HiPer® Ouchterlony Double Diffusion Teaching Kit (Antigen-Antibody Pattern)

Product Code: HTI002

Number of experiments that can be performed: 10

Duration of Experiment: 2 days

Day 1- Protocol: 1 hour

Day 2- Observations: 30 minutes

Storage Instructions:

- The kit is stable for 6 months from the date of receipt
- Store the 10X Assay buffer, Antiserums and Antigens at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

HiMediaLaboratories™

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Aim:

To study the reaction pattern of an antigen with a set of antibodies by Ouchterlony Double Diffusion method.

Introduction:

Immunodiffusion in gels encompasses a variety of techniques, which are useful for the analysis of antigens and antibodies. Gel immunodiffusion can be classified into two groups:

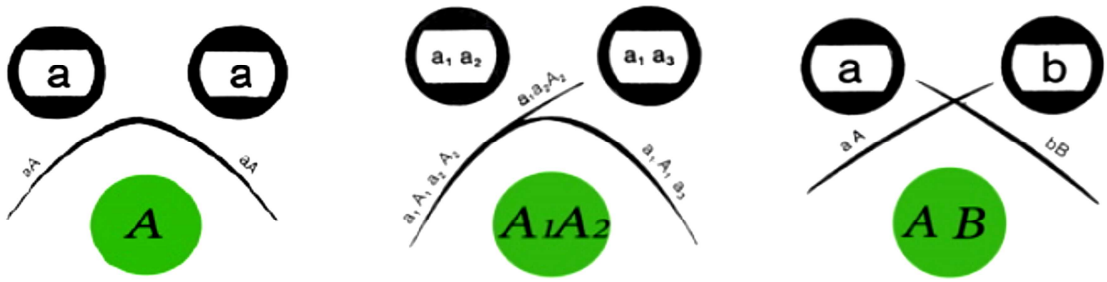
1. Single Immunodiffusion
2. Double Immunodiffusion

In the Ouchterlony double diffusion, both the antigen and the antibody diffuse toward each other in a semisolid medium to a point till their optimum concentration is reached. A band of precipitation occurs at this point. The qualitative Ouchterlony Test can simultaneously monitor multiple Antibody-Antigen system and can be used to identify particular antigens in a preparation. This procedure was developed by Örjan Ouchterlony.

Principle:

When soluble antigen and antibody samples are placed in adjacent wells in agarose gel, they diffuse radially into the agarose gel and set up two opposing concentration gradients between the wells. Once the gradients reach to an optimal proportion, interactions of the corresponding molecules occur and a line of precipitation will form. Using such a technique, the antigenic relationship between two antigens can be analyzed. Distinct precipitation line patterns are formed against the same anti-sera depending on whether two antigens share all antigenic epitopes or partially share their antigenic epitopes or do not share their antigenic epitopes at all. The Ouchterlony test also can be used to estimate the relative concentration of antigens. When an antigen has a relatively higher concentration, the equivalent zone will be formed a little bit away from the antigen well. When an antigen has a relatively lower concentration, the equivalent zone will be formed a little bit closer the antigen well.

The pattern of lines that form can be interpreted to determine the relationship between the antigens and antibodies.



X — **Pattern of Identity**

Y — **Pattern of Partial Identity**

Z — **Pattern of Non Identity**

Fig 1: Antigen- Antibody Patterns formed in Ouchterlony Double Diffusion

Pattern of Identity: X

Pattern of identity occurs when the antigens in the two wells are identical and specific for the antibody in the antiserum present in the third well. The concentration of the two antigens been the same, they will diffuse at the same rate resulting in a smooth line of precipitate. The antibodies cannot distinguish between the two antigens i.e. the two antigens are immunologically identical as shown in Fig 1.

Pattern of Partial Identity: Y

Pattern of partial identity occurs when the antigens in the two wells share some epitopes which are same for both, yet each of the two antigens also have unique epitopes. In this case antiserum contains polyclonal antibodies specific for each epitope. When one of the antigens has some of the same epitopes compared to other, the polyclonal antibody population will respond differently to the two antigens and the precipitin line formed for each antigen will be different. The 'spur' is thought to result from the determinants present in one antigen but lacking in the other antigen (refer to Fig 1).

A similar pattern of partial identity is observed if the antibodies are cross reactive with an epitope on one of the antigen that is similar, but not identical to that present on the other antigen.

Pattern of Non-Identity: Z

Pattern of non-identity occurs when the antigens in the two wells are totally different. They are neither cross reactive, nor do they have any epitopes which are same. In this case the antiserum containing the antibodies is heterogeneous as some of the antibodies react with antigen in one well while some react with antigen present in the other well. So the two antigens are immunologically unrelated as far as that antiserum is concerned (refer to Fig 1).

Kit Contents:

This kit can be used to determine the relationship between the antigens and antibodies using Ouchterlony Double Diffusion technique (Antigen-Antibody Pattern).

