HiPer® Antigen Capture ELISA Teaching Kit

Product Code: HTI012

Number of experiments that can be performed: 4

Duration of Experiment: 2 days

Day1-Coating of wells: 15 minutes
Day2- Protocol, observation and result: 5 hours

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store all the reagents at 2-8°C
- Other kit content can be stored at room temperature (15-25°C)

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Aim:

To determine the antigen concentration by Antigen Capture ELISA method.

Introduction:

Enzyme linked immunosorbent assay or ELISA is a sensitive immunological technique to detect the presence of a specific antigen (Ag) or antibody (Ab) in a biological sample. It utilizes the dual properties of antibody molecules being specific in reactivity and their ability to be conjugated to active molecules such as enzymes. An enzyme conjugated with an antibody reacts with a chromogenic colourless substrate to generate a coloured reaction product. ELISA is extensively used for diagnostic purpose which utilizes the dual properties. It requires an immobilized antigen/antibody bound to a solid support (e.g. microtitre plate or membrane). There are different types of ELISAs for the detection of a protein of interest in a given sample. One of the most sensitive ELISA is Antigen Capture ELISA which can measure the concentration of antigen.

Principle:

In Antigen Capture ELISA method, an antibody is bound to the wells of a microtitre plate. Then one unlabeled antigen and one enzyme labeled antigen (Horseradish peroxidase (HRP)) corresponding to the antibody are added and allowed to complex with the bound antibody. As a result, there is a competition between the labeled and unlabeled antigen for the immobilized antibody. Unreacted antigens are washed out and the amount of labeled antigen bound to the immobilized antibody is detected by using H_2O_2 as substrate and Tetramethylbenzidine (TMB) as a chromogen. HRP acts on H_2O_2 to release nascent oxygen, which oxidizes TMB to TMB oxide, which gives, a blue coloured product. The intensity of the colour is measured using a spectrophotometer at 450 nm. The developed colour is directly proportional to the amount of labeled antigen present in the sample and the amount of labeled antigen that binds is inversely proportional to the amount of unlabeled antigen in the reaction mixture. As a result the estimate of label in the microtitre well decreases with increase in the antigen concentration in the standard or test sample.

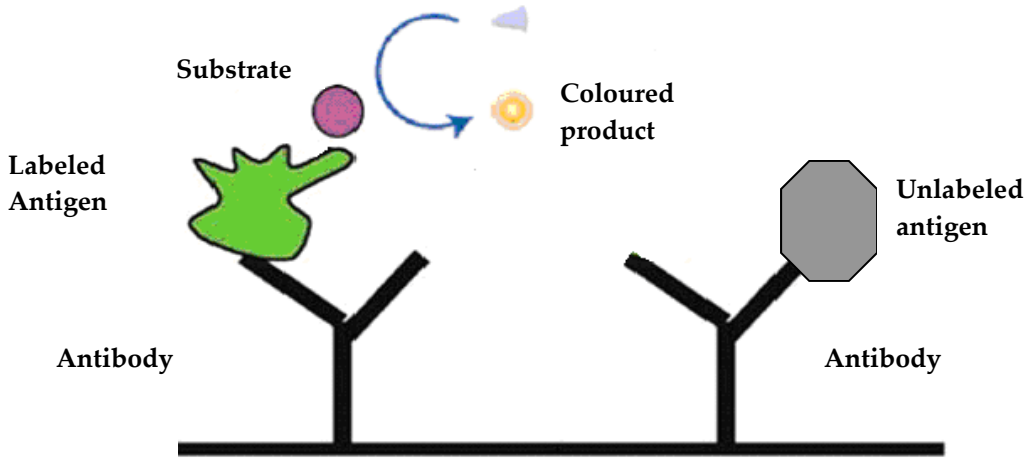


Figure 1: In Antigen Capture ELISA, an antibody is bound to the well and a labeled and unlabeled antigen are allowed to compete for the binding to the antibody. The concentration of antigen can be determined from the intensity of the color developed in the product.

