HiPer® DNA Estimation Teaching Kit

Product Code: HTBC006

Number of experiments that can be performed: 5/20

Duration of Experiment

Protocol: 1 hour

Storage Instructions:

- The kit is stable for 6 months from the date of receipt
- Store DNA Standard and DNA Samples at -20°C
- Store Diphenylamine and Diluent Buffer at Room Temperature (15-25°C)

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Aim:

To determine the concentration of DNA by Diphenylamine (DPA) Method

Introduction:

HiPer® DNA Estimation Teaching Kit is designed for rapid and accurate determination of DNA by Diphenylamine reagent. Estimation of DNA is possible by a number of methods based on the physical or chemical properties of the nucleic acid. A convenient and easy colorimetric method is available on the basis of quantitative reaction of deoxysugar with diphenylamine reagent. The assay can be widely used on relatively crude extracts and in other circumstances where direct measurement of ultraviolet absorbance of denatured DNA is not practical.

Principle:

HiPer® DNA estimation Teaching Kit relies on the colorimetric determination of DNA present in the biological sample. The deoxyribose in DNA under acidic conditions at 100 °C form hydroxylevulinic aldehyde which reacts with Diphenylamine to give a blue colour. The colour intensity is measured at 595 nm which is directly proportional to the concentration of DNA. The reaction is given by free 2- deoxyribose, 2- deoxyxylose and purine deoxy ribonucleotides. Pyrimidine deoxy ribonucleotides scarcely react. Pentoses and RNA also react with the diphenylamine reagent, but not as strongly as DNA. Agar gives a strong green colour and thus cultures grown on agar should be washed thoroughly before processing.

