## **Biofilm Assay**

- 1. 96 well plate was filled with LB Broth 198 uL
- 2. Add pathogen bacteria 2 uL and incubate overnight in static incubator
- 3. Well was filled crystal violet 0,5% 200 uL → incubate 10 minute
  4. Crystal violet was took out → put in to the bottle = reused crystal violet
- 5. Wash the well, soak it in the water and wah for 4 times
- 6. Well is contain crystal violet that pinned in biofilm
- 7. Well was filled 96% ethanol (v/v) and acetate acid 2% 96% absolute ethanol + 2%  $CH_3COOH(v/v)$ 96 uL absolute ethanol + 2 uL acetate acid + 2 uL  $H_2O$
- 8. Wait for 2 minutes and bring it into pass through box
- 9. Elisa reader