

Protocol III: Spectrophotometric monitoring of biofilm formation in bacterial cultures

This protocol describes how to trace the formation of biofilm in bacterial cultures using the bacterial amyloid curli and the carbohydrate cellulose as biofilm markers. As ECtracer™ doesn't influence biofilm formation when used in recommended concentrations, it can be present in growing cultures. We have tested ECtracer™ for tracing biofilm produced by *Salmonella Enteritidis* and *Salmonella Typhimurium* during growth. For these strains we have not observed staining of intracellular or membrane components.

Solutions and Reagents:

ECtracer™ is provided as 1000-fold concentrated solution. The following common reagents are required (not supplied):

- Growth medium
- Phosphate buffered saline (PBS), pH 7.4
- 96-well plate (round bottom) with cover
- Deionized water
- Spectrophotometer

Assay Procedure:

- Dilute ECtracer™ in growth medium 1:1000
- Inoculate supplemented growth medium with bacterial culture.
- Fill the wells of the 96-well plate with 100 µl inoculated medium.
- Fill unused wells with sterile water to avoid drying during incubation.
- Seal the plate with a cover or adhesive seal.
- To maximize the temporal resolution when monitoring the biofilm formation, we recommend to incubate the 96-well plate directly in the spectrophotometer. Alternatively, position the 96-well plate in a standard incubator and move it to the spectrophotometer at regular time intervals for recording.

Spectrophotometer Settings:

- **ECtracer™480 (part of ECtracer™ Mix&Try Kit):** Excite at 430 nm and collect emission at 480 nm. Optional: Record an emission spectrum (450 - 700 nm) with 430 nm excitation.
- **ECtracer™520 (part of ECtracer™ Mix&Try Kit):** Excite at 470 nm and collect emission at 530 nm. Optional: Record an emission spectrum (490 - 700 nm) with 470 nm excitation.
- **ECtracer™630 (part of ECtracer™ Mix&Try Kit):** Excite at 510 nm and collect emission at 635 nm. Optional: Record an emission spectrum (530 - 800 nm) with 510 nm excitation.
- **ECtracer™680:** Excite at 540 nm and collect emission at 680 nm. Optional: Record an emission spectrum (560 - 800 nm) with 540 nm excitation.

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