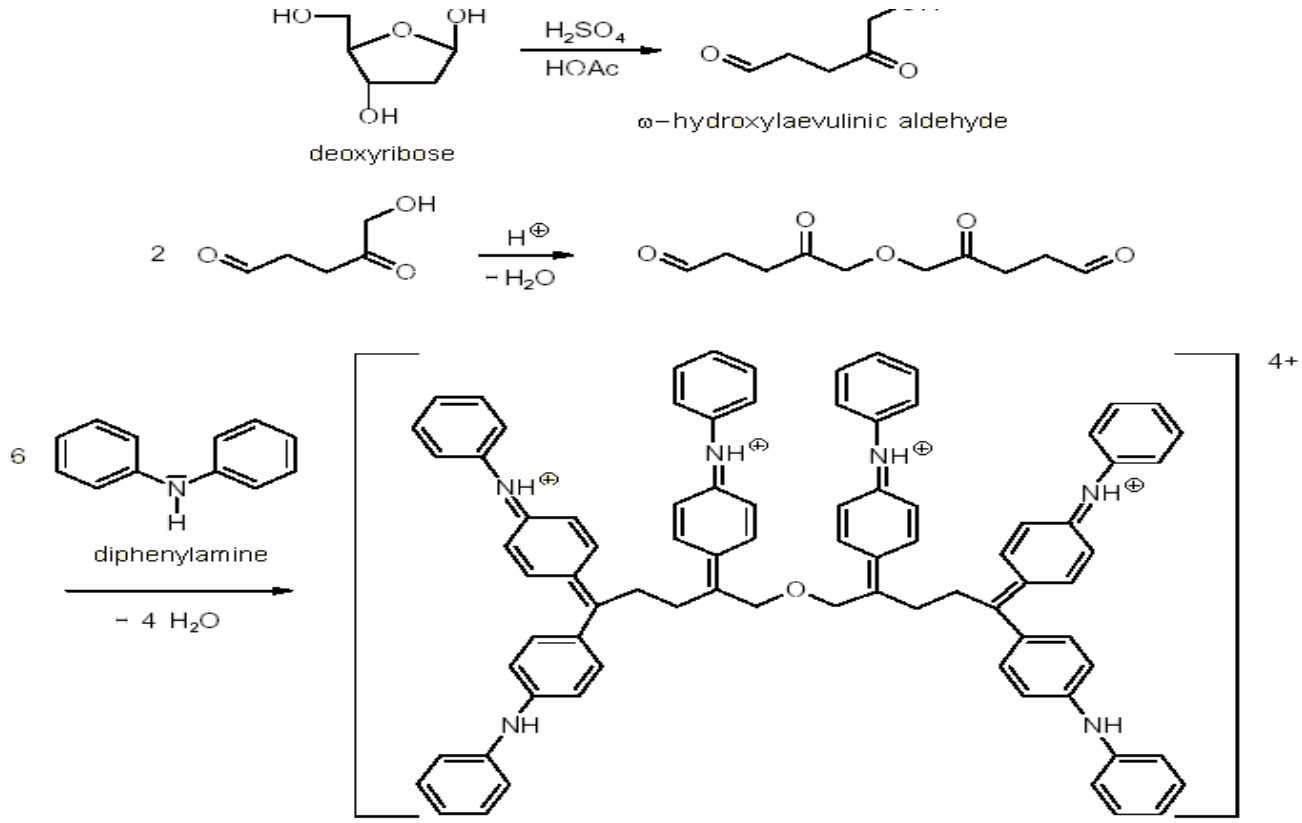


Fig 1: Sequence of reactions for the estimation of DNA by DPA method

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
			5 Expts	20 Expts	
1	TKC210	DNA Standard (10 mg/ml)	0.7 ml	2.3 ml	- 20°C
2	RM520	Diphenylamine	1.8 g	6.3 g	RT
3	TKC211	Diluent Buffer	8 ml	30 ml	RT
4	TKC212	DNA Sample 1	1.2 ml	4.2 ml	- 20°C
5	TKC213	DNA Sample 2	1.2 ml	4.2 ml	- 20°C

Materials Required But Not Provided:

Glass wares: 1 ml and 10 ml Pipettes, cuvettes, test tubes

Reagents: Glacial Acetic Acid, Concentrated Sulphuric Acid, Distilled Water

Other requirements: Spectrophotometer/Colorimeter to determine the absorbance at 595 nm, Micropipette and tips

Storage:

HiPer® DNA Estimation Teaching Kit is stable for 6 months from the date of receipt without showing any reduction in performance. On receipt, store DNA Standard, DNA Samples at - 20°C. Diphenylamine powder and Diluent Buffer can be stored at room temperature.

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. All glasswares should be clean and detergent free otherwise it will interfere with the assay.
3. The unknown and standard samples should be treated identically for accurate results.
4. The assay should be carrid out at the same time and in the same buffer conditions.
5. DNA test Samples provided are of different concentrations. (DNA Sample 1 and 2)
6. **Preparation of DPA Reagent (30 ml)**: Dissolve 0.3 g of DPA in 30 ml of Glacial Acetic Acid. Add 750 µl of concentrated sulphuric acid into it. The Diphenylamine reagent can be prepared in advance. Once prepared, it should be stored at 2-8°C.

Procedure:

1. Make dilutions of DNA standards with concentrations of 300, 250, 200, 150, 100, 50 µg/ 200 ul by transferring respective amount of DNA from the standard DNA solution (10 mg/ml) and adjusting it to a total volume of 200 µl by adding diluent buffer as mentioned below.
2. Add 3 ml of DPA reagent to each test tube including the Blank and Unknown tubes. Mix well.
3. Keep it in a Boiling Water Bath for 15 minutes. Take out the tubes and cool down to RT.
4. Switch on the Spectrophotometer, select the wavelength at 595 nm and let it warm before taking the absorbance (OD). First take the OD of Blank and make it zero.
5. Remove Blank tube and take the OD of all the tubes and record it. Wash the cuvette after taking OD of each sample.

