Estimation of DNA by Diphenylamine method

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Aim: To estimate the concentration of DNA by diphenylamine reaction.

Principle: This is a general reaction given by deoxypentoses. The 2-deoxyribose of DNA, in the presence of acid, is converted to ω-hydroxilevulinic aldehyde, which reacts with diphenylamine to form a blue coloured complex, which can be read at 595 nm.

Requirements:

1. Standard DNA solution- Dissolve calf thymus DNA (200µg/ml) in 1N perchloric acid/buffered saline.

2. Diphenylamine solution- Dissolve 1g of diphenylamine in 100 ml of glacial acetic acid and 2.5 ml of concentrated H_2SO_4. This solution must be prepared fresh

3. Buffered Saline- 0.5 mol/litre NaCl; 0.015 mol/litre sodium citrate, pH 7.

Procedure:

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.
2. Pipette out 1 ml of the given sample in another test tube.
3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
4. Now add 2 ml of DPA reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
5. Mix the contents of the tubes by vortexing / shaking the tubes and incubate on a boiling water bath for 10 min.
6. Then cool the contents and record the absorbance at 595 nm against blank.
7. Then plot the standard curve by taking concentration of DNA along X-axis and absorbance at 595 nm along Y-axis.
8. Then from this standard curve calculate the concentration of DNA in the given sample.

Result: The given unknown sample contains ----µg DNA/ml.

Observations and Calculations

Volume of standard (200 µg/ml) DNA (ml)	Volume of distilled water (ml)	Concentration of DNA (µg)	Volume of DPA reagent (ml)	Incubate in boiling water bath for 10 Min & Cool	A595
0.0	1.0	00	2		0.00
0.2	0.8	40	2		
0.4	0.6	80	2		
0.6	0.4	120	2		
0.8	0.2	160	2		
1.0	0.0	200	2		
1.0 Unknown	0.0	To be Estimated	2