



# Libra S32/S35 Life Science Modes Operation Manual

English

**This section is only a description of the additional Life Science modes now included with every Libra S32/S35 spectrophotometer.**

**For a complete description of all other modes and the general use of the spectrophotometer, please refer to the main manual included on the CD-ROM.**

Life Science mode includes following applications:

- Has stored parameters for DNA, RNA and oligonucleotide quantification and purity checking, cDNA fluorophore label check, nucleic acid wavelength scan and a calculation facility for  $T_m$ ; there is also useful information concerning nucleic acids in general.
- Has stored parameters for Bradford, Lowry, Biuret, BCA and UV methods for protein determination; there is also useful information concerning proteins and amino acids in general.

## Nucleic Acids

Mode	Factor	A260/A280	Use
DNA	50 ng/μl	1.8	DNA quantification and purity checking
RNA	40 ng/μl	2.0	RNA quantification and purity checking
Oligo	33 ng/μl	Sequence dependent	Oligonucleotide quantification and purity checking
cDNA label	50 ng/μl		Fluorescent cDNA and PCR probe quantification for micro-arrays and <i>in-situ</i> hybridisation studies, respectively
Scan	-	-	Spectrum of samples, as well as quantification and purity check of selected wavelengths
Tm	-	-	Calculate theoretical Tm for a nucleotide base sequence
Info	-	-	Information relating to nucleic acids

Nucleic acids can be quantified at 260 nm because it is well established that a solution of DNA or RNA with an optical density of 1.0 has a concentration of 50 or 40 μg/ml, respectively, in a 10mm pathlength cell. Oligonucleotides, as a rule of thumb, have a corresponding factor of 33 μg/ml, although this does vary with base composition.

$$\text{Concentration} = \text{Abs}_{260} * \text{Factor}$$

Extracting nucleic acids from cells is accompanied by protein, and extensive purification is required to separate the protein impurity. The 260/280 ratio gives an indication of purity; it is only this, however, and not a definitive assessment. Pure DNA and RNA preparations have expected ratios of  $\geq 1.8$  and  $\geq 2.0$ , respectively; deviations from this indicate the presence of protein impurity in the sample, but care must be taken in interpretation of results. An elevated absorbance at 230 nm can indicate the presence of impurities as well; 230 nm is near the absorbance maximum of peptide bonds and also indicates buffer contamination since Tris, EDTA and other buffer salts absorb at this wavelength. When measuring RNA samples, the 260/230 ratio should be  $> 2.0$ ; a ratio lower than this is generally indicative of contamination with guanidinium thiocyanate, a reagent commonly used in RNA purification and which absorbs over the 230 – 260 nm range. A wavelength scan of a sample can also be obtained for visual inspection of integrity over the range 200 – 350 nm.

$$\text{Absorbance ratio} = \text{Abs}_{260} / \text{Abs}_{280}$$

cDNA and PCR tagged with fluorescent probes can be scanned up to 850 nm so that both peaks can be used to monitor labelling efficiency.

Background correction at a wavelength totally separate from the nucleic acid and protein peaks at 260 and 280 nm, respectively, is sometimes used to compensate for the effects of background absorbance. The wavelength used is 320 nm and it can allow for the effects of turbidity, high absorbance buffer solution and the use of reduced aperture cells. If your laboratory has not used background correction before, set this option to no.

$$\text{Concentration} = (\text{Abs}260 - \text{Abs}320) * \text{Factor}$$

$$\text{Absorbance ratio} = (\text{Abs}260 - \text{Abs}320) / (\text{Abs}280 - \text{Abs}320)$$

The spectrophotometer calculates concentration, displays 260/280 ratio and compensates for sample dilution. If you wish to use other wavelengths, for example if the peak maximum is at 257 nm or a background correction of 350 nm, use nucleic scan or absorbance ratio modes.

#### *Use of 7 µl volume ultramicrovolume cell*

- If using this cell, please note that it has a 5 mm pathlength, and that these modes assume a 10 mm pathlength cell is used.
- Ensure the cell is filled correctly by holding up to the light using the viewer supplied with the cell; this is to avoid the possibility of the liquid meniscus being in the light beam and causing non-reproducible results.
- Background compensation at 320 nm is useful when using restricted aperture cells such as this

#### *DNA, RNA, Oligo*

- Select if background correction at 320 nm is required using ▶
- Enter the sample dilution factor
- If oligo
  - Enter the conversion factor if known, default is 33 µg/ml. The factor can be calculated from a known base sequence if the concentration is known (see T<sub>m</sub> Calculation).
- Enter the integration time using ▶
  - Default is 1 second, other options are 2, 5 and 0.1 seconds. Use long integration times for very low and very high absorbance readings.
- Save method if required using ▶
- Insert reference and samples, and press **run**
  - If using a single cell holder
    - Insert reference and press **set ref.**
    - Insert sample and press **run** (repeat as required)