Kit Contents:

This kit can be used for the determination of antigen concentration bound to immobilized antibody followed by binding of the antigen to the labelled secondary antigen and its detection by using appropriate substrate.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			4expts	
1	TKC150	Unlabeled antigen (1mg/ml)	0.180 ml	2-8 °C
2	TKC151	Antibody	0.2 ml	2-8 °C
3	TKC152	Test Sample 1	1 ml	2-8°C
4	TKC153	Test Sample 2	1 ml	2-8°C
5	TKC154	Test Sample 3	1 ml	2-8°C
6	TKC155	HRP labeled antigen	0.015 ml	2-8°C
7	TKC156	Blocking Buffer	25 ml	2-8°C
8	TKC157	10X TMB substrate	5 ml	2-8°C
9	TKC158	Coating Buffer	25 ml	2-8°C
10	TKC159	10X Wash Buffer	40 ml	2-8°C
11	TKC160	Stop Solution	240 ml	2-8°C
12	TKC131	Microtitre plate (Detachable)	1 No.	RT

Materials required but not provided:

Glass wares:, Measuring cylinder, Test Tubes
Reagents: Distilled water
Other requirements: Blotting paper, Micropipette, Tips, Spectrophotometer, Cuvettes.

Storage:

HiPer® Antigen Capture ELISA Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. Store all the reagents at 2-8°C. Other kit content can be stored at room temperature.

Important Instructions:

1. Before starting the experiment the entire procedure has to be read carefully.
2. Always wear gloves while performing the experiment.
3. Bring all the buffers to room temperature before the assay.
4. Use 24 wells per experiment. One plate can be used for 4 experiments. Don't reuse the same wells.
5. Dilute only required amount of buffers to 1X with distilled water before use.
6. Blocking buffer BSA in PBS.
7. **Coating buffer:** Carbonate bicarbonate buffer.
8. **Stop solution:** Sulphuric acid.
9. **Preparation of 1X TMB substrate:** Take 0.5 ml of 10X TMB substrate and add 4.5 ml of distilled water to it.
10. **Preparation of 1X Wash Buffer:** Take 5 ml of 10X Wash Buffer and add 45 ml of distilled water to it.

Prepare the reagents as indicated below before starting each experiment:

Preparation of sample diluent: Take 1 ml of blocking buffer and make up the volume to 30 ml with 1X wash buffer. Use this to dilute standard antigen and HRP labeled antigen.

Preparation of dilutions of standard antigen:
Concentration of standard antigen is 1 mg/ml; dilute this to get a range of concentrations using sample diluent, as follows:

No.	Dilutions of Standard Antigen	Concentration of Antigen
1	40µl of 1mg/ml (stock)+ 960 µl of sample diluent	40µg/ml (a)
2	400µl of (a) + 1600 µl of sample diluent	8µg/ml (b)
3	1000 µl of (b)+ 1000 µl of sample diluent	4 µg/ml (c)
4	1000 µl of (c)+ 1000 µl of sample diluent	2 µg/ml (d)
5	1000 µl of (d)+ 1000 µl of sample diluent	1 µg/ml (e)
6	1000 µl of (e)+ 1000 µl of sample diluent	0.5 µg/ml (f)
7	1000 µl of (f)+ 1000 µl of sample diluent	0.25 µg/ml (g)
8	1000 µl of (g)+ 1000 µl of sample diluent	0.125 µg/ml (h)

Procedure:

Day 1: Coating of wells with antibody

1. Dilute 50 µl of antibody with 4.95 ml of coating buffer. Concentration of the diluted antibody is 1µg/ml.
2. Pipette 200 µl of diluted antibody into each wells of the microtitre plate (24 wells). Tap or shake the plate so that the antibody solution is evenly distributed over the bottom of each well.
3. Incubate the wells overnight at 4°C.

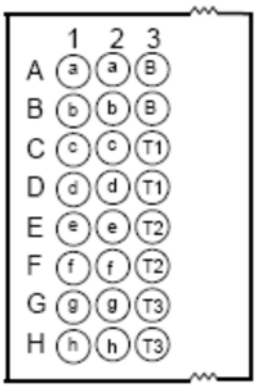
Day 2: a) Blocking the residual binding sites on the wells

4. Discard the well contents. Rinse the wells with distilled water for three times by draining out the water after each rinse.
5. Fill each well with 200 µl of blocking buffer and incubate at room temperature for 1 hour.
6. Rinse the plate three times (as given above) with distilled water. Drain out the water completely by tapping the plate on a blotting paper.

b) Addition of antigen to the wells

7. Prepare standard and test antigen dilutions as given above.

8. Add 100 µl of standard antigen, test samples and 1X wash buffer to the coated wells (in duplicates) as shown in the following picture.



