

NUCLEIC ACID CONCENTRATION VIA UV_{260/280} ABSORBANCE

Rem: Turn on the spectrophotometer and warm up UV bulb.

Rem: Calculate dilution so that OD₂₆₀ is in the range of 1.0 and 0.1 for best results.
(0.05 O.K. on HP spec)

Rem: It takes more dsDNA to yield an OD₂₆₀=1.0 due to hypochromic effect.

(1) Record OD₂₆₀ and OD₂₈₀ measurements.

(2) Calculate estimated concentration of nucleic acid solution using the following equation:

$$\frac{\text{OD}_{260} * \text{dilution factor}}{X} = \mu\text{g}/\mu\text{l}$$

X = 20 for dsDNA

X = 25 for ssDNA and RNA (Also used for natural population total NA's)

X = 50 for oligonucleotides (rough estimate at best!)

Rem: ~5.0 to 2.5 ng/μl minimum for dsDNA.

Therefore OD₂₆₀ = 1.0 corresponds to:

50 ng/μl dsDNA

40 ng/μl ssDNA and RNA (Natural pop NA's)

20 ng/μl oligonucleotides (random!)

Use the OD_{260/280} ratio to determine purity:

DNA = 1.8 - 1.9

RNA = 1.9 - 2.0

For more precise estimates of oligonucleotide concentration use the following molar extinction coefficient:

$$\begin{array}{l} \text{G} = 12010 \\ \text{A} = 15200 \\ \text{T} = 8400 \\ \text{C} = 7050 \end{array} \quad \text{mM} = \frac{\text{OD}_{260} * \text{dilution factor}}{[(\#G's * 12.01) + (\#A's * 15.20) + (\#T's * 8.40) + (\#C's * 7.05)]}$$