Nucleic Acid Quantitation

Quantitation of nucleic acids is commonly performed to determine the concentration of DNA or RNA present in a mixture. It is also used to determine the purity of a DNA or RNA sample. Contamination of a nucleic acid solution by proteins, carbohydrates, and other organic molecules can be determined using a procedure called the A260/280 ratio.

The basis of this test relies on the Beer-Lambert law: A = εbc; where A is absorbance, ε is the molar extinction coefficient, b is the cell path length, and c is the sample concentration. The commonly accepted extinction coefficients for a 1 mg/mL nucleic acid solution at 260 nm and 280 nm are 20 and 10, respectively. In proteins, the extinction coefficient values at 260 nm and 280 nm at a concentration of 1 mg/mL are 0.57 and 1.00, respectively. Therefore, nucleic acid samples would be expected to have a higher absorbance at 260 nm than at 280 nm; in a protein sample, the opposite is true. Using these extinction coefficients, pure nucleic acid samples would have an A260/280 ratio of 2.0, while pure protein samples would have an A260/280 ratio of 0.57.

OBJECTIVES

In this experiment, you will determine the A260/280 ratio of a sample.

MATERIALS

Chromebook or computer

Vernier Spectral Analysis app

UV-VIS spectrophotometer[[1]](#footnote-1)

prepared biological sample containing DNA, RNA and/or protein

buffer solution used to prepare biological sample

PROCEDURE

1. Obtain and wear goggles and gloves.

2. Connect the AC power supply to the UV-VIS Spectrophotometer. Turn the power switch to the ON position.

3. When the LED for the lamp indicator stays green, connect the spectrophotometer to the USB port of a Chromebook or a computer. Launch Spectral Analysis and select Absorbance vs. Wavelength. **Note**: If using Go Direct UV-VIS Spectrophotometer, you can also connect to a mobile device.

4. Prepare a blank by filling the empty quartz cuvette 3/4 full with the buffer solution used to prepare your sample. Calibrate the spectrophotometer.



5. Collect absorbance *vs.* wavelength data.

1. Fill a cuvette with ~3 mL of the biological sample to be tested. Your sample should not be turbid or have any particulates in it.
2. Place the sample in the spectrophotometer.
3. Start data collection. Once the spectrum is displayed, stop data collection.
4. Make sure the absorbance values at 260 nm and 280 nm are between 0.1 and 1.0 absorbance units. Any values outside this range may introduce an error. If your sample is outside this range, dilute or concentrate it and take another spectrum.

6. Using the Examine feature or by scrolling down the data table, find the exact absorbance values at 260 nm, 280 nm, and 340 nm. Record the absorbance values in your data table. **Note**: A value of 340 nm is used to normalize here because there can be contaminants in your solution that scatter the light and create an absorbance offset throughout this wavelength region.

7. Repeat Steps 5–6 two more times (optional). **Note**: Data are stored automatically.

DATA table

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | A260 | A280 | A340 | A260/280 (corrected) |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
|  |  |  | Average |  |

DATA Analysis

1. Correct your A260 and A280 absorbance values for the A340 absorbance value according to the following equation:



Complete this calculation for each run. Record these values in the data table.

2. Calculate the average A260/280 of your three trials. Record this value in the data table.

3. Discuss the purity and composition of your samples.

1. The procedure is written for UV-VIS spectrophotometers from Vernier including the Go Direct UV-VIS Spectrophotometer (order code: GDX-SPEC-UV) and the Vernier UV-VIS Spectrophotometer (VSP-UV). [↑](#footnote-ref-1)