

## HiPer<sup>®</sup> Ouchterlony Double Diffusion Teaching Kit (For Antibody Titration)

**Product Code: HTI003**

**Number of experiments that can be performed: 15**

**Duration of Experiment: 2 days**

Day1- Protocol: 1 hour

Day2- Observations: 30 minutes

### **Storage Instructions:**

- The kit is stable for 12 months from the date of manufacture
- Store the 10X Assay buffer, Test Antiserum and Antigen at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

## Index

<b>Sr. No.</b>	<b>Contents</b>	<b>Page No.</b>
1	Aim	3
2	Introduction	3
3	Principle	3
4	Kit Contents	3
5	Materials Required But Not Provided	3
6	Storage	4
7	Important Instructions	4
8	Procedure	4
9	Observation and Result	5
10	Interpretation	5
11	Troubleshooting Guide	6

### Aim:

To learn the technique of Ouchterlony double diffusion (Antibody Titration).

### Introduction:

Interaction between antigen and antibody at the molecular level forms the basis for several techniques that are useful in modern day scientific studies and in routine clinical diagnosis. These techniques are either based on the use of labeled reagents, a tracer or immunoprecipitation. Ouchterlony double diffusion (ODD) or double immunodiffusion technique is one of the simplest techniques extensively used to check antisera for the presence of antibodies for a particular Ag and to determine its titre.

### Principle:

Precipitation of soluble antigen and antibody complexes as visible lattices in agarose can be utilized to find the 'Titre' value of the given antiserum. In Ouchterlony Double Diffusion technique a standard, uniform concentration of the antigen is subjected to diffusion with serially diluted antiserum samples. This is achieved when antigen sample is applied to a single central well, surrounded with suitable placed wells with serially diluted antiserum samples. Also, the same can be done in parallel rows of wells with constant antigen samples in all the wells of a row with corresponding rows of wells being loaded with the serially diluted antiserum samples. This later arrangement has the advantage with three rows of wells in a same agarose slide where, two sets of antisera samples can be tested against a common antigen. The highest dilution where the reaction between antigen and antibody stops is termed as the 'Titre Value' of the antiserum.

### Kit Contents:

This Kit can be used to determine the titre of the antiserum, by using serially diluted antiserum against the antigen and observing the formation of precipitin line.

**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	MB002	Agarose	1.7 g	RT
2	TKC081	10X Assay buffer	17 ml	2-8°C
3	TKC025	Test Antiserum	0.320 ml	2-8°C
4	TKC080	Antigen	0.17 ml	2-8°C
5	TKC095	Glass plate	3 Nos.	RT
6	TKC083	Gel puncher	1 No.	RT
7	TKC295	Template	3 Nos.	RT

### Materials Required but Not Provided:

**Glass wares:** Conical flask, Measuring cylinder, Beaker

**Reagents:** Distilled water\*, alcohol

**Other requirements:** Incubator (37°C), Microwave or Bunsen burner, Vortex mixer, spatula, Micropipettes, Tips, Moist chamber (box with wet cotton)

### Storage:

HiPer<sup>®</sup> Ouchterlony Double Diffusion Teaching Kit (For Antibody Titration) is stable for 12 months from the date of manufacture without showing any reduction in performance. Store the Assay buffer, Antiserum and Antigens at 2-8°C. Other reagents can be stored at room temperature (15-25°C).

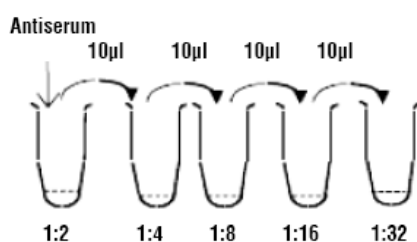
### Important Instructions:

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.

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

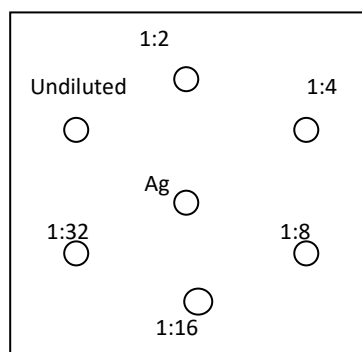
### Procedure:

1. Prepare 10 ml of 1% agarose (as given in important instructions).
2. Cool the solution to 55-60°C and pour 6 ml of the agarose solution on to grease free glass plate 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 gel puncher corresponding to the markings on the template. Use gentle suction to avoid forming rugged wells.
5. Serially dilute the test antiserum up to 1:32 dilution as described below:
  - a. Take 10µl of 1X assay buffer in each of the five vials.
  - b. Add 10µl of test antiserum into the first vial and mix well. The dilution of antiserum in this vial is 1:2.
  - c. Transfer 10µl of 1:2 diluted antiserum from the first vial into the second vial. The dilution is this is 1:4.
  - d. Repeat the dilutions up to fifth vial as shown in figure 1.



**Fig 1: Serial dilutions of antiserum**

- Add 10 $\mu$ l of the antigen to the center well and 10  $\mu$ l each of undiluted, 1:2, 1:4, 1:8, 1:16, 1:32 dilutions of antiserum into the surrounding wells as shown in figure 2.

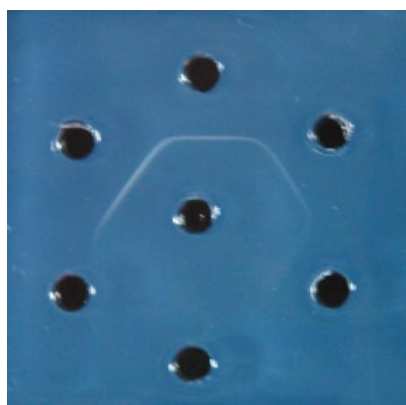


**Fig 2: Template for addition of antigen and antiserum to the wells.**

- Keep the glass plate in a moist chamber overnight at 37°C.
- After incubation, observe for opaque precipitin lines between the antigen and antisera wells.
- Note down the highest dilution at which the precipitin line is formed. This is the titre value of the antiserum.

### Observation and Result:

Observe for presence of precipitin lines between the centre antigen well and the corresponding serially diluted antisera wells and note down the titre value of the test antiserum.



**Fig 3: Precipitin lines observed in Ouchterlony Double Diffusion (Antibody Titration)**

Titre value was found to be 1: 8

**Note:** For sharper/ intense precipitin lines incubation can be done for longer period at 37°C.

### Interpretation:

Precipitin lines will be observed between the centre antigen and corresponding antiserum wells. The highest dilution of the antiserum where the precipitin line is observed is the titre value of the antiserum.









### 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
		Serial dilution of antiserum not carried out properly	Carry out the antiserum dilutions accurately and load the diluted samples in proper order
		Uneven pouring of the 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](mailto: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

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