

## ELISA: Sandwich ELISA Protocol - Standard

## **Materials**

- Maxisorb 96-well plate
- Specific antibody from species A as capture antibody
- · Specific antibody from species B or biotinylated antibody from species A as detector antibody
- Goat anti-species B IgG peroxidase (HRP) conjugated or Streptavidin peroxidase (HRP) conjugated
- · Microplate shaker
- Microplate absorbance reader with filters at 450 nm and a reference wavelength (e.g. 620-650 nm)

## Reagents

- Coating buffer: 0.1 M Na-carbonate, pH 9.6 (store 0.5 M stock at -20°C)
- Washing buffer: Tris buffered saline (TBS) with 0.05% Tween 20 (TBST)
- Blocking buffer: 5% skimmed milk in TBST
- Substrate solution: Tetramethylbenzidine (TMB) reagent for development
- Stop solution: 0.25 M H<sub>2</sub>SO<sub>4</sub> to stop development

## Procedure

- 1. Coat 96-well microplate with 100 µl capture antibody (200-400 ng/well) in coating buffer. Seal the 96-well microplate and incubate overnight at 4°C.
- 2. Block the surface with blocking buffer for 1 h at RT and 700 rpm.
- 3. Wash the plate three times with washing buffer (at least 5 min per wash).
- 4. Apply antigen diluted in **blocking buffer** and incubate for 2 h at RT and 700 rpm.
- 5. Wash three times with washing buffer.
- 6. Apply detector antibody diluted in **blocking buffer** (dilution 1:1000 up to 1:8000) and incubate for 2 h at RT and 700 rpm.
- 7. Wash three times with washing buffer.
- 8. Incubate with HRP-coupled goat anti-species B antibody or HRP-conjugated streptavidin, diluted in blocking buffer (1:5000 1:10000) for 1 h at RT and 700 rpm.
- 9. Wash three times with washing buffer.
- 10. Add 100 µl substrate solution for development.
- 11. Stop the reaction after 5-10 min with 100 µl stop solution and read the absorbance at 450 nm (ref: 620-650 nm).