a to h – various dilutions of standard antigen
T1, T2 and T3 – Three test samples
B – 1X wash buffer (Blank)

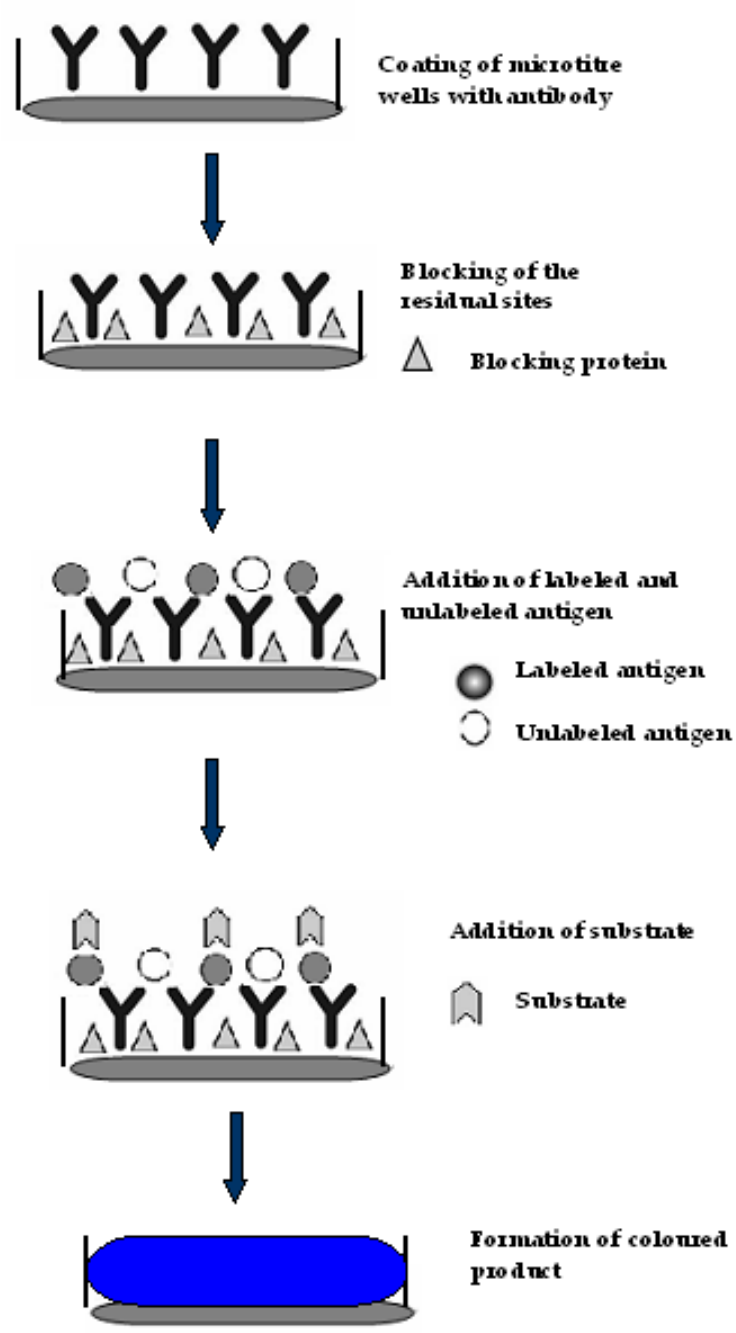
c) Addition of HRP labeled antigen

9. Dilute 3 µl of HRP labeled antigen with 3 ml of sample diluent
10. Add 100 µl of HRP labeled antigen to all the wells.
11. Incubate at room temperature for 30 minutes.
12. Discard the well contents; fill the wells with 1X wash buffer, allow it to stand for 3 minutes, discard the contents. Repeat this step twice.

d) Addition of substrate and measurement of absorbance

13. Dilute required amount of 10 X TMB substrate solutions to 1X using distilled water.
14. Add 200 µl of 1X substrate to each well.
15. Incubate at room temperature for 10 minutes.
16. Add 100 µl of 1X stop solution to each well.
17. Transfer the contents of each well to individual tubes containing 2 ml of 1X stop solution.
18. Prepare substrate blank by adding 200 µl of 1X substrate solution to 2.1 ml of 1X stop solution.
19. Read the absorbance at 450 nm after blanking the spectrophotometer with substrate blank.