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

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			10 expts	
1	MB002	Agarose	1.2 g	R T
2	TKC081	10X Assay buffer	12 ml	2-8 °C
3	TKC069	Antiserum X	0.1 ml	2-8°C
4	TKC070	Antiserum Y	0.1 ml	2-8°C
5	TKC071	Antiserum Z	0.1 ml	2-8°C
6	TKC072	Antigen X1	0.1 ml	2-8°C
7	TKC073	Antigen X2	0.1 ml	2-8°C
8	TKC074	Antigen Y1	0.1 ml	2-8°C
9	TKC075	Antigen Y2	0.1 ml	2-8°C
10	TKC076	Antigen Z1	0.1 ml	2-8°C
11	TKC078	Antigen Z2	0.1 ml	2-8°C
12	TKC082	Glass plate	4 Nos.	R T
13	TKC083	Gel puncher	1 No.	R T
14	TKC079	Template	4 Nos.	R T

Materials Required But Not Provided:

Glass wares: Measuring cylinder, Beaker
Reagents: Alcohol
Other requirements: Incubator (37°C), Microwave or Bunsen burner, Vortex mixer, spatula, Micropipettes, Tips, Moist chamber (box with wet cotton)

Storage:

HiPer® Ouchterlony Double Diffusion Teaching Kit (Antigen- Antibody Pattern) is stable for 6 months from the date of receipt without showing any reduction in performance. Store 10X Assay buffer, Antisera and Antigens at 2-8°C. Other kit contents can be stored at room temperature (15-25°C).

Important Instructions:

1. Before starting the experiment the entire procedure has to be read carefully.
2. Always wear gloves while performing the experiment.
3. **Preparation of 1X Assay Buffer:** To prepare 10 ml of 1X Assay Buffer, add 1 ml of 10X Assay buffer to 9 ml of sterile distilled water.
4. **Preparation of 1% Agarose gel:** To prepare 10 ml of agarose gel, add 0.1g of agarose powder to 10 ml of 1X Assay Buffer, boil to dissolve the agarose completely.
5. Wipe the glass plates with cotton; make it grease free using alcohol for even spreading of agarose.
6. Cut the wells neatly without rugged margins.
7. Ensure that the moist chamber has enough wet cotton to keep the atmosphere humid.

Procedure:

1. Prepare 10 ml of 1% agarose (as given in important instructions).
2. Cool the solution to 55-60°C and pour 5 ml/plate on to grease free glass plates placed on a horizontal surface. Allow the gel to set for 30 minutes.
3. Place the glass plate on the template provided.
4. Punch wells with the help of the gel puncher corresponding to the markings on the template. Use gentle suction to avoid forming of rugged wells.
5. Add 10 µl each of the antiserum and the corresponding antigens to the wells as shown in fig 2.
6. Keep the glass plate in a moist chamber overnight at 37°C.
7. After incubation, observe for opaque precipitin lines between the antigen and antiserum wells.

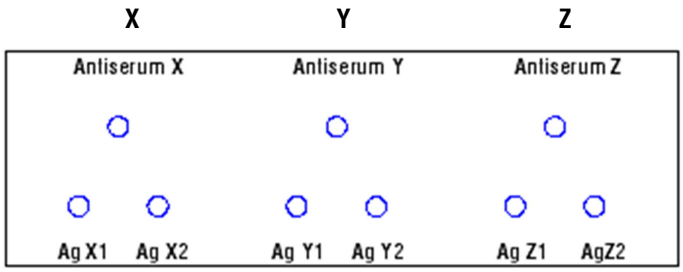


Fig 2: Template for addition of antiserum and antigen to their respective wells

Observation and Result:

Observe for presence of precipitin lines between antigen and antisera wells. Note the pattern of precipitin line observed in each case.

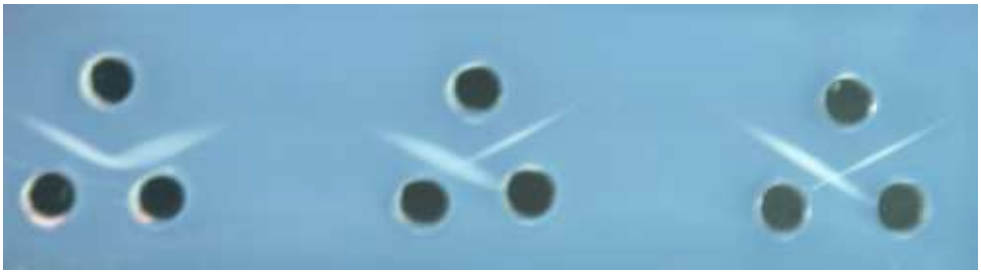


Fig 3: Diagram showing pattern of precipitin lines

Interpretation:

When antigen and antibody meet in optimal proportions a precipitation line is formed. In Ouchterlony Double Diffusion (Antigen Antibody Pattern), three patterns of precipitin lines can be observed.

1. If pattern X or pattern of identity is observed between the antigens and the antiserum, it indicates that the antigens are immunologically identical.

2. If pattern Y or pattern of partial identity is observed, it indicates that the antigens are partially similar or cross-reactive.

3. If pattern Z or pattern of non-identity is observed, it indicates that there is no cross-reaction between the antigens. i.e. the two antigens are immunologically unrelated.

Troubleshooting Guide:

Sr.No	Problem	Probable Cause	Solution
1	No precipitin ring observed	Inadequate filling of the wells	Samples should be loaded directly into the wells without spilling to the sides. Air bubbles should be avoided
		Drying of the agarose gel during incubation	Ensure that the moist chamber has enough moist cotton to avoid drying of the gel
2	Blur or improper precipitin ring observed	Samples not applied properly to the wells	Samples should be loaded directly into the wells without spilling to the sides
		Uneven pouring of gel	Place the glass plate on a flat surface while pouring the gel. Do not move the plate once the gel is poured

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