## *cDNA*

Measurement of the labelling efficiency of fluorescently labelled cDNA probes before 2-colour microarray hybridisation ensures that there is sufficient amount of each probe to give satisfactory hybridisation signals. This data also provides an opportunity to balance the relative intensities of each fluorescent dye by adjusting the concentration of each probe before hybridisation. The cDNA yield is measured at 260 nm while the incorporation of fluorescein, Cy3 and Cy5 are measured at their absorption peaks. This method may also be useful for measuring the yields and brightness of fluorescently labelled *in-situ* hybridisation probes.

The procedure is as follows:

### *Set up*

- Select the fluorophore label from Cy3, Cy5, Fluorescein, other dye using ▶
- Select if background correction is required using ▶
  - If selected, enter wavelength required
    - Default values are 430nm for Cy3/Cy5, 400nm for Fluorescein and 410nm for the user defined other dye
    - It is important that there is no absorbance due to carried over buffer or other components from the purification step at the selected wavelength.
- Enter the amount of starting RNA in ng (up to 99999)
  - RNA can be quantified using the RNA or Scan modes
- Enter the sample dilution factor
- Enter the cDNA conversion factor to be applied to Abs260
- Enter the probe volume in  $\mu\text{l}$

### *Scan*

- Select if scanning is to be enabled; we recommend that scan is always on.
- If yes
  - Enter the start wavelength and press **enter**
    - Only go below 220nm if using quartz cells
  - Enter the end wavelength and press **enter**
  - Select scan speed as appropriate; slow, medium or fast, using ▶
    - We recommend medium; note that the graph for low and medium scans is smoothed.

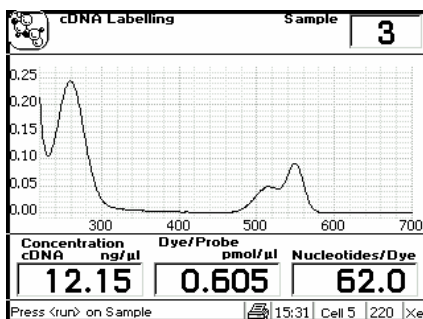
### *Other dye*

- Enter the name of the dye using the alphanumeric keypad
- Enter the absorption maximum (nm) of the dye
- Enter the Extinction Coefficient of the dye ( $1 \mu\text{mol}^{-1} \text{cm}^{-1}$ )
- Save method if required using ▶
  - Several dyes can be investigated if each is saved as a separate method

- Insert reference and samples, and press **run**
  - If using a single cell holder
    - Insert reference and press **set ref**
    - Insert sample and press **run** (repeat as required)

After measurement, the scan will autoscale. Typically, the scan will show 2 peaks, at 260nm for cDNA and around that of the fluorophore selected (488, 550 and 650 nm for fluorescein, Cy3 and Cy5, respectively). Buffer carry over may obscure the peak at 260 nm, and Abs260/Abs230 ratio will be affected. If this is the case, then it is not possible to determine the amount of nucleic acid reliably with this method. However, the determination of fluorophore concentration is still possible (see Results, below).

The results for cDNA concentration (ng/μl), dye/probe concentration (pmo/μl) and nucleotides/dye are presented.



### Results

Use this facility to view the underlying absorbance data that give these results, together with absorbance ratios and the yield of cDNA (% and ng).

If the Abs260/Abs230 ratio < 1.5, the peak at 260nm is poorly defined, and results which use Abs260 in the calculations will not be meaningful (cDNA concentration, nucleotides / dye, cDNA yield % and Total cDNA). A low Abs260/Abs230 ratio may be due to:

- buffer carry over, or
- impurities raising the Abs230 value and affecting the Abs260 value also, or
- the solution may be too dilute.

Note that the dye/ probe (pmol/μl) result is not affected by a poor Abs260/Abs230 ratio.

## *Scan*

The integrity of a nucleic acid sample can be established by inspection of its spectrum over the range 200 – 350 nm. This mode is particularly useful for RNA and oligonucleotide samples. The procedure is as follows:

- Start wavelength is fixed at 200nm. If using a UV transmitting disposable plastic cell such as UViMicro, strange optical effects between 200 and 220 nm may be observed. These can be ignored.
- Enter end wavelength and press **enter** (range is 350 – 600 nm)
- Enter  $\lambda 1$ ; this is used for quantification and absorbance ratio calculations
- Enter  $\lambda 2$ ; this is used for absorbance ratio calculations
- Select if background correction is required using  $\blacktriangleright$ 
  - If yes, enter  $\lambda B$
- Enter the sample dilution factor
- Enter the factor to be applied to  $\lambda 1$
- Save method if required using  $\blacktriangleright$
- Insert reference and samples, and press **run**

$\lambda 1$  can be changed post run using  $\blacktriangleright$ . This is very useful for optimisation; note that concentration and ratio results are updated automatically.

