

## Using the multi-wavelength mode equations for nucleic acid and protein determinations

### ■ Introduction

The multi-wavelength mode of the 67 series spectrophotometers can be used to measure absorbance or transmittance of a sample at up to four different wavelengths. This mode is used for tests where ratios of absorbance values (or difference between absorbance values) at different wavelengths are required. An example of this is the check of DNA and RNA purity. Methods can also be set up to perform DNA and protein concentration calculations using some of the “sum” options in the calculations set up. This protocol details how to set up methods for these simple calculations using the various “sum” options in the multi-wavelength mode.

### ■ $A_{260}/A_{280}$ ratio

A common measurement using the ratio of two wavelengths is to check the purity of DNA and RNA. Pure preparations of DNA and RNA dissolved in TE (pH 8.0) have  $A_{260}:A_{280}$  values of  $\geq 1.8$  and  $\geq 2.0$  respectively. A lower ratio indicates that the sample is significantly contaminated with proteins and/or aromatic substances such as phenol. For DNA, a higher ratio could indicate contamination with RNA. Other factors which affect the ratio are the concentration of the sample and pH<sup>1</sup>. The sample should be of sufficient concentration to give an  $A_{260} \geq 0.1$  for accurate ratio measurements. Acidic conditions give lower ratios and basic conditions can increase ratios by 0.2 to 0.3.

Follow the steps given below to set up a method for  $A_{260}/A_{280}$  ratio measurement:

1. In the multi-wavelength mode method set up, set wavelength #1 to 260nm and wavelength #2 to 280nm.
2. On the **Calculations** tab, specify the sum  $A_1/A_2$  and  $A_1-A_2$ .
3. Set the primary wavelength to wavelength #1 (260nm) and the secondary wavelength to wavelength #2 (280nm).
4. Continue setting up the method as required.
5. Place the sample blank in the sample chamber and close the lid.
6. Press the **Zero** key. This will zero the blank at all the selected wavelengths.
7. Remove the blank and replace it with the sample to be measured. Close the lid.
8. Press the **Read** key. This will read the sample at each wavelength in turn.
9. When completed, the measured absorbance at each wavelength will be displayed, together with the results of the selected calculation.

### ■ Other ratios

The purity of RNA can also be assessed by measuring the  $A_{260}/A_{230}$  ratio. For pure RNA this ratio should be greater than 2.0; a value less than this could indicate the presence of reagent carry-over from the extraction procedure e.g. guanidinium thiocyanate, phenol, TRIzol<sup>®</sup> or other salts.

Follow the steps given above, setting the wavelength #2 to 230nm instead of 280nm.

### ■ **A<sub>320</sub> correction in ratio calculations**

Measurement of nucleic acid samples at an additional reference wavelength of 320nm allows background correction for non-biological factors such as sample turbidity, highly absorbent buffer solutions and the use of reduced aperture cells. The reading at 320nm is subtracted from the readings at 260 and 280 nm and the ratio is calculated:

$$\text{Ratio} = (A_{260} - A_{320}) / (A_{280} - A_{320})$$

For this calculation, the sum K5 x ((K1A1 + K2A2)/(K3A3 + K4A4)) is used:

1. In the method set up, set wavelength #1 to 260nm, wavelength #2 to 320nm, wavelength #3 to 280nm and wavelength #4 to 320nm.
2. On the **Calculations** tab, specify the sum K5 x ((K1A1 + K2A2)/(K3A3 + K4A4)).
3. Set the factors as follows: K1 = 1; K2 = -1; K3 = 1; K4 = -1; K5 = 1.
4. Continue setting up the method as required.
5. Place the sample blank in the sample chamber and close the lid.
6. Press the **Zero** key. This will zero the blank at all the selected wavelengths.
7. Remove the blank and replace it with the sample to be measured. Close the lid.
8. Press the **Read** key. This will read the sample at each wavelength in turn.
9. When completed, the measured absorbance at each wavelength will be displayed, together with the results of the selected calculation.

