# ELISA protocol

## Index



## Reagents

### Coating Buffer (1XPBS Buffer)

8.5g NaCl
1.4g $Na_2HPO_4$
0.2g $NaH_2PO_4$
Adjust pH 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 PBS Buffer
0.05 ml Tween 20
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)



860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-free Tel: 1-877-436-7274 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

# A. Direct ELISA protocol

## Procedure

### Coating

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

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

2. Cover the plate with an adhesive plastic and incubate for 2 hour at 37$^{o}$C or 4$^{o}$C overnight.
3. Remove the antigen coating solution from the wells of plate by flicking the plate over a sink.
4. Wash the plate three times by adding the wells with 200 µl *Washing Buffer.*

### Blocking

5. Add 200 µl of *Blocking Buffer* to block the non-specific binding sites in the coated wells.
6. Cover the plate with an adhesive plastic and incubate for at 1 hour at 37$^{o}$C or 4$^{o}$C overnight.
7. Remove the *Blocking Buffer* from the wells of plate by flicking the plate over a sink.

### Incubation

8. 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

9. Cover the plate with an adhesive plastic and incubate for 30 minutes at 37$^{o}$C.
10. Remove the HRP conjugated antibody solution from the wells of plate by flicking the plate over a sink.
11. Wash the plate five times by adding the wells with 200 µl *Washing Buffer*.

### Detection

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

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

14. Read the absorbance of each well with a plate reader.

# B. Indirect ELISA protocol

## Procedure

### Coating

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

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

2. Cover the plate with an adhesive plastic and incubate for 2 hours at 37$^{o}$C or 4$^{o}$C overnight.



860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-free Tel: 1-877-436-7274 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

3. Remove the antigen coating solution from the wells of plate by flicking the plate over a sink.
4. Wash the plate three times by adding the wells with 200 µl of *Washing Buffer*.

**Blocking**

5. Add 200 µl of *Blocking Buffer* to block the non-specific binding sites in the coated wells.
6. Cover the plate with an adhesive plastic and incubate for at 2 hours at 37$^{o}$C or overnight.
7. Remove the *Blocking Buffer* from the wells of plate by flicking the plate over a sink.
8. Wash the plate three times by adding the wells with 200 µl of *Washing Buffer.*

**Incubation**

9. 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

10. Cover the plate with an adhesive plastic and incubate for 1 hour at 37$^{o}$C or overnight at 4°C.
11. Remove the diluted primary antibody solution from the wells of plate by flicking the plate over a sink.
12. Wash the plate three times by adding the wells with 200 µl of *Washing buffer*.
13. Dilute the HRP-conjugated secondary antibody with *Blocking Buffer* and add 100 µl of the diluted secondary antibody to each well of the plate.
14. Cover the plate with an adhesive plastic and incubate for 30 minutes at 37$^{o}$C.
15. Remove the diluted secondary antibody from the wells of plate by flicking the plate over a sink.
16. Wash the plate five times by adding the wells with 200 µl of *Washing Buffer.*.

**Detection**

17. Add 100 µl of the *TMB Reagent* per well with a multichannel pipete or a multipipete.
18. After sufficient color development, add 100 µl of *Stop Buffer* to the wells.

Note: 15-30 minutes is enough for color development.

19. Read the absorbance of each well with a plate reader.

## C. Competitive ELISA protocol

## Procedure

**Coating**

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

Note: the concentration of coated antibody ranges from 2-5 µg.

2. Cover the plate with an adhesive plastic and incubate for 2 hours at 37$^{o}$C or 4$^{o}$C overnight.
3. Remove the antibody coating solution from the wells of plate by flicking the plate over a sink.
4. Wash the plate three times by adding the wells with 200 µl *Washing Buffer.*

**Blocking**



860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-free Tel: 1-877-436-7274 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

5. Add 200 µl *Blocking Buffer* to block the non-specific binding sites in the coated wells.
6. Cover the plate with an adhesive plastic and incubate for at 1 hour at 37°C or overnight.
7. Remove the *Blocking Buffer* from the wells of plate by flicking the plate over a sink.
8. Wash the plate three times by adding the wells with 200 µl of *Washing Buffer*.

**Competitive Incubation**

9. Dilute the standard/sample in the *Blocking Buffer* and dilute the HRP conjugated antigen in the *Blocking Buffer* at the same time.
10. Mix the standards/sample and HRP-conjugated antigen together and add 100 µL of the diluted mixture to the wells.
11. Cover the plate with an adhesive plastic and incubate for 2 hours at 37°C.
12. Remove the mixture solution from the wells of plate by flicking the plate over a sink.
13. Wash the plate three times by adding the wells with 200 µl of *Washing Buffer*.

**Detection**

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

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

16. Read the absorbance of each well with a plate reader.

## D. Sandwish ELISA protocol

The sandwich ELISA measures the amount of antigen between two layers of antibodies. The antigens to be measured must contain at least two antigenic sites, capable of binding to antibody, since at least two antibodies act in the sandwich. So sandwich assays are restricted to the quantitation of multivalent antigens such as proteins or polysaccharides. Sandwich ELISAs for quantitation of antigens are especially valuable when the concentration of antigens is low and/or they are contained in high concentrations of contaminating protein

### Procedure

**Coating**

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

Note: the concentration of coated antibody ranges from 0.5-10 µg

2. Cover the plate with an adhesive plastic and incubate for 2 hours at 37°C or 4°C overnight.
3. Remove the antibody coating solution from the wells of plate by flicking the plate over a sink.
4. Wash the plate three times by adding the wells with 200 µl of *Washing Buffer*.

**Blocking**

5. Add 200 µl of *Blocking Buffer* to block the non-specific binding sites in the coated wells.



860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-free Tel: 1-877-436-7274 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

6. Cover the plate with an adhesive plastic and incubate for at 2 hours at 37°C or overnight.
7. Remove the *Blocking Buffer* from the wells of plate by flicking the plate over a sink.
8. Wash the plate three times by adding the wells with 200 µl *Washing Buffer*.

**Standard and Samples incubation**

9. Dilute the standard/samples with *Blocking Buffer* and coat appropriate wells of ELISA plate with the standard/samples by pipeting 100 µl of the diluted solution.
10. Cover the plate with an adhesive plastic and incubate for 2 hours at 37°C or 4°C overnight.
11. Remove the standard/samples solution from the wells of plate by flicking the plate over a sink.
12. Wash the plate three times by adding the wells with 200 µl of *Washing Buffer.*

**Incubation with HRP conjugated antibody**

13. 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

14. Cover the plate with an adhesive plastic and incubate for 30 minutes at 37°C.
15. Remove the *Blocking Buffer* from the wells of plate by flicking the plate over a sink.
16. Wash the plate three times by adding the wells with 200 µl of *Washing Buffer*.

**Detection**

17. Add 100 µl of the *TMB Reagent* per well with a multichannel pipet or a multipipet.
18. After sufficient color development add 100 µl of *Stop Buffer* to the wells.

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

19. Read the absorbance of each well with a plate reader.



860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-free Tel: 1-877-436-7274 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com