

HiPer[®] Antibody Capture ELISA Teaching Kit

Product Code: HTI013

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 6 months from the date of receipt
 - 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 antibody concentration by Antibody 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 colorless substrate to generate a coloured reaction product. ELISA is extensively used for diagnostic purpose which utilizes the dual. 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 them is Antibody Capture ELISA which can measure the concentration of an antibody through an indirect ELISA method. This method is very useful for the screening of anti serum for specific antibodies.

Principle:

In Antibody Capture ELISA method first the antigen (capture antigen) is bound to the wells of a microtitre plate. Then primary antibody corresponding to the antigen is added and allowed to complex with the bound antigen. Then wells are washed to remove unbound antibodies and a second antibody (detection antibody) labeled with an enzyme e.g. Horseradish peroxidase (HRP) is allowed to bind to the first antibody i.e. the primary antibody. Unreacted labeled antibodies are washed out and the amount of antibody immobilized to secondary 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 antibody present in sample.

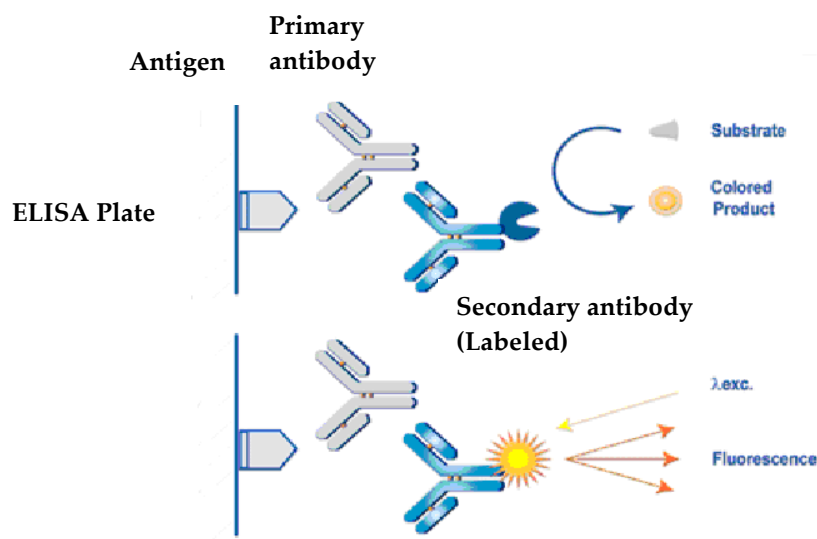


Fig1: In Antibody capture method an antigen is first coated on the wells of an ELISA plate and then subsequently treated with the corresponding primary and HRP-labeled secondary antibody and the concentration of antibody can be determined from the intensity of the developed coloured product

Kit Contents:

This kit can be used for the determination of antibody concentration bound to immobilized antigen followed by binding of the antigen to the labeled secondary antibody 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
			4 expts	
1	TKC161	Coating Antigen(1mg/ml)	0.12 ml	2-8 °C
2	TKC162	Standard Primary Antibody	0.024 ml	2-8 °C
3	TKC163	Test Sample 1	2 ml	2-8°C
4	TKC164	Test Sample 2	2 ml	2-8°C
5	TKC165	Test Sample 3	2 ml	2-8°C
6	TKC166	Secondary Antibody – HRP conjugate	0.02 ml	2-8°C
7	TKC167	Blocking Buffer	30 ml	2-8°C
8	TKC168	10X TMB Substrate	5 ml	2-8°C
9	TKC169	Coating Buffer	30 ml	2-8°C
10	TKC170	10X Wash Buffer	40 ml	2-8°C
11	TKC171	Stop Solution	240 ml	2-8°C
12	TKC131	Microtitre plate (Detachable)	1No.	R T

Materials required but not provided:

Glass wares:, Measuring cylinder, Test tubes

Reagents: Distilled water

Other requirements: Blotting paper, Micropipette, Tips

Storage:

HiPer® Antibody Capture ELISA Teaching Kit is stable for 6 months from the date of receipt 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. Dilute only required amount of buffers to 1X with distilled water before use.
5. Blocking buffer BSA in PBS
6. **Coating buffer:** Carbonate bicarbonate buffer.
7. **Stop solution:** Sulphuric acid.
8. **Preparation of 1XTMB substrate:** Take 0.5 ml of 10XTMB substrate and add 4.5 ml of distilled water to it.
9. **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 antibody and HRP labeled antibody.

