GeneQuant™ 1300

USER MANUAL

biochrom
a division of
Harvard Bioscience, Inc.

Biochrom US
84 October Hill Rd
Holliston, MA
01746-1388
USA

Telephone: 1-508-893-8999
Toll Free: 1-800-272-2775
Fax: 1-508-429-5732
support@hbiosci.com
www.biochromspectros.com

TABLE OF CONTENTS

SAFETY

Spectrophotometer Health & Safety Document including General Operating Instructions are available as a booklet provided with each instrument. The booklet, translated into the European Union languages, is available on the delivered CD. The instructions provide the user with basic use, troubleshooting and how to use the instrument in a safe manner.

CAUTION
This instrument contains a UV source that generates a light beam which traverses the sample cell holder. Do not attempt to divert the beam or operate with casework removed as prolonged exposure to the beam may cause permanent eye damage.

WARNING
High voltages exist inside these units. Repair and maintenance should only be carried out by individuals trained specifically to work on these instruments

WARNING
If the instrument is used in a manner not specified or in environmental conditions not appropriate for safe operation, the protection provided may be impaired and the instrument warranty withdrawn.

Unpacking, Positioning and Installation

- Inspect the instrument for any signs of damage caused in transit. If any damage is discovered, inform your supplier immediately.
- Ensure your proposed installation site conforms to the environmental conditions for safe operation:
  Indoor use only.
  Temperature range 5°C to 40°C. Note that if you use the instrument in a room subjected to extremes of temperature change during the day, it may be necessary to recalibrate (by switching off and then on again) once thermal equilibrium has been established (2-3 hours). A temperature of no more than 4°C/hour is recommended.
  Maximum relative humidity of 80% up to 31°C decreasing linearly to 50% at 40°C
- The instrument must be placed on a stable, level bench or table that can take its weight (< 3.5 kg) so that air can circulate freely around the instrument.
- This equipment must be connected to the power supply with the power adaptor supplied. The adaptor can be used on 90 – 240 V~, 50-60 Hz supplies.
- If the instrument has just been unpacked or has been stored in a cold environment, it should be allowed to come to thermal equilibrium for 2-3 hours in the laboratory before switching on. This will prevent calibration failure as a result of internal condensation.
- Switch on the instrument via the keypad () after it has been plugged in. The instrument will perform a series of self-diagnostic checks.
- Please read through this user manual prior to use.
- Please contact your original supplier in the first instance if you experience technical or sample handling difficulties.

INTRODUCTION

Your spectrophotometer

Your spectrophotometer is a simple-to-use UV/Visible instrument with twin CCD array detectors (1024 pixels). It has no moving parts, which is the basis of the rapid scanning operating system.

The user interface is built around folders which are displayed on the home page when the instrument is switched on. After switch on and calibration, the default home page is "GeneQuant™ 1300" offering the choice of:

Life Science	Standard Life Science methods such as nucleic acid assays, protein assays and cell counting
Applications	General spectroscopic methods
Favorites	A folder to store your more frequently used configured methods
Methods	Contains nine folders that can store less frequently used configured methods (nine methods per folder)
Utilities	Instrument set up (date, time, language, etc)

The instrument is supplied with a program PVC (Print via Computer) on the accompanying CD. PVC can be used to "print through" to a local printer result data obtained over a USB or if a Bluetooth interface is installed over the wireless Bluetooth connection. The data may also be stored as an Excel spreadsheet, as an EMF graphics file, a comma delimited (csv) data file, a tab delimited (txt) data file, RTF word compatible format or in native PVC format for later access.

