



## **Sandwich ELISA Protocol**

### **Materials**

**Affinity-purified rabbit polyclonal or monoclonal IgG as capturing antibody**

**Affinity-purified rabbit polyclonal IgG or biotin labeled affinity purified rabbit IgG as detecting antibody**

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

**HRP-Conjugated anti-rabbit IgG or HRP-Conjugated Streptavidin**

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

**Non-fat dry milk**

### **Method**

1. Coat ELISA plate (96 well plate) with affinity-purified rabbit IgG capturing antibody (4 µg/ml in 50 mM Na<sub>2</sub>CO<sub>3</sub>, pH 9.6), 100 µl/well. Seal the plate and incubate overnight at 4°C.
2. Wash plate 5 times with PBS-T.
3. Block plate with 0.2% non-fat dry milk in PBS at room temperature for 1 hour or at 4°C overnight.

*\*Milk should be thoroughly dissolved. It's recommended to dissolve 0.5 g milk in 50 ml PBS 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. Incubate with testing antigen (100 ng/ml to 0.001 ng/ml in PBS), 100 µl/well, at room temperature for 1 hour.
6. Wash plate 6 times with PBS-T
7. (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.
8. (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.
9. Wash plate 8 times with PBS-T.
10. Develop color using TMB as a substrate (100 µl/well) and incubate at room temperature for 15-30 minutes without shaking.
11. Stop reaction by addition of 2N H<sub>2</sub>SO<sub>4</sub> (100 µl/well). Record the absorbance at 450 nm on a plate reader within 30 minutes of stopping the reaction.