composition.

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

Preparing Reagents

The FilmTracer™ SYPRO® Ruby biofilm matrix stain comes as a ready to use stain and does not require further dilution.

Caution

SYPRO® Ruby biofilm matrix stain is characterized as an irritant due entirely to the solvent system and buffer salts in the product. The heavy metal component of the stain (ruthenium) is not a regulated hazardous heavy metal in the United States. Independent toxicity tests found SYPRO® Ruby stain to be nontoxic at 5000 mg/kg in Sprague-Dawley® rats. Nevertheless, SYPRO® Ruby stain should be handled with care, consistent with good laboratory practices.

Federal regulation in the US does not consider ruthenium or the solvent system and buffers in SYPRO® Ruby biofilm matrix stain to be hazardous waste. Nevertheless, the solvent may be subject to more stringent regulations local to the user, so we recommend that you mix used staining solution with flammable waste and dispose by destructive incineration and not by sewerage or landfill.

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 protocol 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. 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. Immediately add the stain before the biofilm dries.

1.2. Incubate the sample for up to 30 minutes at room temperature, **protected from light**.

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

1.4. 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. Electrophoresis 21, 2509 (2000).

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

Cat. no.	Product Name	Unit Size
F10318	FilmTracer™ SYPRO® Ruby biofilm matrix stain	200 mL
Related Products		
F10317	FilmTracer™ FM® 1-43 green biofilm cell stain	1 mg
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
L10316	FilmTracer™ LIVE/DEAD™ Biofilm Viability Kit	1 kit
S34854	SYTO® 9 green fluorescent nucleic acid stain *5 mM solution in DMSO*	100 µL

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