BOTANY [Hons.]
Fourth Semester
C8P (Practical)

Molecular Biology

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Study 4: DNA estimation by diphenylamine reagent [UV Spectrophotometry]

Principle:

The deoxyribose sugar in DNA reacts in the presence of acid. It forms β-hydroxylevulinaldehyde which in turn reacts with diphenylamine to give a blue colour. This colour is estimated by UV spectrophotometer or by colorimeter at 595 nm.

In DNA, only the deoxyribose of the purine nucleotides react, so that the value obtained represents half of the total deoxyribose present.

Materials required:

Chemicals:

1. Standard DNA solution (0.25mg/ml)
2. Diphenylamine reagent
3. DNA sample in saline citrate buffer
4. Saline citrate buffer (0.15M NaCl, 0.015M sodium citrate, pH 7.0)
5. Glacial acetic Acid
6. Concentrated H_2SO_4
7. Ethanol

Glasswares & Plastic Wares:

1. **Beakers**
2. **Test-tubes**
3. **Cuvettes**
4. **Glass rods**
5. **Stirrer**
6. **Spatula**
7. **Dropper**
8. **Pipetteman**

Equipments:

1. **Measuring balance**
2. **UV spectrophotometer / colorimeter**
3. **Water Bath**

Procedure:

1. ***Preparation of reagent:*** **1.5g of diphenylamine is dissolved in 100ml of glacial acetic acid. It is followed by addition of 1.5ml of conc H_2SO_4. The solution must be stored in a dark glass bottle.**
2. **On the day of use, a fresh solution of ethanol (1ml) is prepared in 50ml of dH_2O (50ml).**

***Assay*:**

3. **0.5ml of this solution is added to each 100ml of the diphenylamine solution.**
4. **A series of dilutions of standard DNA (0.25mg/ml) in saline citrate buffer needs to be prepared to give a concentration of 50-500µg/ml.**
5. **All the samples are prepared in triplicate.**
6. **4ml of diphenylamine reagent is added to 2ml of each dilution of blank, standard and unknown and mixed.**
7. **Tube1 is used as blank and tubes 2 through 7 are**
8. **used for construction of a standard calibration curve for DNA.**
9. **Tubes 8-9 are for unknown samples. (Table1)**
10. **All the tubes are incubated in boiling water for 10 min.**
11. **The test-tubes are cooled and the absorbance read at 595nm against the blank.**
12. **A standard curve is prepared of absorbance A595 vs. quantity of DNA and then the concentration of unknown DNA dissolved in the saline citrate solution is calculated from the standard curve.**

TABLE 1: OBSERVATIONS

SL NO.	DNA		DH_2O	REAGENT	A_{595}
	µl	µg			
1	-	-	2000	4	
2	200	50	1800	4	
3	400	100	1600	4	
4	800	200	1200	4	
5	1200	300	800	4	
6	1600	400	400	4	
7	2000	500	-	4	
8	Unknown (A)			4	
9	Unknown (B)			4	

Calculations: The amount of DNA in the unknown samples is determined by plotting a standard curve of A_{595} on Y-axis and µg of DNA on X-axis.

Precautions:

1. Eye protection and use of a fume hood when preparing DPA reagent is recommended.
2. Diphenylamine is harmful if ingested or inhaled and may irritate skin or eyes if it comes into contact with them. Wash immediately with plain water if it happen.
3. The cuvettes to be handled very carefully.
4. The DPA reagent to be always stored in the dark.

Students please Note:

The relevant figures will be sent to all of your email-IDs.

References/sources used:

1. Jain A., Jain R., Jain S. (2020) Estimation of DNA by Diphenylamine Reaction. In: Basic Techniques in Biochemistry, Microbiology and Molecular Biology. Springer Protocols Handbooks. Humana, New York, NY

Further reading/ Viewing:

1. https://www.youtube.com/watch?v=Q799WUT8VQo
2. https://www.youtube.com/watch?v=b-mlIWb1lDQ
3. Plummer, D.T. (1977) *An Introduction to Practical Biochemistry. Tata McGraw Hill,* Bombay.