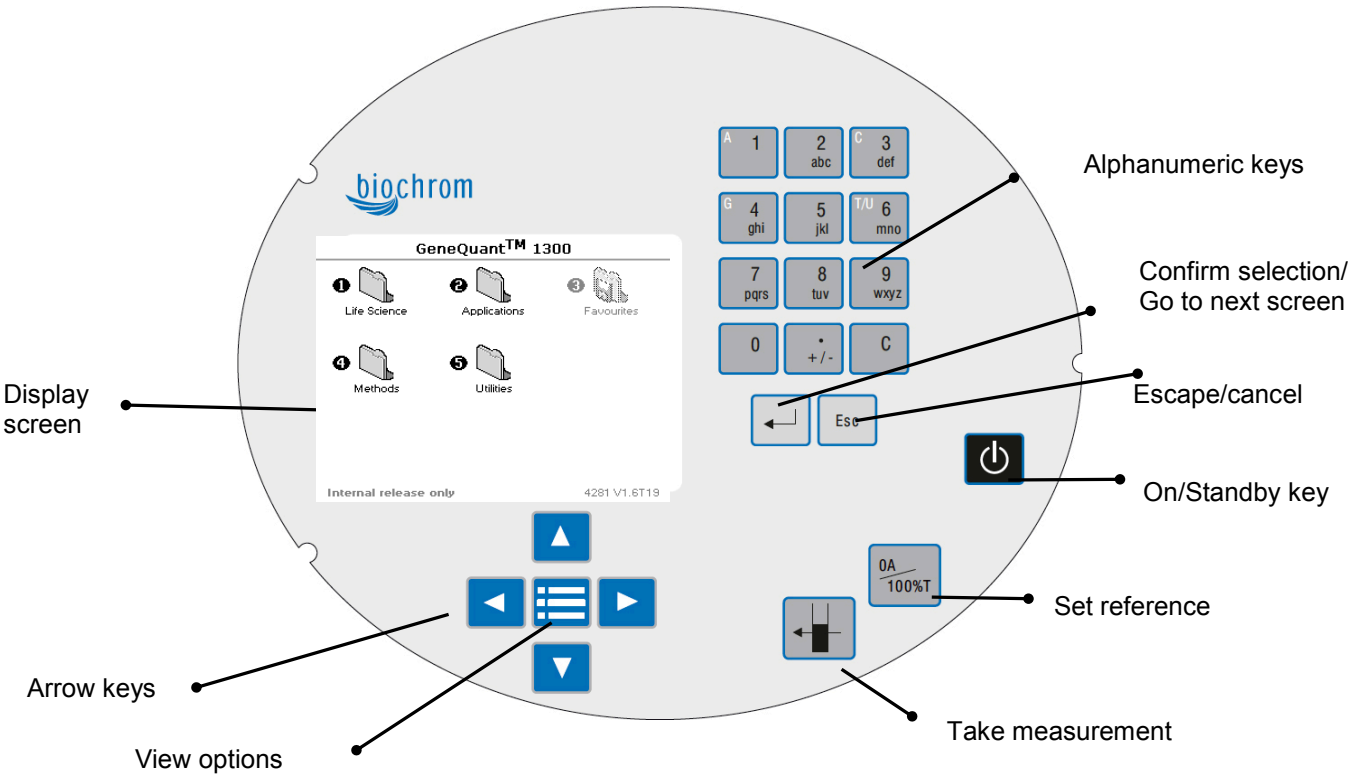
A printer is available for the instrument; this may either be supplied pre-installed or is available as an optional accessory.

Sample handling tips

- Note that the light beam is directed from RIGHT to LEFT through the cell chamber; therefore please ensure the cell is inserted in the correct alignment.
- The cell holder supplied with the instrument accepts standard 10 mm pathlength quartz, glass or plastic cells.
- The optical height is 15 mm, and the minimum volume that can be used is approx. 10µl in a Quartz ultra-micro cell.
- 12 mm test tubes may be used (e.g. for cell cultures), however they are not recommended as higher quality data is produced by using disposable cuvettes for the analysis. If used, align the indicator line on 12 mm test tubes in the same direction to ensure reproducible positioning of the tube. Note that test tubes do not last forever, and that the surface becomes scratched and blemished through repetitive use; if this is the case they should be replaced.

Keypad and display

The back-lit liquid crystal display is very easy to navigate around using the alphanumeric entry and navigation arrow keys on the hard wearing, spill proof membrane keypad.



Key	**Action**
On/off key	Turns the instrument on/off
Arrow keys	Use the four arrow keys to navigate around the display and select the required setting from the active (highlighted) option.
View Options:	View options for that application mode. Some of these are common to all applications and described below. Options unique to an application are described in the relevant section.
Alphanumeric keys	Use these to enter parameters and to write text descriptions where appropriate, or required. Use repeated key presses to cycle through lower case, number and upper case. Leave for 1 second before entering next character. Use C button to backspace and 1 to enter a space.
Escape	Escape from a selection and return to the previous folder.
Set Reference:	Set reference to 0.000 A or 100%T on a reference solution at the current wavelength in the mode selected. When in scan mode, make a reference scan.
OK/Next:	Enter or confirm a selection.
	Take a measurement.

Options  Shows relevant options from within an application. Can also be selected by pressing the relevant number below

Options (select using key pad numbers)

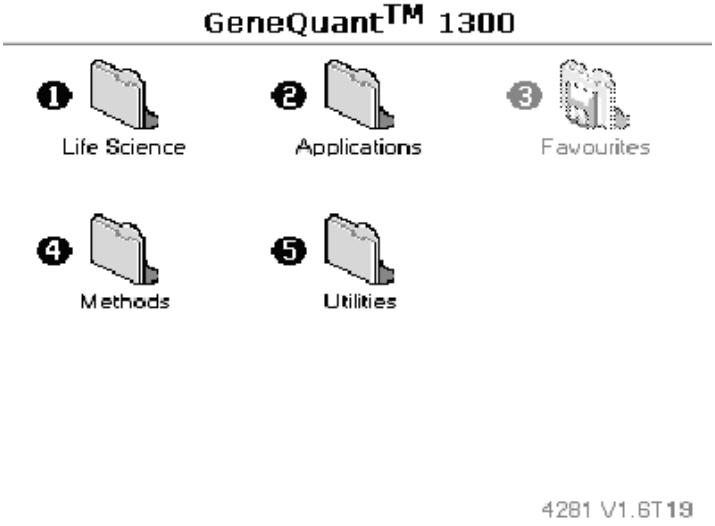
1.	View parameters for the experiment.
2.	Print the results.
3,4,5,6	Described in the application.
7.	Define the sample number you wish to start from.
8.	Save the parameters as a method to a defined folder name with a defined method name.
9.	Toggle auto-print on/off. Default is off.

