Stamatatos Lab
ELISA Protocol

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1. 50ng of protein in 100 μL of NaHCO3 (100mM, pH 9.0) is added to each well of a high binding 96 well plate, incubate O.N. at RT
2. Block the plate with 250ul/well of 3% BSA in PBS, incubate 2hrs at RT
3. Serial dilute the Mab or Sera in PBS + 1% BSA + 0.03% Tween20 (dilution buffer) on the blocked ELISA plate, incubate 1hr at 37^{o}C
4. Wash the plate 4 times
5. Dilute the HRP conjugated secondary Ab 1:3,000 in dilution buffer and add 100ul to each well, incubate 1hr at 37^{o}C
6. Repeat the wash step
7. Add 50μL per well of TMB substrate, incubate 3 min at RT
8. Stop development by adding an equal volume of 1N H_2SO_4
9. Absorbance is measured at 450 nm

To determine the avidity: sera diluted to 1:500 is added to the blocked plate at step 3 and serially diluted ammonium thiocyanate, ranging from 10M to 1M, is then added to the diluted sera and incubated at RT for 20 min on a plate shaker