Protocol III: Biofilm tracking in live cultures using EbbaBiolight 680

This protocol describes how to trace the formation of biofilm in bacterial cultures using EbbaBiolight 680 which binds to the biofilm markers curli and cellulose. As EbbaBiolight 680 does not influence biofilm formation when used in recommended concentrations, it can be present in growing cultures. We have tested EbbaBiolight 680 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. Please make sure that the pH remains constant during your growth experiment. EbbaBiolight 680 works best at pH 7.4

Solutions and Reagents:

EbbaBiolight 680 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 EbbaBiolight 680 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:

- EbbaBiolight 680: Excite at 540 nm and collect emission at 680 nm. Optional: Record an emission spectrum (560 - 800 nm) with 540 nm excitation.