Exit options by pressing Esc, or wait.

Experienced operators can use the numeric keys as a shortcut to the option required without needing to enter the Options menu.

Software style

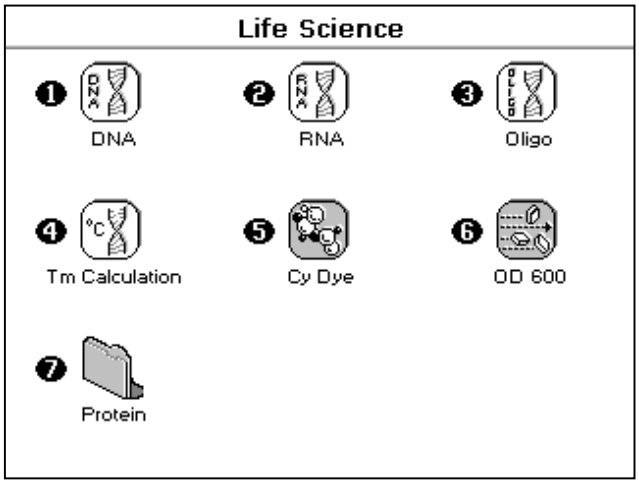
The user interface is built around having folders of files which are displayed on the home page when the instrument is switched on. Different folders are numbered and opened by using the associated number key on the keypad.



Summary Function	Keypad number	Description
	1	Nucleic acids, Proteins and Cell counting
	2	Single wavelength, Concentration, Wavelength scan, Kinetics, Standard Curve, Multiple wavelengths and Ratio
	3	Sub-folder for favorite user selected and configured methods
	4	Nine further sub-folders for configured methods
	5	Instrument set up (date, time, language, etc)

LIFE SCIENCE FOLDER

This contains seven applications/subfolders. Contents of these applicatons/subfolders are detailed below:



Application	Keypad number	Function
DNA	1	Concentration and purity check for DNA samples
RNA	2	Concentration and purity check for RNA samples
Oligo	3	Concentration and purity check for Oligo samples
TM Calculation	4	DNA melting point calculator
CyDye	5	Labeling efficiency measurement
OD 600	6	Cell culture OD600 with correction factor
Protein	7	Folder containing 5 methods of protein determination

DNA, RNA and oligonucleotide characterisation

Nucleic Acid Quantification (NAQ)

- Nucleic acids can be quantified at 260 nm because it is well established that a solution of DNA in a 10 mm pathlength cell with an optical density of 1.0 has a concentration of 50, or 40 µg/ml in the case of RNA. Oligonucleotides have a corresponding factor of 33 µg/ml, although this does vary with base composition; this can be calculated if the base sequence is known.
  Concentration = Abs260 * Factor
- The instrument uses factors 50, 40 and 33 as defaults for DNA, RNA and oligonucleotides, respectively, and compensates for dilution and use of cells which do not have 10 mm pathlength; dilution factor and cell pathlength can be entered.
-

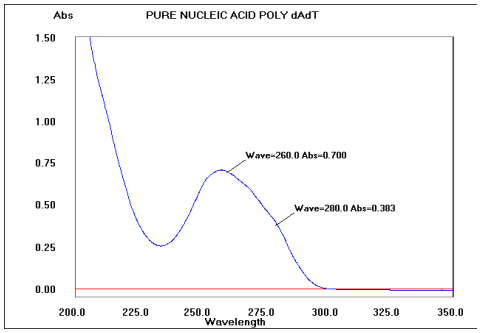
Nucleic Acid Purity Checks

- Nucleic acids extracted from cells are 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 an indication, 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 impurity in the sample, but care must be taken in interpretation of results.
- The 260 nm reading is taken near the top of a broad peak in the absorbance spectrum for nucleic acids, whereas the 280 nm reading is taken on a steep slope (i.e. small changes in wavelength cause large changes in absorbance). Consequently, small variations in wavelength at 280 nm will have a greater effect on the 260/280 ratio than variations will at 260 nm. Thus different instruments of the same and different types may give slightly different ratios due to variations in wavelength accuracy. But each instrument will give consistent results within itself.
- Concentration also affects 260/280 readings. If a solution is too dilute, the readings will be at the instrument's detection limit, and results may vary as there is less distinction of the 260 peak and 280 slope from the background absorbance. This is one reason why the Abs260 value should be greater than 0.1 for accurate measurements.
- 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 This, 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 the nucleic acid is particularly useful for RNA samples.
- The instrument can display 260/280 and 260/230 ratios, and compensates for dilution and use of cells that do not have 10 mm pathlength; dilution factor and cell pathlength can be entered.