### ■ **DNA concentration**

Although it is possible to estimate the concentration of DNA from the A<sub>260</sub> value alone, calculations involving measurements at other wavelengths are generally more accurate as they account for the presence of contaminating compounds. One common method is to use the following equation<sup>2</sup> which includes measurement at a reference wavelength of 320nm to correct for the non-biological factors as described above:

$$\text{Concentration } (\mu\text{g/ml}) = ((\text{Abs}_{\lambda 1} - \text{Abs}_{\text{REF}}) \times \text{Factor}_1 - (\text{Abs}_{\lambda 2} - \text{Abs}_{\text{REF}}) \times \text{Factor}_2) \times \text{dilution factor}$$

Where:

$$\text{Abs}_{\lambda 1} = A_{260}; \text{Abs}_{\text{REF}} = A_{320}; \text{Abs}_{\lambda 2} = A_{280}$$

$$\text{Factor}_1 = 62.9; \text{Factor}_2 = 26$$

$$\text{Dilution factor} = (\text{sample volume} + \text{solvent volume}) / \text{sample volume}$$

Entering the given factor values, the equation above can be re-arranged as follows:

$$\text{Concentration } (\mu\text{g/ml}) = (62.9 \times A_{260} - 62.9 \times A_{320} - 26 \times A_{280} + 26 \times A_{320}) \times \text{dilution factor}$$

The following equation on the 67 series can then be used:

$$(K1A1 + K2A2 + K3A3 + K4A4) \times K5$$

Where:

$$K1 = 62.9; K2 = -62.9; K3 = -26; K4 = 26; K5 = \text{dilution factor}$$

$$A1 = A_{260}; A2 = A_{320}; A3 = A_{280}; A4 = A_{320}$$

Follow the steps given below to set up a method for DNA concentration measurement:

1. In the method set up, set wavelength #1 to 260nm, wavelength #2 to 320nm, wavelength #3 to 280nm and wavelength #4 to 320nm.
2. On the **Calculations** tab, specify the sum  $(K1A1 + K2A2 + K3A3 + K4A4) \times K5$ .
3. Set the factors as follows:  $K1 = 62.9$ ;  $K2 = -62.9$ ;  $K3 = -26$ ;  $K4 = 26$ ;  $K5 =$  dilution factor.
4. Continue setting up the method as required.
5. Place the sample blank in the sample chamber and close the lid.
6. Press the **Zero** key. This will zero the blank at all the selected wavelengths.
7. Remove the blank and replace it with the sample to be measured. Close the lid.
8. Press the **Read** key. This will read the sample at each wavelength in turn.
9. When completed, the measured absorbance at each wavelength will be displayed, together with the results of the selected calculation.

If a reference wavelength at 320nm is not used, then the equation is simplified to:

$$\begin{aligned} \text{Concentration } (\mu\text{g/ml}) &= ((\text{Abs}_{\lambda 1} \times \text{Factor}_1) - (\text{Abs}_{\lambda 2} \times \text{Factor}_2)) \times \text{dilution factor} \\ &= (62.9 \times A_{260} - 26 \times A_{280}) \times \text{dilution factor} \end{aligned}$$

In this instance, the same sum is used. Wavelength #1 is set to 260nm and wavelength #2 to 280nm and the factors  $K1 = 62.9$  and  $K2 = -26$ ;  $K5$  is still the dilution factor.

### ■ Protein concentration (direct UV)

The direct UV method of protein determination has a number of advantages over traditional colorimetric assays in that it does not rely on an external protein standard and the sample is not consumed in the assay. The most common use of this method is to monitor fractions from chromatography columns, or whenever a quick estimation of protein concentration is required. The sample is measured at both 280nm and 260nm and the concentration is calculated using the following formula<sup>2,3</sup>:

$$\text{Concentration (mg/ml)} = (1.55 \times A_{280}) - (0.76 \times A_{260}) \times \text{dilution factor}$$

Follow the steps given below to set up a method for protein concentration measurement:

1. In the method set up, set wavelength #1 to 280nm and wavelength #2 to 260nm.
2. On the **Calculations** tab, specify the sum  $(K1A1 + K2A2 + K3A3 + K4A4) \times K5$ .
3. Set the factors as follows:  $K1 = 1.55$ ;  $K2 = -0.76$ ;  $K5 =$  dilution factor.
4. Continue setting up the method as required.
5. Place the sample blank in the sample chamber and close the lid.
6. Press the **Zero** key. This will zero the blank at all the selected wavelengths.
7. Remove the blank and replace it with the sample to be measured. Close the lid.
8. Press the **Read** key. This will read the sample at each wavelength in turn.

9. When completed, the measured absorbance at each wavelength will be displayed, together with the results of the selected calculation.

■ **References**

1. Wilfinger, W.W., Mackey, K. and Chomczynski, P. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *Biotechniques* **22**: 474-481, (1997).
2. Warburg, O. and Christian, W. *Biochem. Z.* **310**, 384-421, (1941).
3. Layne, E. Spectrophotometric and Turbidimetric Methods for Measuring Proteins. *Methods in Enzymology* **10**: 447-455, (1957).

The protocols described here are for guidance only. Be aware of any hazardous compounds, take precautions where necessary and dispose of any waste in the appropriate manner.