Flowchart:


Coating of microtitre wells with antibody
Blocking of the residual sites
Blocking protein
Addition of labeled and unlabeled antigen
Labeled antigen
Unlabeled antigen
Addition of substrate
Substrate
Formation of coloured product

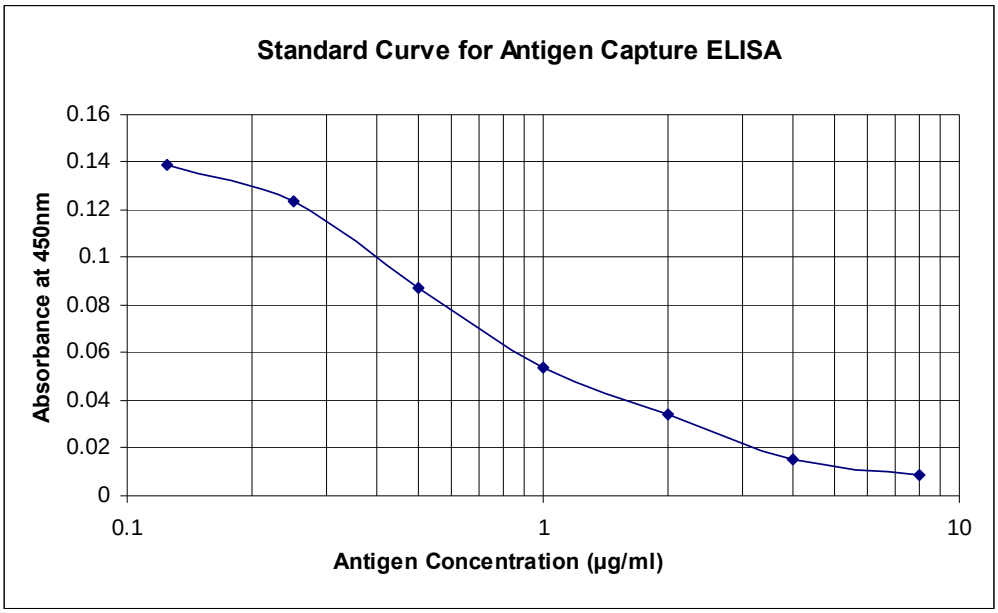
Observation and Result:

Look for the development of blue colour in the wells at the end of the experiment. Read the absorbance at 450 nm after blanking the spectrophotometer with substrate blank and record the readings as follows:

Sample	Concentration (µg /ml)	A_{450}	Average A_{450}
a			
b			
c			
d			
e			
f			
g			
h			
T1			
T2			
T3			
Blank (1X wash buffer)			

Calculation of antigen concentration in test sample:

Calculate the average A450 for each of the samples (standard and test) and plot A450 of standards on Y axis (linear scale) versus the concentration of antigen in µg/ml on X axis (log scale) on a semi-log graph sheet as shown below:



Calculation of antigen concentration:

Calculate the concentration of antigen in mg/ml, in each of the test samples as follows:
Concentration of antigen in the sample:

$$\frac{\text{Concentration in µg/100 µl (from the graph) X Dilution factor}}{10^6} = \_\_\_\_\_ \text{ mg/ml}$$

From the standard curve, determine the concentration of antigen in the test samples and record the readings as below:

Test Sample	Concentration (mg/ml)
1	
2	
3	

Interpretation:

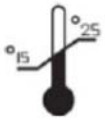
By performing Antigen Capture ELISA, concentrations of the test antigens can be detected from the standard curve. When unlabeled antigen (test antigen or varying amounts of standard antigen) and fixed amount of labeled antigen are added to the antibody immobilized on the wells, the two antigens compete to bind to the immobilized antibody. The amount of labeled antigen bound is then estimated by a chromogenic assay. The amount of labeled antigen that binds to the immobilized antibody is inversely proportional to the amount of unlabeled antigen in the reaction mixture.

Troubleshooting Guide:

Sr.No	Problem	Probable Cause	Solution
1	No signal	Omission of any step	Prepare a check-list for the steps followed
2	High background	Insufficient washing or Secondary antibody concentration is high or Contamination in buffer	Wash plates thoroughly after incubation with Secondary antibody. Decrease the antibody concentration. Use freshly prepared buffer

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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