ELISA Protocols

-Direct ELISA Protocol

Materials

1. Bicarbonate/carbonate coating buffer (100 mM)

Antigen or antibody should be diluted in coating buffer to immobilize them to the wells:

3.03 g Na_2CO_3, 6.0 g $NaHCO_3$, 1000 ml distilled water, pH 9.6.

2. PBS:

1. 16 g Na_2HPO_4, 0.1g KCl, 0.1 g K_3PO_4, 4.0 g NaCl (500 ml distilled water) pH 7.4.

3. Blocking solution:

Commonly used blocking agents are 1% BSA , serum, non-fat dry milk, casein, gelatin in PBS.

4. Wash solution:

Usually PBS or Tris-buffered saline (pH 7.4) with detergent such as 0.05% (v/v) Tween20 (TBST).

5. Antibody dilution buffer:

Primary and secondary antibody should be diluted in 1x blocking solution to reduce Non specific binding.

Method

1. Dilute the antigen to a final concentration of 10 μg/ml in PBS or other carbonate buffer. Coat the wells of a PVC microtiter plate with the antigen by pipeting 100μl of the antigen dilution in the top wells of the plate. Dilute down the plate as required. Seal the plate and incubate overnight at 4℃ or 2 h at room temperature.

2. Wash plate 3 times with PBS.

3. Block the remaining protein-binding sites in the coated wells by adding 200 μl blocking buffer, 5% non fat dry milk/PBS, per well. Alternative blocking reagents include BlockACE or BSA.

4. Cover the plate with an adhesive plastic and incubate for at least 2 h at room temperature or, if more convenient, overnight at 4℃.

5. Wash the plate twice with PBS

6. Add 100 μl of the antibody, diluted at the optimal concentration (according to the manufacturer's instructions) in blocking buffer immediately before use.

7. Cover the plate with an adhesive plastic and incubate for 2 h at room temperature.

8. Wash the plate 5 times with PBS.

9. Dispense 100 µl (or 50 µl) of the substrate solution per well with a multichannel pipet or a multipipet.

10. After sufficient color development (if it is necessary) add 50-100 µl of stop solution to the wells.

11. Record the absorbance at 450 nm on a plate reader within 30 minutes of stopping the reaction.

-Indirect ELISA Protocol

Materials

1. Bicarbonate/carbonate coating buffer (100 mM)

Antigen or antibody should be diluted in coating buffer to immobilize them to the wells:

3.03 g Na_2CO_3, 6.0 g $NaHCO_3$, 1000 ml distilled water, pH 9.6.

2. PBS:

1.16 g Na_2HPO_4, 0.1 g KCl, 0.1 g K_3PO_4, 4.0 g NaCl (500 ml distilled water) pH 7.4.

3. Blocking solution:

Commonly used blocking agents are 1% BSA , serum, non-fat dry milk, casein, gelatin in PBS.

4. Wash solution:

Usually PBS or Tris-buffered saline (pH 7.4) with detergent such as 0.05% (v/v) Tween20 (TBST).

5. Antibody dilution buffer:

Primary and secondary antibody should be diluted in 1x blocking solution to reduce Non specific binding.

Method

1. Dilute antigen to a final concentration of 1-20 µg/mL using PBS or Bicarbonate/carbonate coating buffer. Coat the wells of a PVC microtiter plate with the antigen by pipeting 50 µl of the antigen dilution in the top wells of the plate. Dilute down the plate as required. Seal the plate and incubate overnight at 4℃ or 2h at room temperature.

2. Wash plate 3 times with PBS.

3. Block the remaining protein-binding sites in the coated wells by adding 200 µl blocking buffer, 5% non fat dry milk/PBS, per well. Alternative blocking reagents include BlockACE or BSA.

4. Cover the plate with an adhesive plastic and incubate for at least 2h at room temperature or, if more convenient, overnight at 4℃.

5. Wash the plate 3 times with PBS.

6. Add 100 µl of diluted primary antibody to each well.

7. Cover the plate with an adhesive plastic and incubate for 2h at room temperature.

8. Wash the plate 4 times with PBS.

9. Add 100 µl of conjugated secondary antibody, diluted at the optimal concentration (according to the manufacturer) in blocking buffer immediately before use.

10. Cover the plate with an adhesive plastic and incubate for 1-2h at room temperature.

11. Wash the plate 5 times with PBS.

12. Dispense 100µl (or 50µl) of the substrate solution per well with a multichannel pipet or a multipipet.

13. After sufficient color development (if it is necessary) add 50-100µl of stop solution to the wells.

14. Record the absorbance at 450 nm on a plate reader within 30 min of stopping the reaction.

-Sandwich ELISA Protocol

Materials

1. Bicarbonate/carbonate coating buffer (100mM)

Antigen or antibody should be diluted in coating buffer to immobilize them to the wells:

3.03 g Na_2CO_3, 6.0 g $NaHCO_3$, 1000 ml distilled water, pH 9.6.

2. PBS:

1.16 g Na_2HPO_4, 0.1 g KCl, 0.1 g K_3PO_4, 4.0 g NaCl (500 ml distilled water) pH 7.4.

3. Blocking solution:

Commonly used blocking agents are 1% BSA , serum, non-fat dry milk, casein, gelatin in PBS.

4. Wash solution:

Usually PBS or Tris-buffered saline (pH 7.4) with detergent such as 0.05% (v/v) Tween20 (TBST).

5. Antibody dilution buffer:

Primary and secondary antibody should be diluted in 1x blocking solution to reduce Non specific binding.

Method

1. Coat the wells of a PVC microtiter plate with the capture antibody at a concentration of 1-10 µg/ml in carbonate/bicarbonate buffer (pH7.4). Seal the plate and incubate overnight at 4℃or 2h at room temperature.

2. Wash plate 3 times with PBS.

3. Block the remaining protein-binding sites in the coated wells by adding 200µl blocking buffer, 5% non fat dry milk/PBS, per well.

4. Cover the plate with an adhesive plastic and incubate for at least 1-2h at room temperature or, if more convenient, overnight at 4℃.

5. Add 100µl of appropriately diluted samples to each well. For accurate quantitative results, always

compare signal of unknown samples against those of a standard curve. Standards (duplicates or triplicates) and blank must be run with each plate to ensure accuracy. Incubate for 90 min at 37℃.

6. Wash the plate twice with PBS.

7. Add 100 µl of diluted detection antibody to each well.

8. Cover the plate with an adhesive plastic and incubate for 2h at room temperature.

9. Wash the plate 4 times with PBS.

10. Add 100 µl of secondary antibody conjugated, diluted at the optimal concentration (according to the manufacturer) in blocking buffer immediately before use.

11. Cover the plate with an adhesive plastic and incubate for 1-2 h at room temperature.

12. Wash the plate 5 times with PBS.

13. Dispense 100µl (or 50µl) of the substrate solution per well with a multichannel pipet or a multipipet.

14. After sufficient color development (if it is necessary) add 50-100µl of stop solution to the wells.

15. Record the absorbance at 450 nm on a plate reader within 30 min of stopping the reaction.