Tube No.	**Blank**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Conc. Of DNA (µg)	0.0	50	100	150	200	250	300	DNA sample 1	DNA Sample 2
Amt of Stock (µl)	0.0	5	10	15	20	25	30	200 µl	200 µl
Amt of diluent (µl)	200	195	190	185	180	175	170		
Amt of DPA reagent (ml)	3	3	3	3	3	3	3	3	3
Keep in Boiling Water Bath for 15 minutes and cool									
Absorbance at 595 nm									

6. Plot a Standard Curve of absorbance at 595 nm on "Y" axis versus concentration of DNA µg/200 µl on "X" axis.
7. Record the value "x" of Unknown from graph corresponding to the optical density reading of the test.

Determination of DNA Concentration in Unknown Sample:

DNA concentration in unknown sample can be calculated using following formula:

$$\textbf{DNA Concentration in Unknown Sample} = \frac{\textbf{Concentration of Unknown in "µg"}}{\textbf{Volume of sample in "µl"}} \times \textbf{1000 µg/ml}$$

Observation and Result:

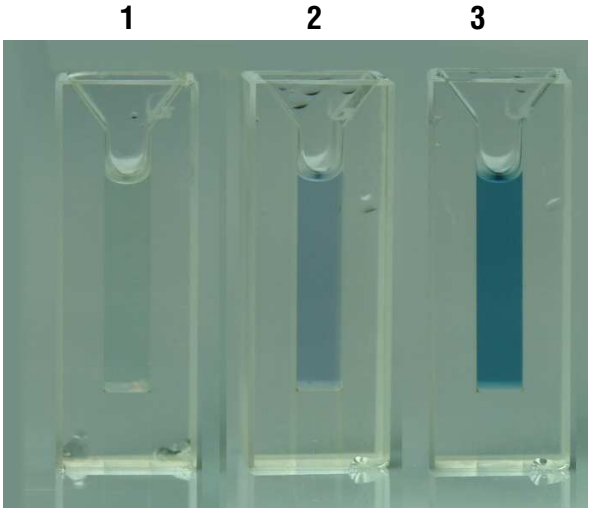
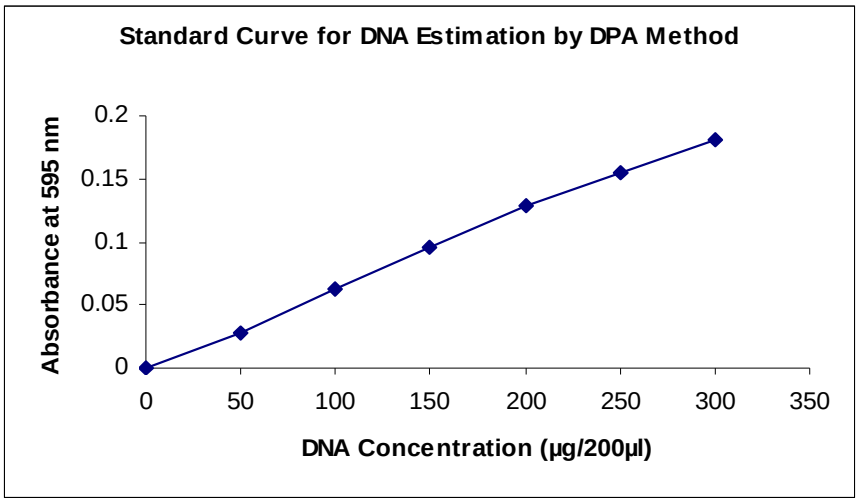


Fig 2: DNA Estimation by DPA method – showing increasing amounts of DNA concentration



Interpretation:

The DPA method is carried out by preparing a set of solutions with known DNA concentrations and mixing them with DPA reagent. A standard curve can be made and the concentration of unknown DNA sample can be derived from the standard curve.

Troubleshooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1	Standards and Samples give lower OD values than expected although the Blank is ok	DPA reagent was not stored properly	Always store reagent at 2-8°C
		Reagent was cold while performing the reaction	Allow reagent to come to RT before starting the reaction
		Absorbance was not measured at correct wavelength	Measure absorbance at correct wavelength as mentioned 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