## *Tm Calculation*

$T_m$ , the theoretical annealing temperature, can be calculated for a primer or oligonucleotide if the base sequence and its concentration in solution are known; this is useful for PCR and sequencing studies. The parameter is calculated on the basis of thermodynamic calculations for each base in the nucleotide chain in relation to its neighbours (Breslauer et al, Proc. Natl. Acad. Sci. USA, 83, 3746). The procedure is as follows:

- Select mode, DNA or RNA, using  $\blacktriangleright$  (T is replaced by U in RNA)
- Enter the molar concentration of buffer / salts
- Enter the concentration of primer / oligo in  $\mu\text{g/ml}$  (or  $\text{ng}/\mu\text{l}$ )
- Enter the base sequence using the keypad (keys 1, 4, 7, • for C, G, T/U, A, respectively)
  - Note that the length (in mer) and the molecular weight of the oligo are shown as the sequence is entered; once 10 mer is reached the concentration in  $\text{pmol}/\mu\text{l}$ , conversion factor ( $\mu\text{g/ml}$ ) and theoretical  $T_m$  ( $^{\circ}\text{C}$ ) are displayed

## *Info*

General information relating to cell selection, formulae for mass to moles conversion and the codon dictionary is available.

## **Protein**

Determination of the amount of protein in solution can be conveniently determined using colorimetric methods in conjunction with a UV/Visible spectrophotometer. A number of methods are available, four of which are included as built in options on the instrument; note that the wavelengths used can vary with manufacturers' kits. Generic UV methods and information relating to proteins and amino acids are also included.

### **Bradford**

This method depends on quantitating the binding of a dye, Coomassie Brilliant Blue, to an unknown protein and comparing this binding to that of different amounts of a standard protein, usually bovine serum albumin (BSA). It is designed to quantify 1 to 10  $\mu\text{g}$  of protein. Protein determinations in the range 10 – 100  $\mu\text{g}$  may be carried out by increasing the volume of the dye solution 5-fold, and using larger tubes.

### **Lowry**

The Lowry method depends on quantifying the colour obtained from the reaction of Folin-Ciocalteu phenol reagent with the tyrosyl residues of an unknown protein and comparing this colour value to those derived from a standard curve of a standard protein, usually BSA at 750nm. This assay is designed to quantify 1 to 20  $\mu\text{g}$  of protein. Protein determinations in the range 5 – 100  $\mu\text{g}$  may be carried out by increasing all volumes 5-fold, and using larger tubes.

### **Biuret**

This method depends on the reaction between cupric ions and peptide bonds in an alkali solution, resulting in the formation of a complex absorbing at 546nm. The quantification is linear up to 130mg/ml when absorbance is measured after a standardised reaction time, and the method can be applied to routine analyses of serum and urine.

### **BCA**

This method also depends on the reaction between Cupric ions and peptide bonds, but in addition combines this reaction with the detection of Cuprous ions using bicinchonic acid (BCA), giving an absorbance maximum at 562nm. The analysis has a working range of 1-2000  $\mu\text{g/ml}$ .

### **UV methods**

This includes estimation of protein concentrations in nucleic acid solutions, and empirical methods for protein determination at 205, 280 and 215, 225 nm.

### **Information**

Information relating to proteins and amino acids.

## ***Bradford, Lowry, Biuret, BCA***

These all follow the same format; wavelengths, numbers of standards and other parameters can be edited, including concentrations, which can vary from one manufacturer's kit to another. The protocols can therefore be modified to suit your needs; for example, position 1 can be a reference blank (assumed to be 0.000 absorbance, 0.000 concentration), position 2 the background set to 0.000 concentration units, with subsequent standards as appropriate (the method can then be saved). The procedure is as follows:

A choice of 3 curve fit methods is provided for the standards:

- Linear regression – the best straight line through the data points, calculated using a least squares fit (requires a minimum of 3 data points), and the linearity (quality of line fit) are calculated (see Appendix)
- Linear interpolation – joins up consecutive data points by a series of straight lines
- Spline – calculates and fits the best curved line through the data points using a natural cubic spline fitting method (requires a minimum of 4 data points)

### *Set up*

- Enter the wavelength
- Select the curve type from linear regression, linear interpolation, spline using ▶
- Enter the number of standards, maximum is 9
- Enter the number of replicates for each standard, maximum is 3
- Enter the number of replicates for the samples, maximum is 3
- Select units using ▶

### *Concs*

- Enter the concentrations of the standards in increasing order
- Select the integration time using ▶
  - Default is 0.1 second, other options are 1, 2, and 5 seconds. Use long integration times for very low and very high absorbance readings.
- Save method parameters if required using ▶
  - To save the actual standard curve plot of concentration – absorbance data, return to this mode by pressing **mode** after running the set of standards, and then save the method parameters and data together.

