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_2SO_4 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).