# Indirect ELISA protocol

Statement: This experimental protocol serves as a general guidance document, and the reagents mentioned herein may be substituted according to specific circumstances. Regardless of the situation, we strongly recommend conducting preliminary experiments prior to formal implementation to verify the applicability and feasibility of the protocol within your laboratory environment.

**Coating**

1. Dilute the antigen to a final concentration of 10 μg/mL in carbonate buffer, 100 μL per well. Cover the plate with a piece of self-adhesive sealing film for microplate and incubate at 4 ˚C overnight.
2. Wash the plate 3 times with 1x PBS 0.1% (v/v) Tween 20. Remove any remaining wash buffer by patting the plate on a paper towel.

**Blocking**

3. Block the remaining protein-binding sites in the coated wells by adding 300 μL blocking buffer (1%~3% BSA-PBS). Cover the plate with a piece of self-adhesive sealing film for microplate and incubate at 37℃ for 1 hour or room temperature for 2 hours.
4. Wash the plate 3 times with 1x PBS 0.1% (v/v) Tween 20. Remove any remaining wash buffer by patting the plate on a paper towel.

**Antibody Incubation**

5. Dilute the primary antibody appropriately in 1x PBS (or 0.02%~0.2% BSA-PBS), 100 μL per well. Cover the plate with a piece of self-adhesive sealing film for microplate and incubate at 37℃ for 1 hour.

*Note: Although 1 hour is usually enough to obtain a strong signal, if a weak signal is obtained, stronger staining will often observed when incubated overnight at 4°C.*

6. Wash the plate 3 times with 1x PBS 0.1% (v/v) Tween 20. Remove any remaining wash buffer by patting the plate on a paper towel.
7. Dilute the HRP conjugated secondary antibody at the optimal concentration (according to the manufacturer) in 1x PBS (or 0.02%~0.2% BSA-PBS), 100 μL per well. Cover the plate with a piece of self-adhesive sealing film for microplate and incubate at 37℃ for 30 min.
8. Wash the plate 3 times with 1x PBS 0.1% (v/v) Tween 20. Remove any remaining wash buffer by patting the plate on a paper towel.

**Detection**

9. Add 100 μL of the TMB substrate solution to each well, incubate at 37°C for 10 min, protected from light.

*Note: Please observe carefully during the chromogenic reaction to avoid underdevelopment or overdevelopment..*

10. Add 50 μL of stop solution to each well to terminate the chromogenic reaction.
11. Read the absorbance at 450 nm and 630 nm for each well using a microplate reader.