ELISA protocol

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Reagents

Coating Buffer (1XPBS Buffer)

8.5 g	NaCl
1.4 g	Na_2HPO_4
0.2 g	NaH_2PO_4
1000 ml	ddH_2O

Adjust to pH 7.4

Store at 4 °C

Washing Buffer

0.5 ml	Tween 20
1000 ml	PBS Buffer

Store at 4 °C

Blocking Buffer

100 ml	Washing Buffer
1 g	BSA

Store at 4 °C

Stop Buffer

8.3 ml	12 mol/L HCl
91.7 ml	ddH_2O

Store at 4 °C

TMB Reagent (GenScript Cat.No M00078)

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A. Direct ELISA protocol

Procedure

Coating

1. Dilute the antigen with *Coating Buffer* and coat appropriate wells of ELISA plate with the antigen by adding 100 µl of the diluted antigen solution.

   Note: The concentration of coated antigen ranges from 1-10 µg/ml.

2. Cover the plate with an adhesive plastic and incubate at 37 °C for 2 hours or at 4 °C overnight.
3. Wash the plate with 200 µl of *Washing Buffer* for three times.

Blocking

4. Add 200 µl of *Blocking Buffer* to block the non-specific binding sites in the coated wells.
5. Cover the plate with an adhesive plastic and incubate at 37 °C for 1 hour or at 4 °C overnight.
6. Wash the plate with 200 µl of *Washing Buffer* for three times.

Incubation

7. Dilute the HRP-conjugated antibody with *Blocking Buffer* and add 100 µl of the diluted antibody to each well of the plate.

   Note: The concentration of the conjugated antibody is based on the manufacturer's instructions.

8. Cover the plate with an adhesive plastic and incubate at 37 °C for 30 minutes.
9. Wash the plate with 200 µl of *Washing Buffer* for five times.

Detection

10. Add 100 µl of *TMB Reagent* per well with a multichannel pipette.
11. After sufficient color development, add 100 µl of *Stop Buffer* to the wells.

    Note: 10~15 minutes is enough for color development.

12. Read the absorbance of each well using 450 nm.

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B. Indirect ELISA protocol

Procedure

Coating

1. Dilute the antigen with *Coating Buffer* and coat appropriate wells of ELISA plate with the antigen by adding 100 µl of the diluted solution.
   Note: The concentration of coated antigen ranges from 1 -10 µg/ml.
2. Cover the plate with an adhesive plastic and incubate at 37 °C for 2 hours or at 4 °C overnight.
3. Wash the plate with 200 µl of *Washing Buffer* for three times.

Blocking

4. Add 200 µl of *Blocking Buffer* to block the non-specific binding sites in the coated wells.
5. Cover the plate with an adhesive plastic and incubate at 37 °C for 1 hour or at 4 °C overnight.
6. Wash the plate with 200 µl of *Washing Buffer* for three times.

Incubation

7. Dilute the primary antibody or antiserum with *Blocking Buffer* and add 100 µl of the diluted antibody to each well of the plate.
   Note: The concentration of primary antibody is based on the manufacturer's instructions.
8. Cover the plate with an adhesive plastic and incubate at 37 °C for 1 hour or at 4 °C overnight.
9. Wash the plate with 200 µl of *Washing Buffer* for three times.
10. Dilute the HRP-conjugated secondary antibody with Blocking Buffer and add 100 µl of the diluted secondary antibody to each well of the plate.
11. Cover the plate with an adhesive plastic and incubate at 37 °C for 30 minutes.
12. Wash the plate with 200 µl of *Washing Buffer* for five times.

Detection

13. Add 100 µl of the *TMB Reagent* per well with a multichannel pipette.
14. After sufficient color development, add 100 µl of *Stop Buffer* to the wells.
    Note: 15-30 minutes is enough for color development.
15. Read the absorbance of each well using 450 nm.

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C. Competitive ELISA protocol

Procedure

Coating

1. Dilute the antibody with Coating Buffer and coat appropriate wells of ELISA plate with the antibody by adding 100 µl of the diluted solution.

   Note: The concentration of coated antigen is 1 µg/ml.

2. Cover the plate with an adhesive plastic and incubate at 37 °C for 2 hours or at 4 °C overnight.
3. Wash the plate with 200 µl of *Washing Buffer* for three times.

Blocking

4. Add 200 µl of *Blocking Buffer* to block the non-specific binding sites in the coated wells.
5. Cover the plate with an adhesive plastic and incubate at 37 °C for 1 hour or at 4 °C overnight.
6. Wash the plate with 200 µl of *Washing Buffer* for three times.

Competitive Incubation

7. Dilute the standard/samples in the Blocking Buffer and dilute the HRP conjugated antigen in the Blocking Buffer at the same time.
8. Mix the standards/sample and HRP-conjugated antigen together and add 100 µl of the diluted mixture to the wells.
9. Cover the plate with an adhesive plastic and incubate at 37 °C for 2 hours.
10. Wash the plate with 200 µl of *Washing Buffer* for three times.
11. Add HRP-conjugated secondary antibody to the wells and incubate at 37 °C for 30 minutes.
12. Wash the plate with 200 µl of *Washing Buffer* for three times.

Detection

13. Add 100 µl of the *TMB Reagent* per well with a multichannel pipette.
14. After sufficient color development add 100 µl of *Stop Buffer* to the wells.

    Note: 10-15 minutes is enough for color development.

15. Read the absorbance of each well using 450 nm.
16. Calculate the concentration of the sample from standard curve.

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D. Sandwich ELISA protocol

Procedure

Coating

1. Dilute the antibody with Coating Buffer and coat appropriate wells of ELISA plate with the antibody by adding 100 µl of the diluted solution.

   Note: The concentration of coated antibody ranges from 0.5-10 µg/ml.

2. Cover the plate with an adhesive plastic and incubate at 37 °C for 2 hours or at 4 °C overnight.
3. Wash the plate with 200 µl of *Washing Buffer* for three times.

Blocking

4. Add 200 µl of *Blocking Buffer* to block the non-specific binding sites in the coated wells.
5. Cover the plate with an adhesive plastic and incubate at 37 °C for 1 hour or at 4 °C overnight.
6. Wash the plate with 200 µl of *Washing Buffer* for three times.

Standard and Samples incubation

7. Add 100 µl of appropriately diluted samples or standards to each well.
8. Cover the plate with an adhesive plastic and incubate at 37 °C for 2 hours or at 4 °C overnight.
9. Wash the plate with 200 µl of *Washing Buffer* for three times.

Incubation with HRP-conjugated antibody

10. Dilute the HRP-conjugated antibody with *Blocking Buffer* and add 100 µl of the diluted antibody to each well of the plate.

    Note: the concentration of incubated antibody is based on the manufacturer's instructions

11. Cover the plate with an adhesive plastic and incubate at 37 °C for 30 minutes.
12. Wash the plate with 200 µl of *Washing Buffer* for three times.

Detection

13. Add 100 µl of the *TMB Reagent* per well with a multichannel pipette.
14. After sufficient color development add 100 µl of *Stop Buffer* to the wells.

    Note: 10~15 minutes is enough for color development.

15. Read the absorbance of each well using 450 nm.
16. Calculate the concentration of the sample from standard curve.

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