Bacterial Biofilm Matrix Stain

Introduction

Biofilms present significant challenges for fluorescent labeling and subsequent imaging due to their complex architecture. These microbial communities exhibit heterogeneous thickness across surfaces, creating barriers to stain penetration. Within biofilms, bacterial cells exist in diverse physiological states influenced by varying microenvironmental conditions. Additionally, biofilms contain numerous undefined components, such as the extracellular polymeric matrix, whose composition varies with species and growth conditions.

Bacterial Biofilm Matrix Stain is designed for labeling biofilms. This stain effectively labels a broad spectrum of protein classes, including glycoproteins, phosphoproteins, lipoproteins, calcium-binding proteins, and fibrillar proteins. Bacterial Biofilm Matrix Stain exhibits excitation and emission maxima at 450 nm and 610 nm, producing a red fluorescence signal. It has been validated for staining the matrix of Pseudomonas aeruginosa and certain E. coli strains. The product is a valuable tool for comprehensive biofilm analysis.

Storage

Store at room temperature away from light and moisture, stable for at least 9 months.

Protocol

Here we take biofilms grown on glass CDC reactor coupons as an example, and other types of staining can be optimized depending on the specific experiment.

1. Gently add 200 μL (or other appropriate volume) of Bacterial Biofilm Matrix Stain to the biofilms without disturbing the biofilms. Add quickly and do not allow the biofilms to dry.
2. Incubate at room temperature for up to 30 min, protected from light. The optimal incubation time can be adjusted according to the specific experiment.
3. Gently rinse the sample with filter-sterilized water. Remove the staining solution and rinse water from the base of the supporting material.
4. Gently place the coupon in a 6 cm dish and gently add filter-sterilized water to cover the coupon surface by 1-3 mm. Subsequent observe using a water immersion objective (Ex/Em=450/610 nm).

***Note:**

a) Staining in water is recommended as phosphates in the buffer may interfere with staining.

b) If there are multiple samples to be stained, it is recommended to stain only 1-2 samples at a time. Because stain might be

drawn from cells over time as they sit in water. Image immediately as soon as possible after rinsing.

c) When using CDC reactor coupons, it is recommended to use glass coupons rather than polycarbonate coupons, as polycarbonate has autofluorescence and a rough surface that can interfere with imaging.

Note

1. This product contains irritating substances. Please pay attention to protection when using.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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