Spectrophotometry

IMBB 2014
Spectrophotometry

• Nucleic acids absorb ultraviolet light (UV) in a specific spectral pattern depending on base composition.

• The more light absorbed by the sample, the higher the nucleic acid concentration in the sample.

• The peak absorbance of DNA & RNA is at 260 nm ($A_{260}$)
Spectrophotometry: Application in nucleic acid analysis

• Measure the concentration of DNA or RNA in solution.

• Measure purity relative to contaminating proteins and organic and inorganic compounds e.g. phenol.
Conversion factor for measuring DNA concentration

Optical density (A260) of 1.0 = 50 ng/μl dsDNA (for a path length = 1 cm)

Therefore, the conversion factor is 50
Practical use: Concentration

Example:

- Absorbance at 260 nm (A260) = 0.4
- Path length = 1 cm

Path length x A260 x conv. factor (50)

= 1 x 0.4 X 50

= 20 ng/µl
Measuring protein contamination: A260:280 ratio

- The peak absorbance of protein is at 280 nm
- The ratio A260/280 is used to assess protein contamination of nucleic acids
- For pure DNA, A260/280 is ~1.8 and for pure RNA A260/280 is ~2
- A low ratio may be the result of a contaminant absorbing at 280 nm or less
- Note: it takes a relatively large amount of protein contamination to significantly affect the 260:280 ratio in a nucleic acid solution
Measuring chemical contamination: A260:230 ratio

- Contamination with residual chemicals from extraction procedures may affect the A260/230 ratio.

- Expected A260/230 values are commonly in the range 2.0-2.2

- A low ratio may be the result of a contaminant absorbing at 230 nm or less
NanoDrop Spectrophotometer

- Fast- measurement time < 5 sec
- Small sample volume: 0.5 – 2 μL
- Measures DNA, RNA, proteins, cell suspensions
- Measures up to 15,000 ng/μl with no sample dilution
- Low cost operation - no extra consumables
- Stores digital data on computer
NanoDrop spectrophotometer

Sampling arm

Lower measuring pedestal
NanoDrop spectrophotometer: How does it work?

Fiber optic cable in measurement arm

Sample (1-2 µL)

Receiving fiber (fiber optic cable)

Spectrophotometer

Xenon lamp
NanoDrop reading for pure DNA
NanoDrop 1000
http://www.youtube.com/watch?v=ZodxPBlyKvQ

NanoDrop Microvolume Quantitation of Nucleic Acids
http://www.youtube.com/watch?v=FiGZnNs2xXY