Use of Background Correction

- 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. The instrument can use background correction.
- If it is used, there will be different results from those when unused, because Abs320 is subtracted from Abs260 and Abs280 prior to use in equations:
  Concentration = (Abs 260 - Abs 320) * Factor
  Abs ratio = (Abs 260 - Abs 320) / (Abs 280 - Abs 320)
  Abs ratio = (Abs 260 - Abs 320) / (Abs 230 - Abs 320)
- If your laboratory has not used background correction before, set this option to NO.
- The use of background correction can remove variability due to handling effects of low volume disposable cells.

Spectral scan of nucleic acid



Note:

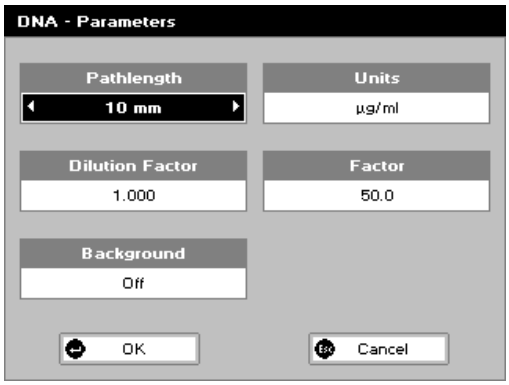
- absorbance maximum near 260 nm and absorbance minimum near 230 nm
- flat peak near 260 nm and steep slope at 280 nm
- very little absorbance at 320 nm

Operation of the instrument for Nucleic Acid measurements is described in the following sections

DNA and RNA are very similar, whilst in Oligo it is possible to calculate the factor from the composite bases by entering the proportions of the 4 bases.

1: DNA

The procedure is as follows:



Step 1

Press 1 to select DNA mode.

Step 2

Select path length using the left and right arrows. Options are 5 or 10 mm.

Press the down arrow.

Step 3 (dilution factor known)

Enter the dilution factor using the keypad numbers. Range 1.00 to 9999. Use the C button to backspace and clear the last digit entered.

OR

Step 3 (calculate dilution factor)

Press to enter the dilution factor screen (see second parameter screen to the left).

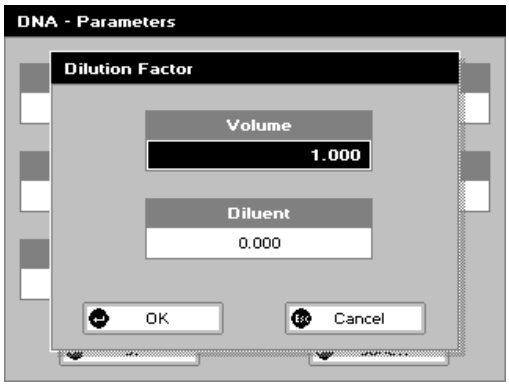
Enter the volume of the sample using the keypad numbers. Range 0.01 to 9999.

Press the down arrow.

Enter the volume of the diluent using the keypad numbers. Range 0.01 to 9999.

Press OK to calculate the dilution factor and return to the Parameters screen.

OR Press Cancel to cancel the selections and return to the Parameters screen.



Step 4

Select whether the background correction at 320 nm is used or not with the left and right arrows.

Press the down arrow.

Step 5

Select the units of measurement using the left and right arrows. Options: µg/ml, ng/µl, µg/µl.

Press the down arrow.

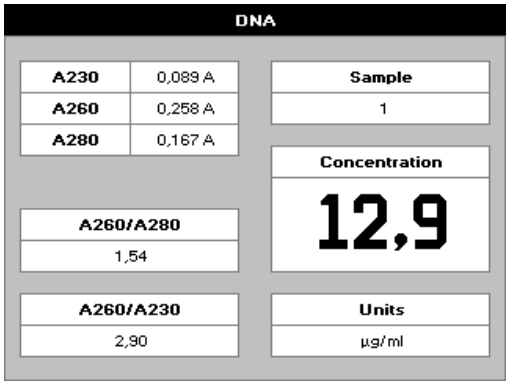
Step 6

Enter the factor using the keypad numbers. Default value is 50, range is 0.01 to 9999.

Step 7

Press OK to enter the Results screen and begin taking measurements.

OR Esc to return to the Life Science folder.



Results Screen

Step 8

Insert the reference sample. Press 0A 100%T. This will be used for all subsequent samples until changed.

