NET quantification protocol using SYTOX Green (Life Technologies)

- PMNs are isolated from pellet using Ficoll gradient, followed by sedimentation using 5% dextran.
- PMNs are resuspended at 4 million/ml in DMEM.
- Cells are plated in 96 wells of a black plate – 200K cells in a volume of 50 ul.
- Bacteria are added to the appropriate wells (50-100ul); *Usually have triplicates for each sample.
    - Positive control: 0.5% Triton X for 1min
    - Negative controls: DMEM alone, Untreated PMNs
- Plate is incubated at 37 degrees for 2 hours
- Sytox green is added to the wells at 5uM (15ul). Protein standards also added.
- Fluorescence measured in 5-10 minutes on the fluorometer. (485/528)