HiPer® Dot ELISA Teaching Kit

Product Code: HTI015

Number of experiments that can be performed: 15

Duration of Experiment: Approximately 1 hour 30 minutes

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store the 10X Assay buffer,Test Serum, Antibody-HRP conjugate, TMB substrate and Dot ELISA Strips at 2-8°C
- Other kit content can be stored at room temperature (15-25 °C)

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Aim:

To learn the technique of Dot ELISA for the detection of an antigen.

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 chormogenic 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 common ELISA is dot ELISA which can visually detect the presence of an antigen very quickly. The nitrocellulose dot technique was first developed for screening large number of hybridoma antibodies in 1983.

Principle:

There are various forms of ELISA for the detection of antigen or antibody based on antibody-antigen interactions. Dot ELISA, a qualitative ELISA test, can be performed very quickly with the end detection done visually. Because of its relative speed and simplicity, the dot ELISA is an attractive alternative to standard ELISA. In Dot-ELISA, small volumes of antibodies are immobilized on a protein binding membrane (Nitrocellulose) and the other antibody is linked to an enzyme Horse radish peroxidase (HRP). The test antigen at first reacts with the immobilized antibody and later with the enzyme-linked antibody. The amount of enzyme linked antibody bound is determined by incubating the strip with an appropriate substrate (Hydrogen peroxide, H_2O_2) and a chromogen [Tetramethylbenzidine (TMB)]. HRP acts on H_2O_2 to release nascent oxygen, which oxidizes TMB to TMB oxide, which gives, a blue coloured product. The latter precipitates onto the strip in the area of enzyme activity and appears as a coloured dot, hence the name Dot-ELISA. The results can be visualized in naked eye. The enzyme activity is indicated by intensity of the dot, which is directly proportional to the antigen concentration.

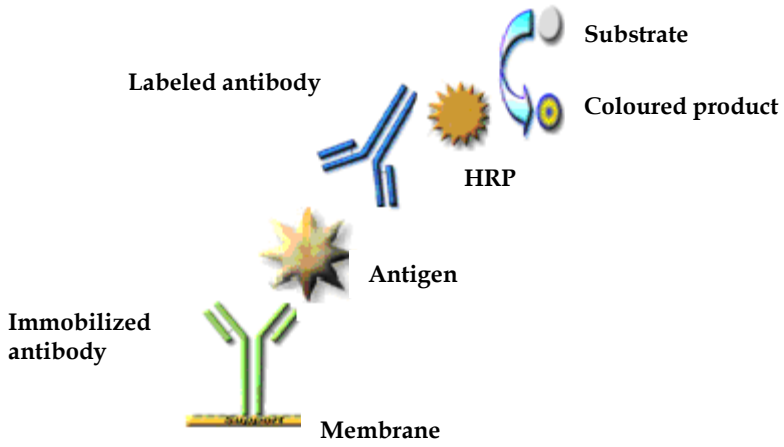


Figure 1: In Dot ELISA, an antibody is immobilized on a membrane and the test antigen is first allowed to react with immobilized antibody and then to the HRP-labeled antibody. The amount of HRP-labeled

antibody bound is measured by treating the membrane strip with an appropriate chromogenic substrate which is converted to a coloured precipitate and appears as a dot on the membrane

Kit Contents:

This kit can be used to detect the presence of a test antigen by immobilized antibody bound to the membrane 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
			15 expts	
1	TKC201	Dot ELISA Strips	15 Nos.	2-8°C
2	TKC202	Test Serum	0.035 ml	2-8°C
3	TKC203	10X Assay Buffer	20 ml	2-8°C
4	TKC204	Antibody-HRP Conjugate	0.035 ml	2-8°C
5	TKC205	TMB/H_2O_2	20 ml	2-8°C
6	PW1139	Collection Tubes, Polypropylene (2.0 ml)	15 Nos.	RT

Materials required but not provided:

Glassware: Test tubes
Reagents: Distilled water
Other requirements: Micropipette, Tips

Storage:

HiPer® Dot 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. Dilute required amount of 10X Assay Buffer to 1X with distilled water, before use.
4. Do not cross-contaminate reagents.
5. Never leave the reagents at room temperature.
6. Ensure that all the three zones of the strip are immersed in solution.
7. **Assay buffer:** Phosphate buffered saline – Tween (PBST).