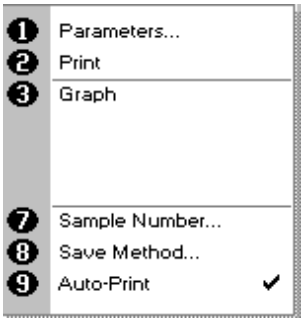
Step 9

Insert sample and press . This measures at the selected wavelengths and displays the results. The ratio of wavelengths 1 and 2 absorbencies are calculated (both corrected by the background wavelength value if selected). Gives concentration based on absorbance at wavelength 1.

Repeat step 9 for all samples.

Press Esc to return to the Life Science folder.

Press to display available Options which are described below.

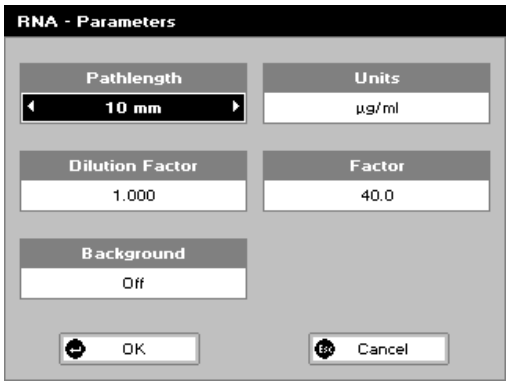


Options (select using key pad numbers)

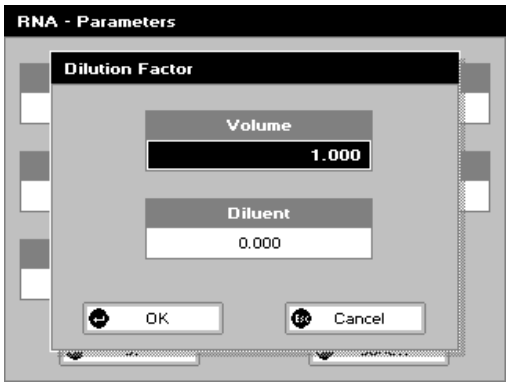
1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. The graph shows a wavescan plot across the range 220 nm to 320 nm with cursors denoting 230, 260, 280 and (if background correction selected) 320 nm.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save ↵.
9. Auto-print – toggles auto-print on/off.

Exit options by pressing Esc or wait.

2: RNA
The procedure is as follows:



Step 1
Press 2 to select RNA mode.
Step 2
Select path length using the left and right arrows. Options are 5 or 10 mm.
Press the down arrow.
Step 3 (dilution factor known)
Enter the dilution factor using the keypad numbers. Range 1.00 to 9999. Use the C button to backspace and clear the last digit entered.
OR
Step 3 (calculate dilution factor)
Press to enter the dilution factor screen (see second image to the left).
Enter the volume of the sample using the keypad numbers. Range 0.01 to 9999.
Press the down arrow.
Enter the volume of the diluent using the keypad numbers. Range 0.01 to 9999.

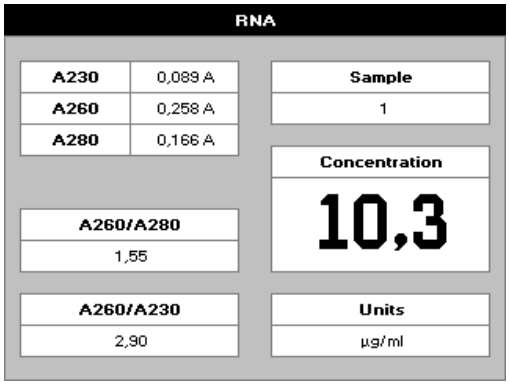


Press OK to calculate the dilution factor and return to the Parameters screen.

OR Press Esc to cancel the selections and return to the Parameters screen.
Step 4
Select whether the background correction at 320 nm is used or not with the left and right arrows.
Press the down arrow.
Step 5
Select the units of measurement using the left and right arrows. Options: µg/ml, ng/µl, µg/µl.
Press the down arrow.
Step 6
Enter the factor using the keypad numbers. Default value is 40, range is 0.01 to 9999.
Step 7

Press OK to enter the Results screen and start taking measurements

OR Esc to return to the Life Science folder.



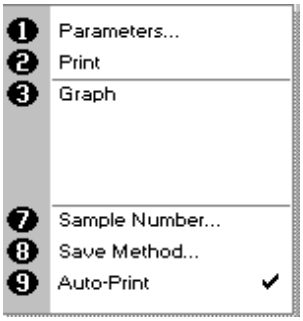
Results Screen
Step 8

Insert the reference sample. Press 0A 100%T. This will be used for all subsequent samples until changed.
Step 9

Insert sample and press . This measures at the selected wavelengths and displays the results. The ratio of wavelengths 1 and 2 absorbencies are calculated (both corrected by the background wavelength value if selected). Gives concentration based on absorbance at wavelength 1.
Repeat step 9 for all samples.

Press Esc to return to the Life Science folder.

Press to display available Options which are described below.



