ADMEbio
for proteomic research

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Sandwich ELISA

I. Reagents

- Coating Buffer: 50 mM Carbonate/Bicarbonate buffer (pH 9.5)
- Samples-protein or peptide
- Double-distilled water (ddH2O)
- Wash Buffer-PBS containing 0.05% Tween-20 (PBST): 10 mM phosphate buffer, pH 7.4, 150 mM NaCl, 0.05% Tween-20, 0.1% thimerosal
- Blocking Buffer: 3-5% skimmed milk/PBST or 1-3% BSA/PBST stored at 4°C
- Sera/primary antibody
- Second antibody: use according to product specifications
- TMB Peroxidase substrate: Reagent A and B and TMB stop solution

II. Procedure

- Coat 100µl of each sample (protein at 2-5µg/ml or peptide at 10µg/ml) into appropriate wells of the ELISA plate.
- Incubate the plate at 4°C overnight for protein and at room temperature overnight for peptide in humidity Chamber.
- Wash: Empty the plate and wash the plate 3 times with 280µl of dd.H2O.
- Block: Block the plate with 240µl of blocking buffer at room temperature for 2 hours in humidity Chamber.
- Wash: Empty the plate and wash the plate 3 times with 280µl of dd.H2O.
- Primary Antibody:
- Add 100µl of sera/antibody in blocking buffer at recommended dilution.
- Incubate at RT for 1 hour in humidity Chamber.
- Wash: Empty the plate and wash the plate 3 times with 280µl of PBST followed by 3X wash with 280µl of dd.H2O.
- Secondary Antibody:
- Add 100 µl of second antibody in blocking buffer to appropriate wells.
- Incubate at room temperature for 1 hour in humidity Chamber.
- Wash: Empty the plate and wash the plate 3 times with 280µl of PBST and then wash 3 times with 280µl of dd.H2O.
- Add TMB-reagent A solution, 80µl, and reagent B solution, 80µl, into appropriate wells.
- Incubate the plate at room temperature for 5 minutes.
- Add 80µl of TMB stop solution to appropriate wells to stop reaction.
- Measure absorbance at optical density (OD) 450nm in a microplate reader within 10 minutes.