JENWAY Cole-Parmer®

7415 SPECTROPHOTOMETER

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

Introduction

The multi-wavelength mode of the 7415 spectrophotometer can be used to measure absorbance or transmittance of a sample at up to four different wavelengths. This mode can be 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.

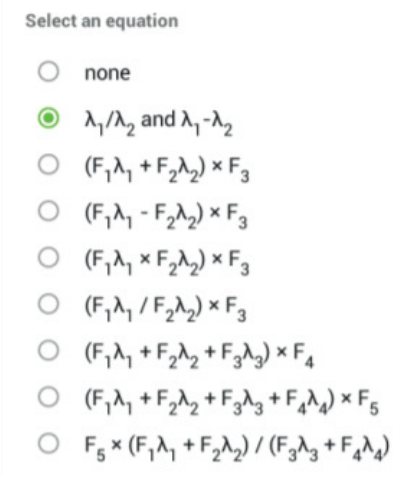
Methods can also be set up to perform DNA and protein concentration calculations using some of the equation parameters and factors in the calculations set up. Selecting an appropriate equation and entering the required factors enables the user to set up methods for most of the common methods for nucleic acid and protein analysis.

A260/A280 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 ≥1.8 and ≥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[1]. The sample should be of sufficient concentration to give an A_{260} ≥ 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. Open Multi-wavelength mode and set wavelength 1 to 260nm and wavelength 2 to 280nm.
2. Touch *Equation* to open the equation parameters then touch *Equation* on the parameter screen to open the list of available equations (see opposite).
3. Select λ_1/λ_2 and λ_1-λ_2 and touch *Apply* to accept.
4. Place the sample blank in the sample chamber and close the lid.
5. Touch *Blank*. This will zero the instrument at all the selected wavelengths.
6. Remove the blank and replace it with the sample to be measured. Close the lid.
7. Touch *Sample*. This will read the sample at each wavelength in turn.



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® or other salts. Follow the steps given above, setting the wavelength 2 to 230nm instead of 280nm.

A_{320} 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:

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

For this calculation, the formula $F_5 \times (F_1\lambda_1 + F_2\lambda_2) / (F_3\lambda_3 + F_4\lambda_4)$ is used:

1. Open Multi-wavelength mode and set wavelength 1 to 260nm, wavelength 2 to 320nm, wavelength 3 to 280nm and wavelength 4 to 320nm.
2. Touch *Equation* to open the equation parameters then touch *Equation* on the parameter screen to open the list of available equations.
3. Select $F_5 \times (F_1\lambda_1 + F_2\lambda_2) / (F_3\lambda_3 + F_4\lambda_4)$ and touch *Apply* to accept.
4. Set the factors as follows: $F_1 = 1$; $F_2 = -1$; $F_3 = 1$; $F_4 = -1$; $F_5 = 1$ and touch *Apply* to accept.
5. Place the sample blank in the sample chamber and close the lid.
6. Touch *Blank*. This will zero the instrument at all the selected wavelengths.
7. Remove the blank and replace it with the sample to be measured. Close the lid.
8. Touch *Sample*. This will read the sample at each wavelength in turn.

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_{260} value alone (where 1 A_{260} unit of dsDNA = 50µg/ml in H_2O), 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[2] which includes measurement at a reference wavelength of 320nm to correct for the non-biological factors as described above:

Concentration (µg/ml) = $62.9(A_{260}-A_{320}) - 36(A_{280}-A_{320})$ x dilution factor

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

1. Open Multi-wavelength mode and set wavelength 1 to 260nm, wavelength 2 to 320nm, wavelength 3 to 280nm and wavelength 4 to 320nm.
2. Touch *Equation* to open the equation parameters then touch *Equation* on the parameter screen to open the list of available equations.
3. Select $(F_1\lambda_1 + F_2\lambda_2 + F_3\lambda_3 + F_4\lambda_4) \times F_5$.
4. Set the factors as follows: $F_1 = 62.9$; $F_2 = -62.9$; $F_3 = -36$; $F_4 = 36$; F_5 = dilution factor or 1. Set the units to µg/ml and touch *Apply* to accept.
5. Place the sample blank in the sample chamber and close the lid.
6. Touch *Blank*. This will zero the instrument at all the selected wavelengths.

7. Remove the blank and replace it with the sample to be measured. Close the lid.
8. Touch *Sample*. This will read the sample at each wavelength in turn.

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:

Concentration (µg/ml) = (62.9 x A_{260}) – (36 x A_{280}) x dilution factor

In this instance, the same formula is used. Wavelength 1 is set to 260nm and wavelength 2 to 280nm and the factors F_1 = 62.9 and F_2 = -36; F_5 remains the dilution factor.

• Protein concentration

Like nucleic acids, it is possible to estimate the concentration of proteins from the A_{280} value alone – the direct UV method - (where 1 A_{280} unit of proteins = 1mg/ml). This is often used when a quick estimation is required or when monitoring fractions from chromatography columns. Proteins can also be determined using a number of different colorimetric protein assays e.g. BCA, Bradford etc. If it is likely the sample is contaminated with nucleic acids then the following formula[2,3] can be used to determine protein concentration:

Concentration (mg/ml) = 1.55(A_{280}-A_{320}) – 0.76(A_{260}-A_{320}) x dilution factor

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

1. Open Multi-wavelength mode and set wavelength 1 to 280nm, wavelength 2 to 320nm, wavelength 3 to 260nm and wavelength 4 to 320nm.
2. Touch *Equation* to open the equation parameters then touch *Equation* on the parameter screen to open the list of available equations.
3. Select ($F_1\lambda_1$ + $F_2\lambda_2$ + $F_3\lambda_3$ + $F_4\lambda_4$) x F_5.
4. Set the factors as follows: F_1 = 1.55; F_2 = -1.55; F_3 =-0.76; F_4 = 0.76; F_5 = dilution factor or 1. Set the units to mg/ml and touch *Apply* to accept.
5. Place the sample blank in the sample chamber and close the lid.
6. Touch *Blank*. This will zero the instrument at all the selected wavelengths.
7. Remove the blank and replace it with the sample to be measured. Close the lid.
8. Touch *Sample*. This will read the sample at each wavelength in turn.

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:

Concentration (µg/ml) = (1.55 x A_{280}) – (0.76 x A_{260}) x dilution factor

In this instance, the same formula is used. Wavelength 1 is set to 280nm and wavelength 2 to 260nm and the factors F_1 = 1.55 and F_2 = -0.76; F_5 is still the dilution factor.

• 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).