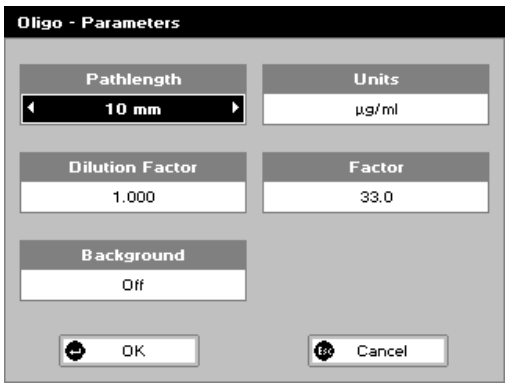
Options (select using key pad numbers)

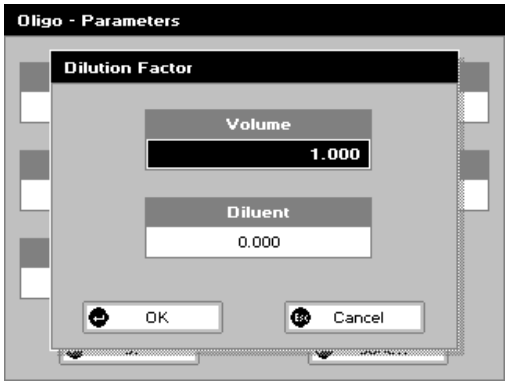
1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. The graph shows a wavescan plot across the range 220 nm to 320 nm with cursors denoting 230, 260, 280 and (if background correction selected) 320 nm.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save ↵.
9. Auto-print – toggles auto-print on/off.

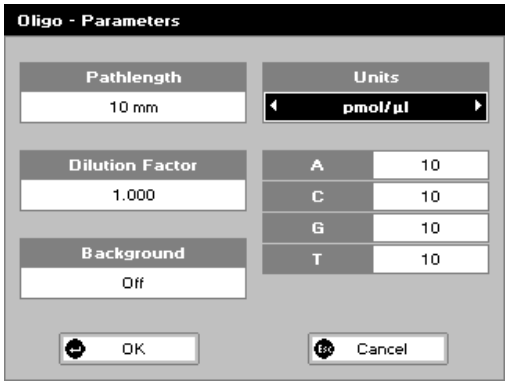
Exit options by pressing Esc, or wait.

3: Oligo

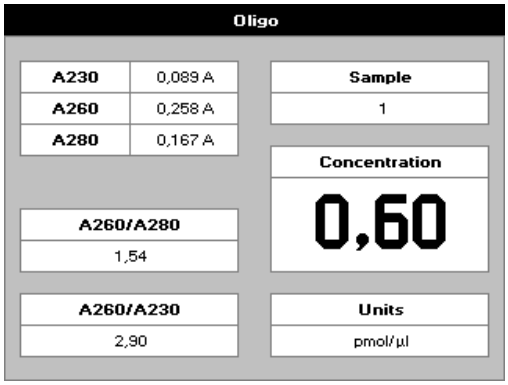
The procedure is as follows:







Step 1
Press 3 to select Oligo mode.
Step 2
Select path length using the left and right arrows. Options are 5 or 10 mm.
Press the down arrow.
Step 3 (dilution factor known)
Enter the dilution factor using the keypad numbers. Range 1.00 to 9999. Use the C button to backspace and clear the last digit entered.
OR
Step 3 (calculate dilution factor)
Press to enter the dilution factor screen.
Enter the volume of the sample using the keypad numbers. Range 0.01 to 9999.
Press the down arrow.
Enter the volume of the diluent using the keypad numbers. Range 0.01 to 9999.
Press OK to calculate the dilution factor and return to the Parameters screen.
OR Press Esc to cancel the selections and return to the Parameters screen.
Step 4
Select whether the background correction at 320 nm is used or not with the left and right arrows.
Press the down arrow.
Step 5
Select the units of measurement using the left and right arrows. Options: µg/ml, ng/µl, µg/µl and pmol/µl. If pmol/µl is selected the factor changes to a selection table denoting the ratios of the 4 bases in the structure.
Press the down arrow.
Step 6 (units not pmol/µl)
Enter the factor using the keypad numbers. Default value is 33, range is 0.01 to 9999.
OR
Step 6 (units pmol/µl)
Enter the proportions of bases present using the keypad numbers and up and down arrows to move between boxes. Default is 10 for each, range is 0 to 9999.
Step 7
Press OK to enter the Results screen and start taking measurements
OR
Esc to return to the Life Science folder.



Results Screen
Step 8

Insert the reference sample. Press 

This will be used for all subsequent samples until changed.

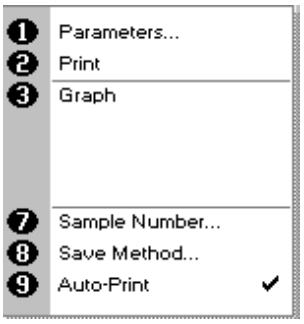
Step 9

Insert sample and press  . This measures at the selected wavelengths and displays the results. The ratio of wavelengths 1 and 2 absorbencies are calculated (both corrected by the background wavelength value if selected). Gives concentration based on absorbance at wavelength 1.
Repeat step 9 for all samples.

