Nucleic Acid Quantitation

before you begin

* This experiment is compatible with the Go Direct UV-VIS Spectrophotometer (order code: GDX-SPEC-UV) and the Vernier UV-VIS Spectrophotometer (VSP-UV). It is recommended that you read the user guide for your device before beginning this, or any, experiment with the instrument.
* This experiment requires the most recent version of the data-collection software you are using (e.g., Logger *Pro*, LabQuest 2 App, LabQuest 3 App, or Spectral Analysis). Download the latest version of your software at <www.vernier.com/downloads>

Experiment Notes

1. Biological samples can be unstable. They should be prepared freshly for this experiment or stored properly.

2. This experiment is designed only to analyze a biological sample. The preparation and concentrations your students use are up to you. You may want to do a trial run to confirm the absorbance values at 260 nm and 280 nm are less than 1.0. Absorbance values higher than 1.0 begin to lose linearity and could therefore generate erroneous results.

3. A value of 340 nm is used as a correction factor here because there can be contaminants in your solution that scatter the light and create an absorbance offset throughout this wavelength region. The value in a pure nucleic acid sample should be zero. If you are more concerned about electronic noise, you may want to instruct your students to pick the absorbance value at 800 nm to normalize.

4. Negative values could result if an incorrect solution was used as a blank. Alternatively, these values could arise due to fluorescence of a dye in the solution.

Sample Data

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | A260 | A280 | A340 | A260/280 (corrected) |
| 1 | 0.93 | 0.47 | 0.01 | 2.00 |
| 2 | 0.86 | 0.42 | 0.01 | 2.07 |
| 3 | 0.88 | 0.47 | 0.01 | 1.89 |
|  |  |  | Average | 1.99 |

Answers to the DATA Analysis Questions

1. Answers will vary. See sample data above.

2. Answers will vary. See sample data above.

3. Answers will vary. For pure DNA, A260/280 is 1.8 and for pure RNA, A260/280 is 2.0. Industrial samples, such as the DNACON used in the sample data, are prepared to resemble the absorption of pure RNA.

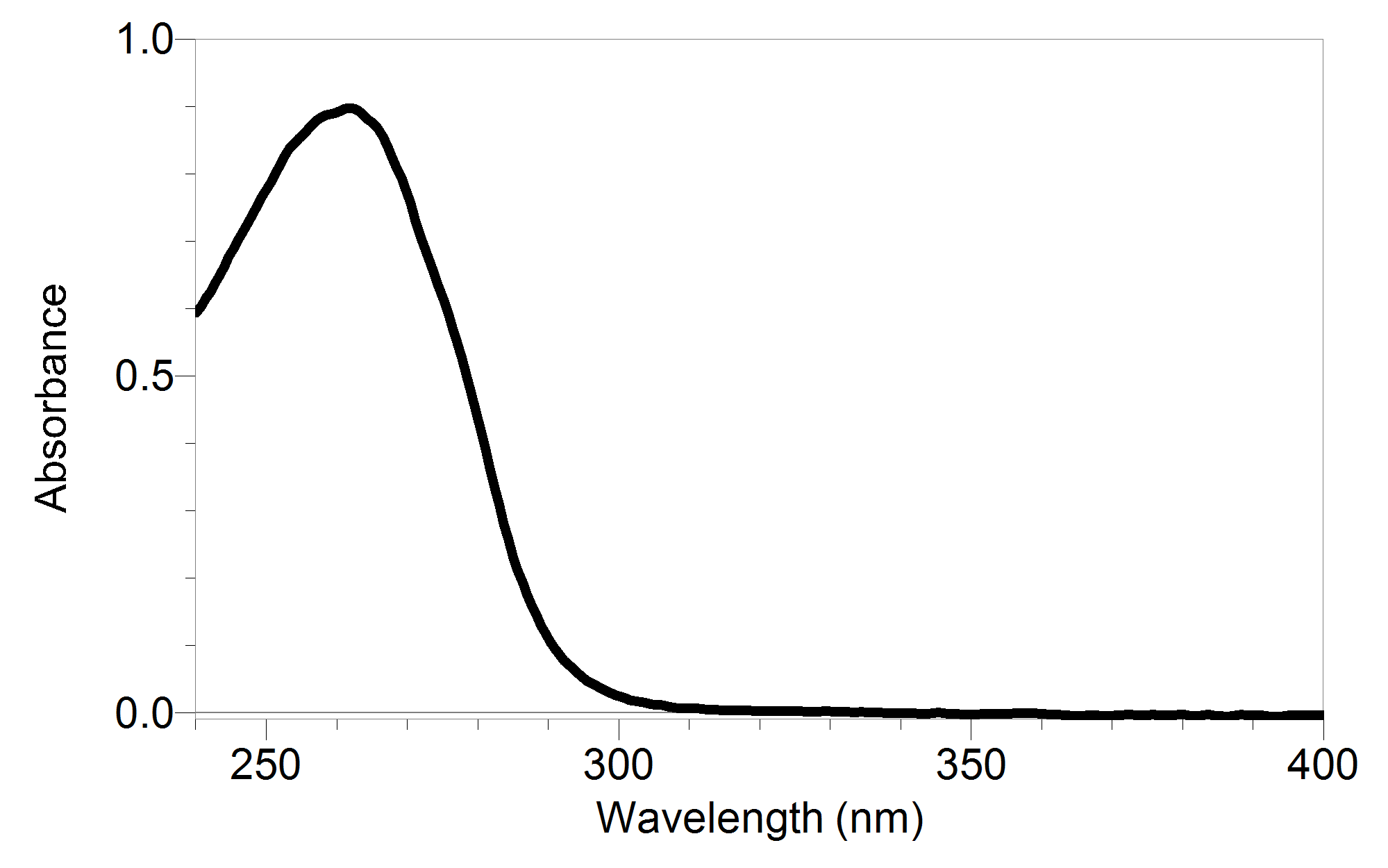
Sample graphs

Figure 1 Absorbance spectrum of Starna DNACON 260/280