### *Running Standards*

- Insert reference and standards, and press **run**
  - A reference is always required in position 1, and is assumed to be zero absorbance and zero concentration
  - To include a zero concentration standard, include this in the number of standards to be entered and enter 0.00 for concentration; use another blank when required to enter standard 1
  - Standards should be loaded in order of increasing concentration
  - Replicates and means are shown on the display as unfilled and filled squares, respectively

### *Running Samples*

- When the instrument has standards on the display, it expects samples to be run
- Press **run** after the standards have been run or a method has been recalled
  - If loading new standards, insert reference and standards and select yes
  - If loading samples, insert reference and samples and select no
- Samples have to be run separately and individually
- If a sample absorbance is within 10% of the ends of the calibration curve, the curve will be extrapolated linearly from the end points to accommodate this; if this is done, it is indicated on the display and on the print out

### *Graph*

This facility enables scaling of the results, and defines how they are to be presented on the LCD and print out.

- Enter the maximum absorbance to be shown on the display
- Enter the minimum absorbance to be shown on the display
- Select if automatic scaling of the results to fit the display is required post run

### *Standards*

This facility enables the concentrations and absorbances of the standards to be viewed, together with mean absorbance with standard error % (SE) if replicated were used. If linear regression curve fit has been selected, the slope, intercept and linearity of the regression are shown.

## *UV methods*

### **Protein Impurity**

The presence of nucleic acid in a protein solution can have a significant effect due to strong nucleotide absorbance at 280 nm. To compensate for this by measuring Abs 260, the equation of Christian and Warburg for the protein crystalline yeast enolase (Biochemische Zeitung 310, 384 (1941)) can be applied:

$$\text{Protein (mg/ml)} = 1.55 * \text{Abs 280} - 0.76 * \text{Abs 260}$$

This equation can be applied to other proteins if the corresponding factors are known. To customise the equation for a particular protein, the absorbances at 260 and 280 nm should be determined at known protein concentrations to generate simple simultaneous equations; solving these provides the two coefficients. In cases where Factor 2 is found to be negative, it should be set to zero since it means there is no contribution to the protein concentration due to absorbance at 260 nm. The use of background correction at 320 nm is optional.

$$\text{Protein (mg/ml)} = (\text{Factor 1} * \text{Abs 280}) - (\text{Factor 2} * \text{Abs 260})$$

Set Factor 2 = 0.00 for direct  $\lambda$ 280 UV protein measurement; Factor 1 is based on the extinction coefficient of the protein. If BSA (bovine serum albumin) is an acceptable standard, setting Factor 1 = 1.115 will give linear results from 0 to 0.8 mg/ml protein.

$$\text{Protein (mg/ml)} = 1.115 * \text{Abs 280}$$

Rapid measurements such as this at Abs 280 are particularly useful after isolation of proteins and peptides from mixtures using spin and HiTrap columns by centrifuge and gravity, respectively.

The procedure is as follows:

- Select if background correction at 320 nm is required using **▶**
- Enter Factor 1
- Enter Factor 2
- Save method if required using **▶**
- Insert reference and samples, and press **run**

## 280, 205 nm method

Protein concentration (mg/ml) can be determined using the empirical formula below (Scopes, R.K. (1974) Measurement of protein by spectrometry at 205 nm, *Anal. Biochem.* 59, 277 – 282):

$$\text{Protein (mg/ml)} = 27 + (120 * \text{Abs280} / \text{Abs205})$$

The procedure is as follows:

- Save method if required using ▶
- Insert reference and samples, and press **run**

## 215, 225 nm method

Protein concentration (µg/ml) can be determined using the empirical formula below (Waddell, W. J. (1956) A simple UV spectrophotometric method for the determination of protein, *J. Lab. Clin. Med.* 48, 311 – 314):

$$\text{Protein (µg/ml)} = 144 * (\text{Abs215} / \text{As 225})$$

The procedure is as follows:

- Save method if required using ▶
- Insert reference and samples, and press **run**

## Info

Conversion data relating Abs280 to concentration (mg/ml) for a range of common proteins and general information on amino acids is available.