Aim 1 To Estimate the Amount of DNA in given Sample

Introduction

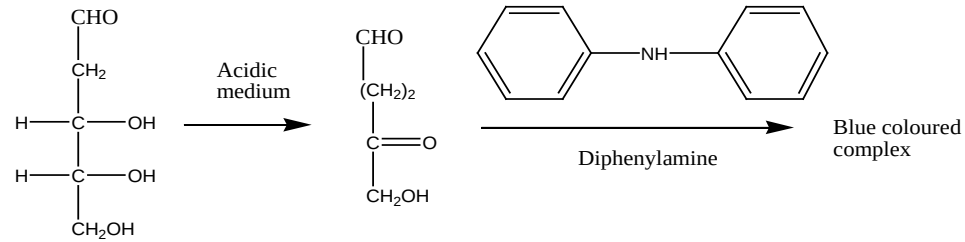
When DNA is treated with diphenylamine, a blue coloured compound is formed under the acidic condition that shows maximum absorption at 595 nm. In general, this reaction is used for 2-deoxy pentose, not for DNA. Under the acidic condition, the deoxypentose sugar of DNA is converted into ω-hydroxy levulinic aldehyde which is highly reactive with diphenylamine and produced a blue coloured complex.

In this reaction, only deoxyribose of purine nucleotides are released and the value is obtained which is half of the total deoxyribose present in the sample.

Reagents

1. Saline buffer (pH 7.0, 0.015 M sodium citrate and 0.15 M NaCl).
2. DNA (1 mg/ml in the saline buffer).
3. Diphenylamine reagent (DPA) (Dissolve 10g diphenylamine in 25 ml of concentrated H_2SO_4 and 1L of glacial acetic acid). Fresh solution should be prepared.

Reaction



2-deoxyribose ω-hydroxy levulinic aldehyde

Procedure

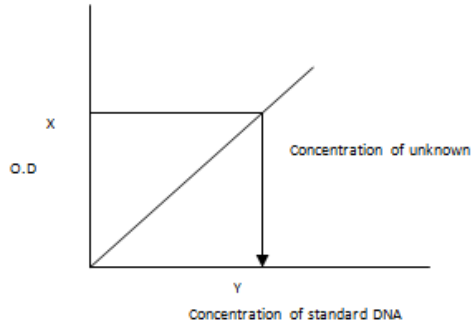
1. Different volumes of standard DNA solution are pipetted out into series of test tubes.
2. 0.5 ml and 1.0 ml of the test sample is pipetted out in test tubes.
3. One test tube is taken as blank.
4. Add saline buffer as shown in observation table to make final volume 2 ml.
5. Add 4 ml of diphenylamine reagent (DPA) to each tube and mix well.
6. Put them in boiling water bath for 10 minutes.
7. Cool and read the absorbance at 595 nm.

Then, a standard curve is drawn and concentration of the unknown sample is calculated.

Observation Table

S. No.	DNA volume (ml)	DNA Conc. (mg)	Buffer volume (ml)	DPA reagent (ml)		O.D. 595 nm
Blank	-	-	2.0	4	Put it on water bath for 10 minutes	
1	0.2	0.2	1.8			
2	0.4	0.4	1.6			
3	0.6	0.6	1.4			
4	0.8	0.8	1.2			
5	1.0	1.0	1.0			
6	1.2	1.2	0.8			
7	1.4	1.4	0.6			
8	1.6	1.6	0.4			
9	1.8	1.8	0.2			
10	2.0	2.0	-			
Unknown	0.5		1.5			
	1.0		1.0			

Standard curve



Calculations

O.D. of sample - X

From standard curve

Suppose X, O.D. corresponds to y mg of DNA

Unknown sample taken – 0.5 ml

So 0.5 ml of the unknown sample contains y mg of DNA.

DNA (mg %) = Y/0.5*100

Alternatively, the concentration can be calculated by the formula.

O.D. test……

O.D. Standard -…….

DNA (mg %) = (O.D. test/ O.D. standard X concentration of standard / volume of sample in ml)* 100

The value resulted by reaction of deoxyribose of purine nucleotides represents half of the total deoxyribose in the sample. The actual value can be obtained by multiplying DNA concentration with 2.

Precautions

1. All apparatus should be clean and dry.
2. Fresh DPA reagent should be used.
3. DNA sample should be weighed accurately.
4. The solution should be carefully pipetted out.
5. Do not pipette DPA reagent via mouth as its fumes are harmful.