Anti-PA14 IgG ELISA Protocol

Day #1

1. **Bacterial Lysis and Protein Assay**
    a. Transfer 1ml of bacterial sub-culture into 1.5ml Eppendorf
    b. Spin for 5' at 12,000 rpm
    c. Wash with 1 ml PBS; spin for 5' at 12,000 rpm
    d. Freeze and thaw the bacterial pellet ~3 times using dry ice
    e. Re-suspend the pellet in 100ul PBS and freeze thaw ~3 times
    f. Add another 900ul PBS, re-suspend well and measure protein concentration using Micro BCA Protein Assay Kit (dilute sample 1:100 or 1:10)
2. **ELISA- Coating and Blocking**
    a. Coat each well with 100ul PA14 lysate (10ug/ml, diluted in PBS) or PBS alone
    b. Incubate for 2hr at 37C
    c. Wash 3X with PBS + 0.05% Tween
    d. Add 300ul PBS+5% Normal Goat Serum (NGS) and incubate o/n at RT

Day #2

3. **ELISA Development**
    a. Wash coated ELISA plate 3X with PBS + 0.05% Tween
    b. Add 100ul patient serum diluted 1:100 in PBS+1% NGS
    c. Incubate for 1hr at RT
    d. Wash 3X with PBS + 0.05% Tween
    e. Add 100ul goat-anti-human HRP-labeled 2' ab (1:50,000 dil. in PBS+1% NGS) and incubate for 1hr at RT; take out Substrate Reagents and Stop soln. to warm up at RT, keep in the dark.
    f. Wash 3X with PBS + 0.05% Tween
    g. Add 100ul substrate (R&D, Substrate Reagent A: Substrate Reagent B 1:1)
    h. Incubate at RT in the dark for ~15min
    i. Add 50ul stop solution and read absorbance at 450nm and 570nm (for correction).