Press 

to return to the Life Science folder

Press  to display available Options which are described below.



Options (select using key pad numbers)

1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. The graph shows a wavescan plot across the range 220 nm to 320 nm with cursors denoting 230, 260, 280 and (if background correction selected) 320 nm.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save  .
9. Auto-print – toggles auto-print on/off.

Exit options by pressing 

, or wait.

4. Tm Calculation

This utility calculates the theoretical melting point from the base sequence of a primer. It is done using nearest neighbour thermodynamic data for each base in the nucleotide chain in relation to its neighbour (Breslauer et al, Proc.Natl. Acad. Sci. USA Vol 83, p3746 1986). The data obtained are useful both in characterization of oligonucleotides and in calculating T_m for primers used in PCR experiments.

The ACGT/U sequence entered into the utility is used to calculate the theoretical T_m , the theoretical absorbance (Absorption units/μmol) and the conversion factor (μg/ml). This is because the stability of a bent and twisted sequence of bases such as an oligonucleotide is dependent on the actual base sequence; the calculated thermodynamic interactions between adjacent base pairs have been shown to correlate well with experimental observations.

This utility uses matrices of known, published thermodynamic values and extinction coefficients to calculate T_m and the theoretical absorbance/factor respectively of an entered base sequence.

T_m

This is calculated using the equation:-

$$T_m = \frac{\Delta H \times 100}{\Delta S + [\ 1.987 \times \log_e(c/4 + 53.0822)} - 273.15 + 16.6 \log [salt]$$

where matrices

ΔH and ΔS are the enthalpy and entropy values summed from respective 2x4x4 nearest neighbour

c is the Primer concentration of oligonucleotide (pmoles/μl) in the calculated T_m or the measured concentration in measured T_m. In the latter case concentration is obtained from the equation:

$$c = \frac{\text{Abs(260nm) x calculated factor x pathlength multiplier x 10000}}{\text{Calculated MW}}$$

Calculated factor and MW are defined below

[salt] is the buffer molarity plus total molarity of salts in the hybridization solution (moles/l)

Weights for ΔS are indexed by adjacent paired bases.

A similar equation applies to Weights for ΔH, again indexed by adjacent bases.

Note that bivalent salts may need normalizing using a multiplying factor of 100 because of their greater binding power

Theoretical Absorbance

The Theoretical Absorbance is based on a calculation as follows:

For each adjacent pair of bases (nearest neighbours) an extinction coefficient weight is accumulated using a 4x4 table (one for either DNA or RNA). This total weight is then doubled. Then for each internal base a counterweight is subtracted using another 1x 4 table. The end bases are excluded from the latter summation

That is:

$$\text{Total Extinction Coefficient E} = \Sigma\ (2 \times \text{aTable[base\_type][base(n)][base(n+1)]}) - \Sigma\ (\text{tTable[base\_type][base(n]]})$$

Conversion factor

The Conversion Factor is given by = $\frac{\text{Molecular weight}_{ABCDE}}{\Sigma E_{ABCDE}}$

where

$$E_{ABCDE} = [\, 2 \times (E_{AB} + E_{BC} + E_{CD} + E_{DE}) - E_B - E_C - E_D \,]$$

- The molecular weight (MW) of a DNA oligonucleotide is calculated from:
-

MW (g/mole) = [(dA x 312.2) + (dC x 288.2) + (dG x 328.2) + (dT x 303.2.)] + [(MW counter-ion) x (length of oligo in bases)]

(for RNA oligonucleotide, (dT x 303.2) is replaced by (dU x 298.2)

The MW calculated using this equation must be adjusted for the contribution of the atoms at the 5' and 3' ends of the oligo.

For phosphorylated oligos: add: [17 + (2 x MW of the counter-ion)]
For non-phosphorylated oligos: subtract: [61 + (MW of the counter-ion)]

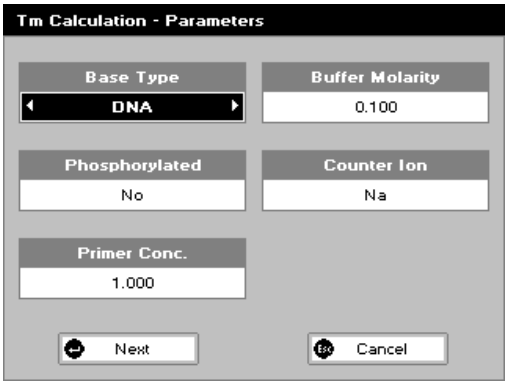
The MW (g/mole) of the most common oligo counter-ions are

