Protocol for Direct ELISA

Materials

Affinity purified rabbit IgG or Biotin labeled affinity purified rabbit IgG

HRP-conjugated Rabbit anti-IgG or HRP-streptavidin

HRP substrate TMB: Kirkegaard & Perry Laboratories, 50-76-02 and 50-65-02) or equivalents

Phosphate buffered saline-Tween 20 solution (PBS-T): 8.0 g sodium chloride, 1.3 g dibasic sodium phosphate, 0.2 g monobasic sodium phosphate in 1.0 liter distilled water, pH 7.4 containing 0.05 % (v/v) Tween-20

Non-fat dry milk

Method

1. Coat ELISA plate (96 well plate) with testing antigen (10 µg/ml to 0.01 ng/ml in 50 mM Na_2C0_3, pH 9.6, adjust based on the reactivity of antibody, 100 µl/well. Seal the plate and incubate overnight at 4°C.

2. Wash plate 3 times with PBS-T (0.05 % Tween-20 in PBS).

3. Block plate with 0.2% non-fat dry milk in PBS at room temperature for 1 hour at 4°C overnight.
   ** Milk should be thoroughly dissolved. It's recommended to dissolve 0.5 g milk in 50 ml PBS (1%) for at least 30 minutes at room temperature with rotation or stirring, then dilute to 0.2 %, and keep rotating or stirring for another 10-15 minutes at room temperature.*

   ***If high background is experienced, 1% milk in PBS could be applied for both blocking and antibody dilution.*

4. Wash plate 3 times with PBS-T.

5. (a) Incubate with biotinylated, affinity-purified rabbit IgG (0.1-0.5 µg/ml in PBS, 100 µl/well) at

room temperature for 1 hour, followed by washing 6 times with PBS-T, then go to 8a.

(b) Alternatively, incubate with affinity purified rabbit IgG (0.2-1 μg/ml in PBS, 100 μl/well) at room temperature for 1 hour, followed by washing 6 times with PBS-T, then go to 8b.

6. (a) Incubate with HRP-Streptavidin (1:4000-10,000 dilutions) in 0.2 % milk-PBS, 100 μl/well, at room temperature for 1 hour.

(b) Incubate with HRP-anti-rabbit IgG (1:3,000-10,000 dilutions of 1 mg/ml or 0.25 μg/ml) in PBS, 100 μl/well, at room temperature for 1 hour.

7. Wash plate 8 times with PBS-T.

8. Develop color using TMB as a substrate (100 μl/well) and incubate at room temperature for 15-30 minutes without shaking.

9. Stop reaction by addition of 2N H_2S0_4 (100 μl/well). Record the absorbance at 450 nm on a plate reader within 30 minutes of stopping the reaction.