Preparation of dilutions of standard Antibody:

Concentration of standard antibody is 800µg/ml; dilute this to get a range of concentrations using sample diluent, as follows:

No.	Dilutions of Standard Antibody	Concentration of Antibody
1	6µl of 800 µg /ml (stock)+ 1500 µl of sample diluent	3200 ng/ml (a)
2	1000 of (a) + 1000 µl of sample diluent	1600 ng/ml (b)
3	1000 µl of (b)+ 1000 µl of sample diluent	800ng/ml (c)
4	1000 µl of (c)+ 1000 µl of sample diluent	400 ng/ml (d)
5	1000 µl of (d)+ 1000 µl of sample diluent	200 ng/ml (e)
6	1000 µl of (e)+ 1000 µl of sample diluent	100 ng/ml (f)
7	1000 µl of (f)+ 1000 µl of sample diluent	50 ng/ml (g)
8	1000 µl of (g)+ 1000 µl of sample diluent	25 ng/ml (h)

Procedure:

Day 1: Coating of wells with antigen

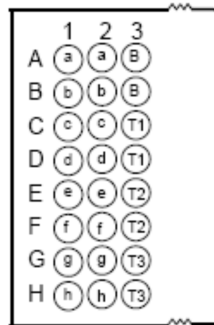
1. Dilute 30 µl of Antigen with 5.97 ml of coating buffer. Concentration of the diluted capture antigen is 5 µg/ml.
2. Pipette 200 µl of diluted capture antigen into each of the microtitre well (24 wells). Tap or shake the plate so that the capture antigen solution is evenly distributed over the bottom of each well.
3. Incubate the microtitre wells overnight at 4°C.

Day 2: 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.

Addition of antigen to wells

7. Prepare standard and test antibody dilutions as given above.
8. Add 200 μ l of standard antibody, test samples and 1X PBST (wash buffer) to the coated wells (in duplicates).



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

9. Incubate at room temperature for 30 minutes.
10. Discard the well contents; fill the wells with 1X PBST, allow it to stand for 3 minutes, discard the contents.
Repeat this step twice.

