Indirect ELISA

All steps are carried out at room temperature unless otherwise indicated. Recipes for all solutions are listed at the end of the protocol.

Antigen Coating

1. Dilute purified antigens to a final concentration of 0.2 μg/ml in antigen-coating buff er and add 100μl of diluted antigen to each well of a 96-well ELISA plate.

2. Carefully cover the plate with adhesive plastic and incubate at 4°C overnight.

Blocking

3. Empty the wells of antigen-coating buff er and wash twice with 200 μl PBST buff er for 5 minutes each time.

4. Add 200 μl blocking buff er per well to block residual protein-binding sites. Cover the plate with adhesive plastic and incubate for 1-2 hour(s) at 37°C.

Antibody incubationg

5. Dilute your primary antibody of choice with blocking buff er in a series e.g. 1:500, 1:1000, 1:2000, 1:4000 and so on, empty the wells of blocking buff er and then add 100 μl of each dilution per well. Repeat in duplicate, or triplicate, for accuracy. Cover the plate with adhesive plastic and incubate for 1 hour at 37°C.

6. Empty the wells and wash 3 times with 200 μl PBST buff er for 5 minutes each time.

7. Dilute the HRP-conjugated secondary antibody with blocking buff er at an optimal concentration (a dilution factor within 1:10,000-1:100,000 is recommended) and add 100 μl of secondary antibody solution to each well. Cover the plate with adhesive plastic and incubate for 1 hour at 37°C.

8. Empty the wells and wash 3 times with 200 μl PBST buff er for 5 min each time.

Antibody incubationg

9. Add 100 μl TMB substrate (mix equal volumes of TMB buffer A and buffer B) to each well with a multichannel pipette. Color development should peak after 15 minutes, at which time it should be stopped by adding 100μl of 2M H_2SO_4 per well. Read absorbance at 450nm.

Solutions

Blocking buffer (100 ml)

5% non-fat dry milk 5g, add PBST buffer to 100ml

Antigen-coating buffer (1000ml)

100mM $NaHCO_3$ 8.4g, adjust pH to 9.6, add ddH_2O to 1000ml

PBST buffer (1000ml)

10mM Na_2HPO_4	1.42g
1.8mM NaH2PO4	0.22g
140mM NaCl	8.19g
0.2% Tween 20	2ml
Adjust pH to 7.4	
Add ddH_2O to 1000 ml	

TMB buffer A (500ml)

NaAc•$3H_2O$	13.6g
Citric Acid	1.6g
30% H_2O_2	0.3ml
Add ddH_2O to 500 ml	

TMB buffer B (500ml)

TMB (dissolved in 3ml DMSO)	0.15g
EDTA-2Na	0.2g
Critic Acid	0.95g
Glycerol	50ml
Add ddH_2O to 500 ml	

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