Sandwich ELISA is a sensitive and accurate way of quantifying antigen concentration in a certain sample type. It is named sandwich ELISA because the antigen of interested is captured by two different antibodies which target the different epitopes within the same protein. The concentration is then quantified by measuring the color change caused by the enzymatic reactions using a spectrometer.

Material:

- 96-well ELISA plate reader
- 96-well microtiter plate (Costar 3369 (Corning) - EIA/RIA Plate)
- Capture antibodies and detection antibodies
- Coating buffer: 1X PBS
- 10X PBS: 1.37M NaCl, 27mM KCl, 100mM Na_2HPO_4, 20mM KH_2PO_4. Adjust pH to 7.4
- Wash Buffer: 1X PBS + 0.005% Tween-20
- Blocking Buffer: 1X PBS + 1%BSA

Procedures:

1. Coat 96-well ELISA plate with antibody overnight at room temperature (RT) or 4℃ for 1-2 days.
   ※ *Antibody should be coated with the concentration of 1-10 µg/ml with 50 µl/well diluted in PBS.*
2. Wash antibody coated plate 3 times with PBST (PBS+0.005% Tween20).
3. Block with 1%BSA in PBS for 30min at RT (200 µl/well).
4. Prepare standard serial dilutions in PBS.
5. Add samples and standards into microtiter plate (50 µl/well) and incubate for 2hrs at RT.
6. Wash 3 times with PBST.
7. Add biotinylated Capture antibody and incubate for 1hr at RT.
   ※ *Concentration at 1-10 µg/ml with 50 µl/well in diluted PBS.*
8. Wash 5 times with PBST.
9. Add strepavidin-HRP in PBST (50 µl/well) and incubate for 45min at RT.
10. Wash 5 times with PBST.
11. Add TMB substrate: 50 µl/well and watch for color change.
12. Stop reaction with 0.5 M H_2SO_4 (Stop Solution) at 50 µl/well.