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Protocol IV: Spectrophotometric quantification of biofilm in colony resuspensions

This protocol describes how to quantify the biofilm markers curli and cellulose in colony re-suspensions. 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:

ECtracer™ 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 ECtracer™ 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:

- **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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