B.Sc. Biotechnology

Experiment- DNA Quantification and Quality check using A260/A280 Ratio.

Aim- (a) Calculate the concentration of ds DNA sample (**OD-0.8 at 260nm**) by using standard plot method and by using formula (**DNA Conc = Conversion factor x OD x Dilution factor**)

Dilution factor = 100

Using a conversion factor :

- one OD at 260 nm is equivalent to

Nucleic Acid	Concentration (µg/ml) per A_{260} Unit
ds DNA	50
ss DNA	33
ss RNA	40

Readings to plot Standard Plot-

Concentration	Absorbance
50ng/ul	0.978
25ng/ul	0.521
12.5ng/ul	0.253
6.25ng/ul	0.125

(b) Compare the Quality of 3 samples and comment on the contamination it might have?

Absorbance	Sample1	Sample2	Sample 3
260 nm	0.8	0.5	0.9
280 nm	0.44	0.4	0.4

Purity of DNA:

$\frac{A_{260}}{A_{280}}$ **, we get purity of DNA**

In this we check purity of DNA, whether it is contaminated with RNA and protein or not.

If ratio = 1.6 – 2.0 (Pure DNA)

If ratio < 1.6 (Proteins / Phenol contamination presents)

If ratio > 2.0 (RNA contamination present)