BT 210 BIOCHEMISTRY LAB

ESTIMATION OF DNA BY DIPHENYLAMINE REACTION

Principle:

The deoxyribose in DNA in the presence of acid forms β-hydroxylevulinaldehyde which reacts with diphenylamine to give a blue colour with a sharp absorption maximum at 595nm. In DNA, only the deoxyribose of the purine nucleotides react, so that the value obtained represents half of the total deoxyribose present.

METHODOLOGY

a) Materials required:

1. **Equipments**:
   - Spectrophotometer
   - Water bath

2. **Chemicals/reagents**:
   - Standard DNA solution (0.25mg/ml)
   - Diphenylamine reagent
   - DNA sample in saline citrate buffer
   - Saline citrate buffer (0.15M NaCl, 0.015M sodium citrate, pH 7.0)
   - Glacial acetic Acid
   - Concentrated H_2SO_4
   - Ethanal

3. **Glasswares and others**:
   - Test tubes
   - Pipettes
   - Graduated cylinder

b) Procedure:

Preparation of reagent: Dissolve 1.5g diphenylamine in 100ml of glacial acetic acid. Add 1.5ml of conc H_2SO_4. Store the solution in a dark glass bottle. On the day of use, prepare a fresh solution of ethanal (1ml) in dH_2O (50ml). Add 0.5ml of this solution to each 100ml of the diphenylamine solution.

Caution: Wear eye protection and use a fume cupboard when preparing this reagent. Diphenylamine is harmful if ingested or inhaled and may irritate skin or eyes if it comes into contact with them.

c) Assay:

1. Prepare a series of dilutions of standard DNA (0.25mg/ml) in saline citrate buffer to give a concentration of 50-500μg/ml.
2. Prepare all the samples in triplicate.
3. To 2ml of each dilution of blank, standard and unknown add 4ml of diphenylamine reagent and mix. Tube1 is used as blank and tubes 2 through 7 are used for construction of a standard calibration curve for DNA. Tubes 8-11 are for unknown samples. (Table1)
4. Incubate all the tubes in boiling water for 10 min.
5. Cool the tubes and read the absorbance at 595nm against the blank.
6. Construct a standard curve of absorbance A_{595} vs. quantity of DNA and then calculate the concentration of unknown DNA dissolved in the saline citrate solution.

Table1:

Sl No	DNA		DH_2O (µl)	Reagent (ml)	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	
10.	Unknown (C)	-	-	4	
11.	Unknown (D)	-	-	4	

Calculation: Determine the slope y/x from the standard curve, which gives the A_{595} per unit of DNA (µg). Hence determine the amount of DNA in the unknown sample.

References:

1. Plummer, D.T. (1977) *An Introduction to Practical Biochemistry*. Tata McGraw Hill, Bombay.
2. J. Jayaram. (1981) *Laboratory Manual in Biochemistry*. New Age International Ltd. New Delhi.
3. Burton, K. (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochemical Journal*. **62**: 314-323.