Protocol IV: Biofilm quantification in colony re-suspensions using EbbaBiolight 680

This protocol describes how to quantify the biofilm markers curli and cellulose in colony re-suspensions using EbbaBiolight 680. We have tested this procedure with Salmonella Enteritidis and Salmonella Typhimurium strains. For these strains we have not observed staining of intracellular or membrane components.

Solutions and Reagents:

EbbaBiolight 680 is provided as 1000-fold concentrated solution. The following common reagents are required (not supplied):

- Agar plates
- Phosphate buffered saline (PBS), pH 7.4
- 96-well plate (round bottom)
- Spectrophotometer

Assay Procedure:

- Grow bacterial colonies on an agar plates under biofilm forming conditions. Notice: no morphotyping is required for this procedure.
- Dilute EbbaBiolight 680™ in PBS 1:1000
- Add 100 μl into each well of a 96-well plate.
- Pick bacterial colonies from the agar plate and resuspend thoroughly into each of the pre-filled wells.
- Place the plate in a spectrophotometer to quantify biofilm in the colony resuspensions.

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.