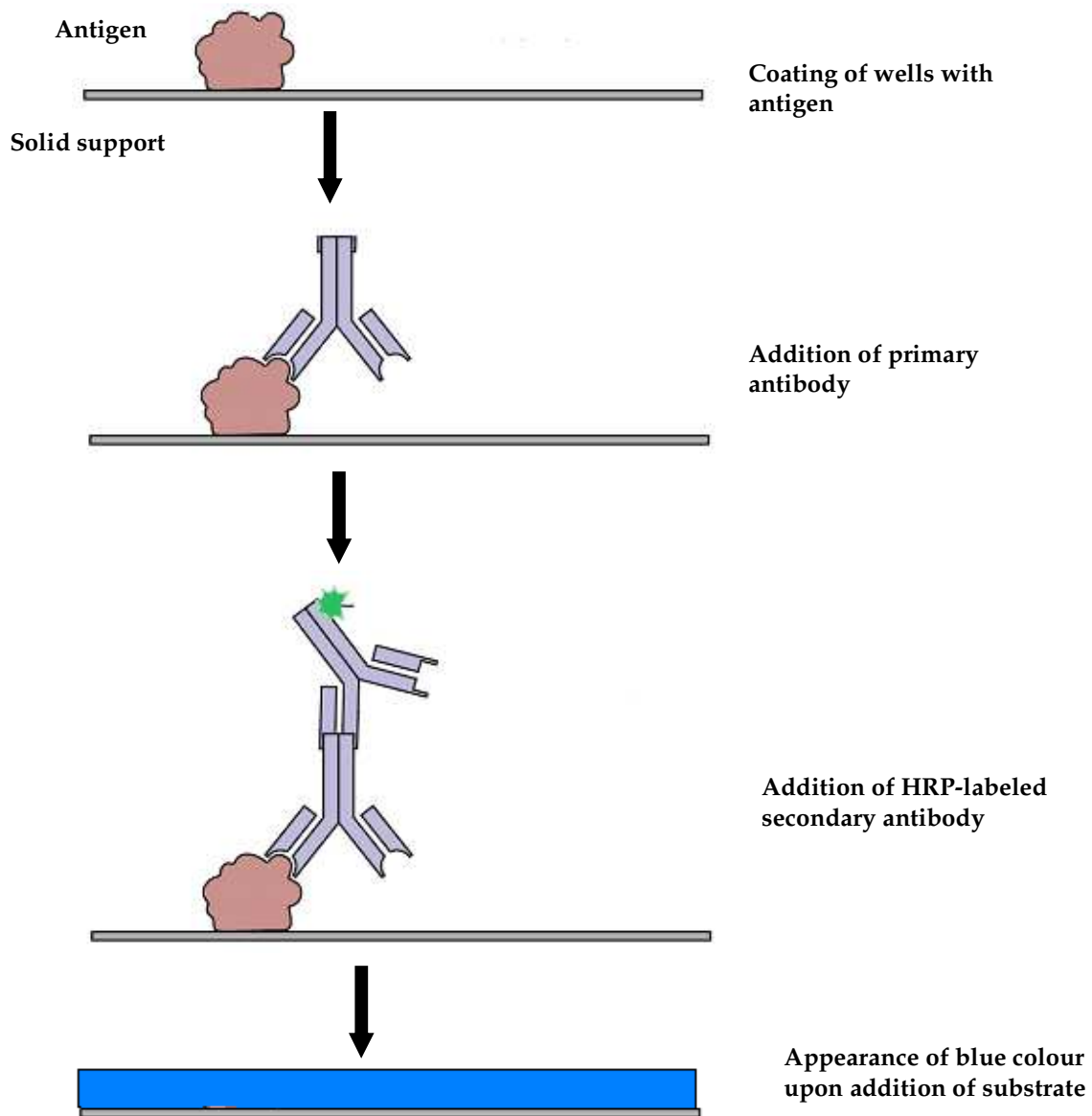
Addition of HRP labeled antibody

11. Dilute 5 μ l of Secondary Antibody-HRP conjugate with 5 ml of sample diluent.
12. Add 200 μ l of diluted HRP labeled antibody to all the wells.
13. Incubate at room temperature for 30 minutes.
14. Discard the well contents and rinse the wells 3 times with 1X PBST (wash buffer).

Addition of substrate and measurement of absorbance

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

Flowchart:



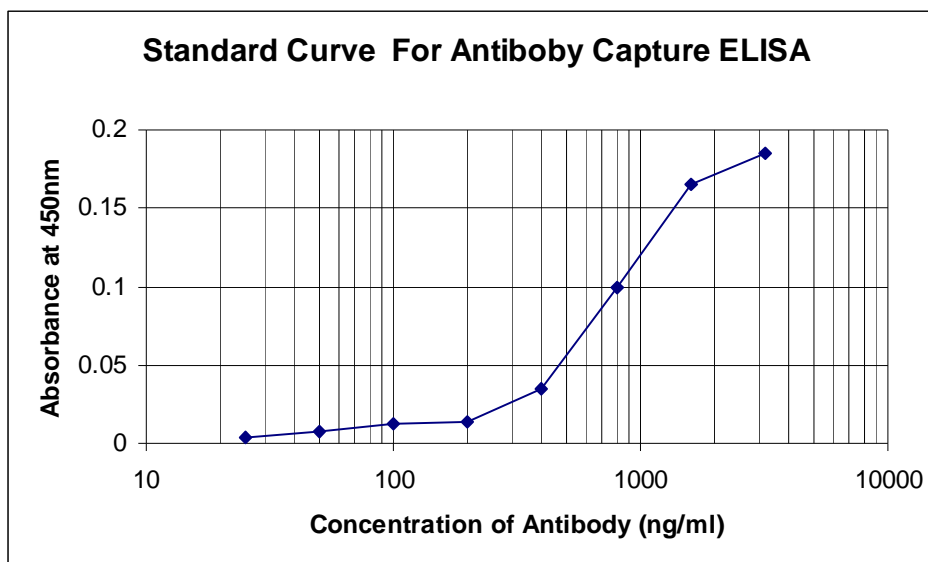
Observation and Result:

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 (ng/ml)	A ₄₅₀	Average A ₄₅₀
a			
b			
c			
d			
e			
f			
g			
h			
T1			
T2			
T3			
Blank (1XPBST)			

Calculation of antibody 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 antibody in ng/ml on X axis (log scale) on a semi-log graph sheet as shown below:



Calculation of antibody concentration:

Calculate the concentration of antibody in mg/ml, in each of the test samples as follows:

Concentration of antibody in the sample:

$$\frac{\text{Concentration in ng/200 } \mu\text{l (from the graph) X Dilution factor}}{10^6} = \text{_____ mg/ml}$$

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

Test Sample	Concentration (mg/ml)
1	
2	
3	

Interpretation:

When an antigen (immobilized on the solid support) is treated with the corresponding primary antibody (test or varying concentrations of standard antibody), the amount of bound antibody can be detected using a secondary antibody conjugated to HRP which forms a blue coloured product upon reaction with TMB. The intensity of the blue colour is directly proportional to the amount of secondary antibody conjugate bound to the primary antibody and is measured spectrophotometrically. By performing Antibody Capture ELISA, concentrations of the test antibodies can be detected from the standard curve

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