

HiPer[®] Quantitative Precipitin Assay Teaching Kit

Product Code: HTI011

Number of experiments that can be performed: 10

Duration of Experiment: 2 hours 45 minutes

Storage Instructions:

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

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Aim:

To determine the antibody concentration by using quantitative precipitin assay.

Introduction:

Quantitative Precipitin Assay is a very simple method that is routinely used in the analysis of antibody and antigen interactions and for the determination of antibody or antigen concentration in a given sample. This method is based upon the interaction of antibody and antigen to form a protein complex which forms a precipitate in a specific buffer. The assay was described by Heidelberger in 1897.

Principle:

When an antigen and its corresponding antibody are combined in certain proportions, it forms a precipitate. Quantitative precipitin assay is an immunological method for measuring the concentration of precipitable antibodies present in a given sample. When increasing amounts of antigen are added to a constant amount of antibody in aqueous solution, they react to form a visible precipitate which can be measured spectrophotometrically. This precipitate is formed when there is a zone of equivalence i.e. perfect concentration of both antibody and antigen in the solution. The precipitated antibody is plotted as a function of antigen added. This method is very simple and reliable for the determination of antibody concentration in a given sample.

Kit Contents:

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	TKC222	Antigen	8 ml	2- 8 °C
2	TKC223	Antibody (Test)	6.6 ml	2- 8 °C
3	TKC224	10X Assay Buffer	10 ml	RT
4	TKC225	1M NaOH	8 ml	RT
5	PW1139	Collection Tubes, Polypropylene (2.0 ml)	60 Nos.	RT

Materials Required But Not Provided:

Reagents: Distilled water

Other requirements: Centrifuge, Spectrophotometer, Incubator, Micropipette, Tips, Cuvette

Storage:

HiPer® Quantitative Precipitin Assay Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. Store antigen and antibody at 2-8 °C. Other kit contents 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. **Preparation of 1X Assay Buffer:** Add 9 ml distilled water to 1 ml of 10X Assay Buffer and mix it properly. The diluted buffer should be stored at 2-8°C.
4. **Preparation of 0.1M NaOH (10 ml):** Add 9 ml distilled water to 1 ml of 1 M NaOH and mix it properly.

Procedure:

1. Take six tubes and label them accordingly. Add antigen (1.0 mg/ml), 1X assay buffer and test antiserum as mentioned in the following table:

Tube No	Antigen (in μ l)	Antigen Concentration (in μ g)	1X Assay Buffer (in μ l)	Test Antibody (in μ l)
1	0	-	300	100
2	50	50	250	100
3	100	100	200	100
4	150	150	150	100
5	200	200	100	100
6	250	250	50	100

2. Mix all the contents of the tubes and incubate at 37°C for an hour followed by incubation at 4°C for an hour.
4. Centrifuge the tubes at 10,000-12,000 rpm for 10 minutes.
5. Aspirate the supernatant carefully (Discard as much as supernatant as possible without disturbing the pellet. Wash the precipitate by resuspending the pellet with 1 ml 1X Assay Buffer.
6. Centrifuge at 10,000-12,000 rpm for 10 minutes.
7. Aspirate the supernatant carefully and dissolve the pellet in 1 ml of 0.1M NaOH.
8. Take OD of the solutions at 280 nm using 1 ml of 0.1M NaOH as blank.

Observation and Result:

Calculate the protein content of all the tubes from the following formula:

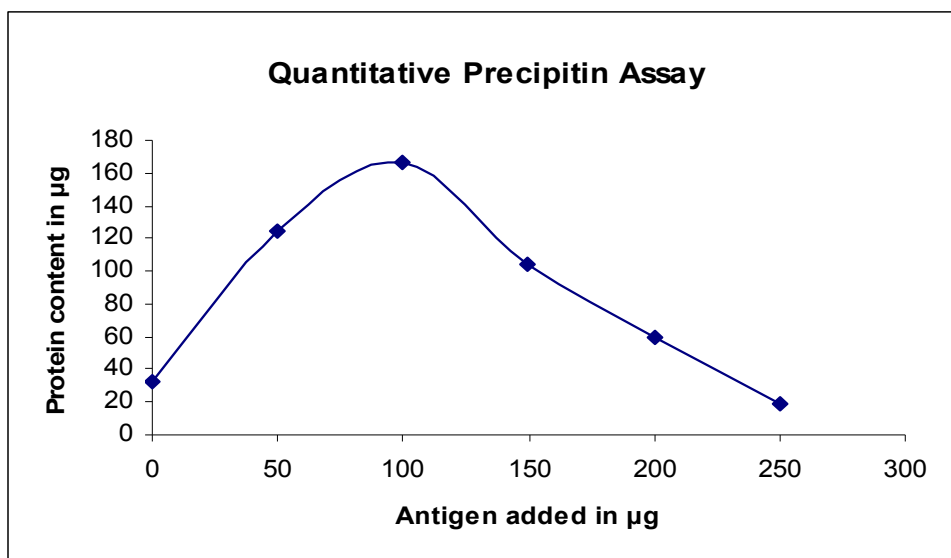
$$\text{Protein content in the precipitate} = (A_{280} \times M)/1.4 \text{ mg}$$

Where **M** is the amount of NaOH added in 'ml' and 1.4 is the Extinction Coefficient.

Record the absorbance values and the protein content as follows:

Tube No.	Absorbance (at 280 nm)	Protein content In μg
1		
2		
3		
4		
5		
6		

Plot the amount of protein (in the precipitate) in μg /tube on Y axis and corresponding antigen added (in μg) on X axis as follows:



From the graph, get the A_{280} values and the corresponding amount of antigen at the point of equivalence which is the point where the maximum amount of precipitate forms.

Calculate the amount of antibody (test) as follows:

$$\frac{(Pm-Ag) \text{ mg/ml}}{V}$$

Where,

Pm - Amount of maximum precipitate in μg

Ag - Amount of antigen in μg added in the tube

V - Volume of antiserum taken in μl

Interpretation:

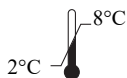
Quantitative Precipitin Assay is the basis of all quantitative antigen-antibody interactions. The amount of an unknown antibody can be determined when constant amount of antibody is mixed with increasing amount of antigen. The amount of test antibody can be easily determined by measuring the amount of precipitation.

Troubleshooting Guide:

Sr.No	Problem	Probable Cause	Solution
1	No zone of equivalence	Antigen or antibody not added properly	Follow the procedure exactly as written in the brochure
		Antigen and antibody are not stored properly	Store antigen and antibody at the temperature specified in the brochure

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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02/2025

PIHTI011_0/0222

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