# Whole Blood Assay Protocol

1. Start bacterial overnight culture (16-18 hrs) in 5ml lysogeny broth (LB) at 37ºC for 16-18 hours
2. Sub-culture bacteria from the overnight culture 1:30 in LB
3. After 2 hrs, take 1ml of the subculture in a 1.5ml eppendorf tube and wash bacteria:
    a. Centrifuge 12,000rpm for 2 mins
    b. Get rid of supernatant.
    c. Resuspend the pellet in 1ml PBS
    d. Centrifuge 12,000rpm for 2 mins
    e. Resuspend the pellet in 1ml RPMI
4. To measure OD, dilute bacteria 1:10 (100ul of the washed bacteria + 900ul media). First blank the spectrophotometer with pure RPMI at OD 600nm. Then measure the OD of the diluted bacteria.

5. Calculate the MOI appropriate for the whole blood assay in the volume of 125ul. Assume 1 million cells/ml in whole blood.
    - MOI = multiplicity of infection, MOI 1 = 1 bacteria per 1 cell, MOI 10 = 10 bacteria per 1 cell for example

6. Prepare LPS for use as positive control:
    a. Dilute sonicated rough *E. coli* J5 LPS (Sigma, stock = 5mg/ml) to 0.1ng/ml in RPMI – total volume 125ul
7. If using BPI:
    a. Pre-incubate whole blood with desired concentration of BPI (diluted in RPMI) for 30min at 37ºC before adding stimulant (bacteria/LPS)
    b. Note: FINAL volume of BPI + stimulant = 125ul
8. Draw blood from a consenting healthy donor using a heparin-coated collection tube
9. Add 125ul of bacteria or LPS to the designated well of a 96-well flat bottom plate at the desired MOI (1 and 10)
10. Aliquot 125ul of blood into each well
11. Incubate for 4 hrs at 37ºC
12. Centrifuge the plate at 2500rpm for 5 mins
13. Save the supernatant (1.5 ml eppendorf tube or 96-well plate) and store in -80ºC until use in TNF-alpha-ELISA.
14. Measure TNF-alpha by using a human TNF-a kit from R&D Systems (catalog: DY210) according to the protocol provided by R&D Systems
    a. Note: use samples at 1:5 dilution in the ELISA