**ELISA (enzyme linked immunosorbent assay)**

**Materials**
- High binding ELISA plates. For example Disposable Sterile ELISA Plates from CORNING (Disposable Sterile ELISA Plates, Cat # 25801).
- Antigen
- Primary antibody
- HRP-conjugated secondary antibody
- Phosphate buffered saline (PBS)
- Wash solution: PBS/0.05% Tween20
- BSA blocking solution: PBS/Tween, 1.0% bovine serum albumin
- BSA incubation solution: PBS/Tween, 0.1% BSA
- Substrate: BD OptEIA Substrate Reagent A, Substrate Reagent B (BD Biosciences, Cat#51-2606KC)

**Coating the Antigen:**
- Dilute the antigen in 1X PBS to 2-10 ug/ml.
- Add 100ul per well. Incubate the plate for 1 hour at 37°C or at 4°C overnight. (Cover the plates with lid or parafilm)
- Rinse wells 3X with PBS/Tween 20, using squirt bottle.

**Blocking**
- Add 250ul of 1% BSA Blocking solution in each well 1 hour at room temperature (RT°C).
- Rinse 3X with PBS/Tween.

1. **Antibody**
   - Dilute primary Ab to appropriate concentration in BSA incubation solution (1:500 – 1:5000).
   - Add 100ul to each well, incubate for 1 hour at RT°C. (Do every test in duplicates, also the controls).
   - Rinse 3X with PBS/Tween.

2. **Antibody**
   - Dilute HPR-conjugated secondary Ab to 1:2500 – 1:10000 in 0.1% BSA incubation solution.
   - Add 150ul per well for 1 hour at RT°C.
   - Rinse 4X with PBS/Tween (it's necessary to wash very carefully with PBS after this incubation to avoid unspecific staining because of free Peroxidase-conjugated antibodies)

**Development**
- Mix equal volume of substrate-A and substrate-B, add 100ul to each well for 20-30 min.
- Stop the reaction with 50ul of 2M H₂SO₄.
- Measure the plates at 450 nm with an ELISA reader.