Protocol II: Optotracing of biofilm in tissue sections using EbbaBiolight 680

This protocol describes how to stain for the biofilm markers curli and cellulose in tissue sections. **EbbaBiolight** can be used to stain tissue sections prepared by the most common techniques like paraffin embedding and freezing. We recommend fixation in ice-cold ethanol, but fixation in 4% paraformaldehyde works as well. 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. As **EbbaBiolight** is only fluorescent when bound, you can consider to omit washing steps when working with sensitve tissues.

Solutions and Reagents:

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

- Ethanol, 95% (-20°C) or 4% PFA
- Phosphate buffered saline (PBS), pH 7.4
- Deionized water
- Glass coverslips
- Mounting medium

Assay Procedure:

- Fix infected cells or tissue sections with method of choice. We recommend fixation with ice-cold ethanol (5 min) at room temperature.
- Rehydrate tissue sections in a mix of ethanol and deionized water (1:1) for 5 min. The rehydration step may need to be repeated with lower ethanol ratio depending on the tissue.
- Equilibrate sections in PBS for 5 min.
- Dilute EbbaBiolight in PBS 1:1000.
- Apply diluted EbbaBiolight generously. Use enough liquid (ca 0.5 ml) to prevent the sections from drying out during incubation. Incubate for 30 min.
- Wash 2 x 5 min in PBS (Optional).
- Mount tissue sections and seal the coverslip onto the slide to prevent drying.

Fluorescence Microscopy:

- EbbaBiolight 680 is excited at 561 nm (standard laser line) and emission is detected using a standard PI (Propodium Iodide), mCherry or Cy3.5 filter set. Optional: An excitation range of 530-565 nm and a detection range of 600-800 nm may be applied depending on available laser lines and filter sets.

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