**Indirect ELISA**

1. **Coat plate with antigen**
   - 2 hr RT or 4°C overnight

2. **Wash plates** with PBS 0.2% Tween 20 4 times

3. **Block** with 5% serum or BSA 2 hr or overnight 4°C

4. **Incubate with primary antibody**
   - 2 hr RT or 4°C overnight

5. **Wash plates** with PBS 0.2% Tween 20 4 times

6. **Add conjugated secondary antibody**
   - Incubate 1 - 2 hr

7. **Wash plates** with PBS 0.2% Tween 20 4 times

8. **Enzymatic detection**
   - Follow manufacturers recommendations

9. **Substrate**
   - Colored product

10. **Read absorbance** on ELISA plate reader and analyze results