BIOLUMINOR

TRITC-d-Lys

Table 1 Contents and storage

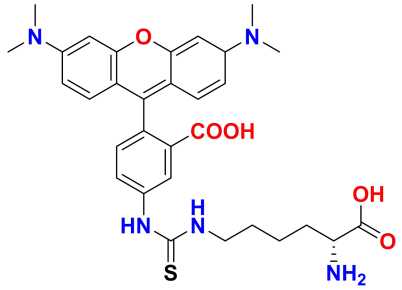
Material	Amount	Concentration	Storage	Stability
TRITC-d-Lys	5mg	0.1 mM stock solution in anhydrous DMSO	• ≤ –20°C • Desiccate • Protect from light	

Spectral characteristic of the fluorescent probe: **Ex~560, Em~580**

Introduction

By the use of the peptidoglycan biosynthetic machinery that metabolic incorporating various non-natural d-amino acids into the peptidoglycan of diverse bacteria. A chemical biology approach that enable rapid and covalent incorporation and detection of a fluorescently derivatized peptidoglycan component during cell wall synthesis in real time, in a wide range of live bacterial species. We employ metabolic incorporation of d-lysine conjugated Tetramethylrhodamine isothiocyanate (TRITC) into bacterial peptidoglycan for *in situ* probing live bacteria.

The probe is made of a Tetramethylrhodamine and d-Lysine (Fig 1), bacterias building its cell wall by the use of d-amino acids so as attache the fluorescein into the cell wall covalently. The probe not only can label gram-negative bacteria but also can label gram-positive bacteria in real time. It can be used for research and exploration infection and pathogenesis of microorganisms.



Chemical Formula: $C_{31}H_{37}N_5O_5S$

Exact Mass: 591.2515

Fig 1. Chemical structures of TRITC-d-Lys

Guidelines for Use

Before opening, allow the vial to warm to room temperature and then briefly centrifuge the vial in a micro centrifuge to deposit the DMSO solution at the bottom of the vial.

The concentration of probe for optimal staining will vary depending on the application. Here we suggest some initial conditions to use as a guideline. The staining conditions may need to be modified depending upon the particular bacteria type to the probe, among other factors.

1.1 *In vitro* staining TRITC-d-Lys into *E. coli* or *S. aureus*

E. coli or *S. aureus* were respectively grown at 37 °C in LB medium until OD_{600} reached 0.6. The medium was diluted to OD_{600}=0.3 with fresh medium containing TRITC-d-Lys (Final concentration: 0.1 mM, usually adding 10 μL of stock solution to per mL cell medium). The diluted bacteria were further incubation at 37°C until OD_{600} = 1.0-1.5. The bacteria were centrifuged, washed with LB medium three times, and then resupended in sodium phosphate buffer (100 mM, pH 7.4) or cell culturing medium of interest. The cells were subjected to confocal fluorescence microscopy analysis .

(10 mM stock solution of TRITC-d-Lys: 5 mg in 0.845 ml water or DMSO)