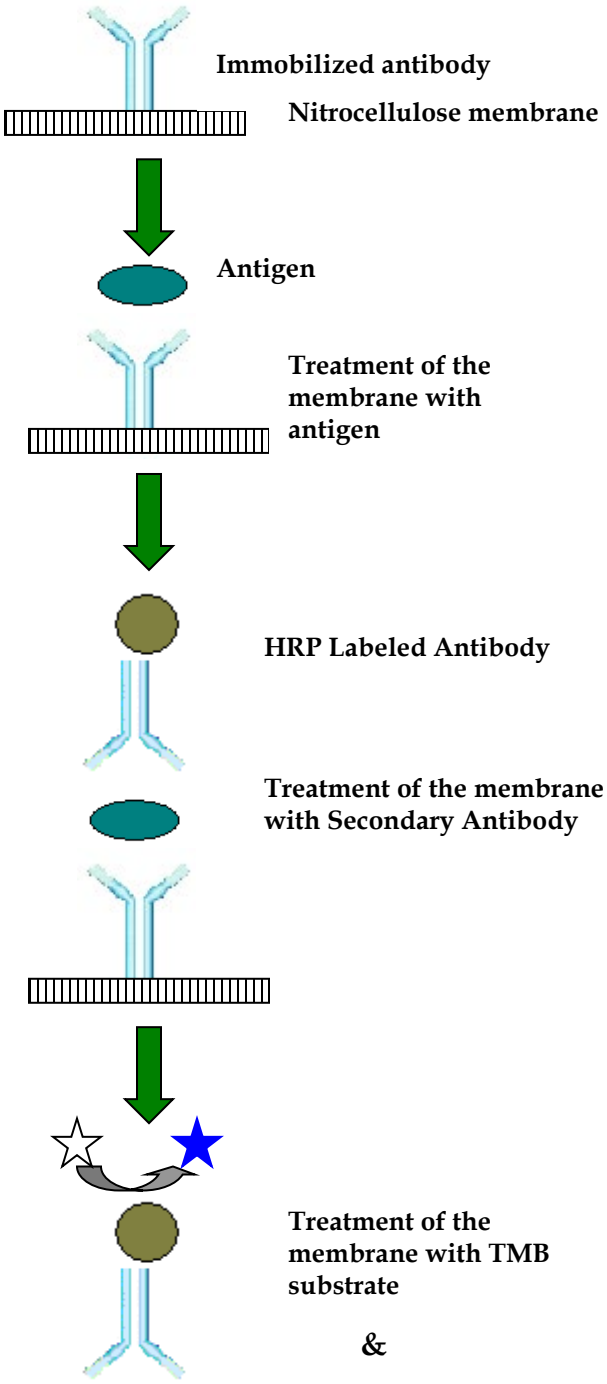
Procedure:

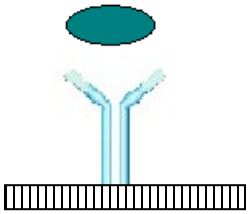
1. Take 2 ml of 1X Assay Buffer in a test tube and add 2 µl of the test serum sample. Mix thoroughly by pipetting. Insert a Dot-ELISA strip into the tube.
2. Incubate the tube at room temperature for 20 minutes. Discard the solution.
3. Wash the strip two times by dipping it in 2 ml of 1X Assay Buffer for about 5 minutes each. Replace the buffer each time.
4. Take 2 ml of 1X Assay Buffer in a fresh test tube, add 2 µl of HRP conjugated antibody to it. Mix thoroughly by pipetting. Dip the ELISA strip into it and allow the reaction to take place for 20 minutes.

5. Wash the strip as in step # 3 for two times.
6. In a collection tube (provided in the kit) take 1.3 ml of TMB/H_2O_2 and dip the ELISA strip into this substrate solution.
7. Observe the strip after 5 - 10 minutes for the appearance of a blue spot.
8. Rinse the strip with distilled water*.

* Molecular Biology Grade Water is recommended (Product Code: ML064)

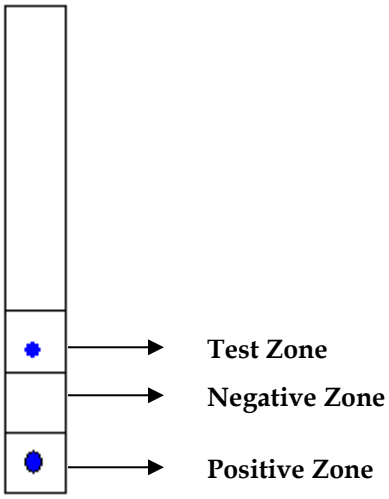
Flowchart:





Observation and Result:

Look for the appearance of the blue dot as shown below:



Record your observations as follows:

Zone	Spot
Positive Zone	
Negative Zone	
Test Zone	

Denote +ve : on appearance of a blue spot and -ve : on absence of a blue spot.

Interpretation:

Spot in the positive control zone and no spot in the negative control zone indicates proper performance of test. In the negative control zone the immobilized antibody is not present and the region is blocked with an inert protein. Therefore, there is no reaction when the reagents are added and no spot can be seen. In the test zone an antibody (specific to the test antigen, serum) is immobilized on it and then blocked with an inert protein. The test serum binds to this region and the HRP-labeled antibody binds to serum which when reacts with substrate develops blue dot. In the positive control zone, the test serum binds to the immobilized antibody and the HRP-labeled antibody binds to serum which when reacts with substrate develops blue dot.

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

Symbol:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIHTI015
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