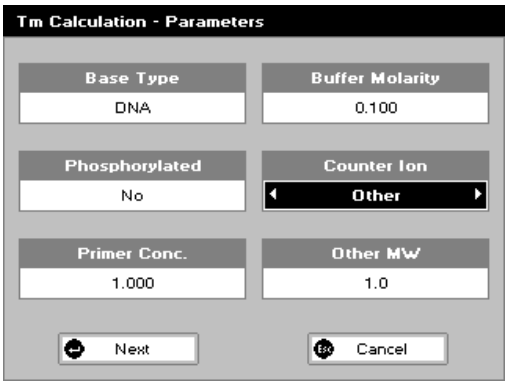
Na (sodium)	23.0
K (potassium)	39.1
TEA (triethylammonium),	102.2
Other	1

Calculated molecular weight: a weight is added for each base looked up from a table. The weight of the counter ion is added for every base from a small table for the known ions. If phosphorylated then the system adds 17.0 plus two counter ions otherwise it subtracts 61.0 and one ion.

Theoretical Absorbance: For each adjacent pair of bases (nearest neighbours) a weight is accumulated using a table. For each internal base a weight is subtracted using another table. Separate tables are used for DNA and RNA.

Calculated factor: This is just the calculated molecular weight divided by the theoretical absorbance.



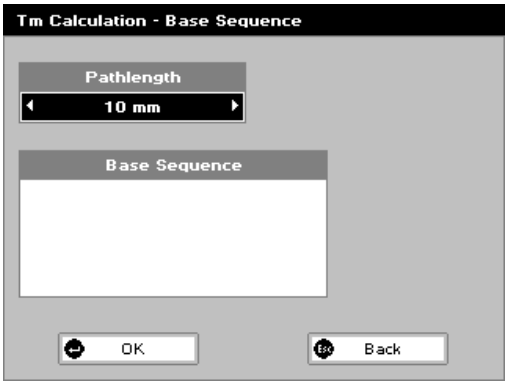


Step 1
Press 4 to enter the Tm Calculation parameters screen.
Step 2
Select the base type: DNA or RNA.
Press the down arrow.
Step 3
Select whether the sample is phosphorylated or not: yes or no.
Press the down arrow
Step 4
Enter the primer concentration using the keypad numbers.
Range 0.000 to 99.9
Press the down arrow.
Step 5
Enter the buffer molarity using the keypad numbers.
Range 0.000 to 10
Press the down arrow.
Step 6
Select the counter ion: Na, K, TEA or Other.

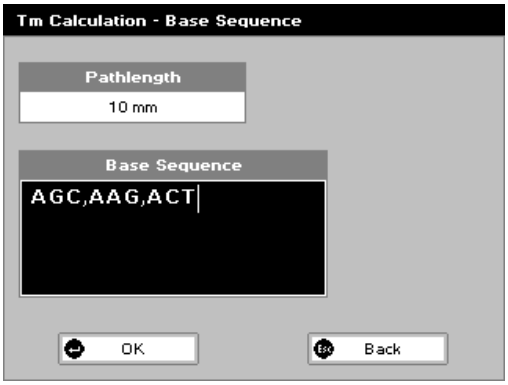
Step 7 (if Other selected)
Enter the molecular weight of the counter ion used.
Step 8

Press Next ↵ to select these parameters and go on to the Base Sequence screen
OR

Press Cancel Esc to return to the Life Science folder.



Step 9
Select the pathlength of the sample cells: 5 or 10 mm.
Press the down arrow

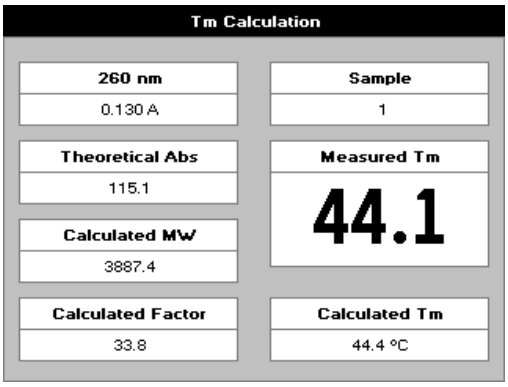


Step 10
Enter the known base sequence triplets using the number keys: 1 for A, 3 for C, 4 for G and 6 for T or U.

Step 11

Press OK ↵ to select these parameters and start to measure Tm
OR

Press Cancel Esc to return to the Parameters screen.



Results Screen
Step 12

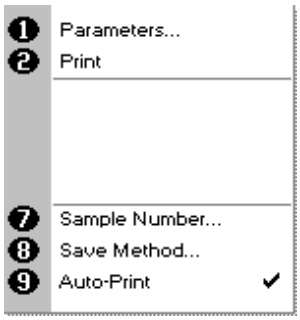
Insert the reference sample. Press  . This will be used for all subsequent samples until changed.

Step 13

Insert sample and press  . The unit measures the absorbance and uses this information to calculate the Measured Tm.
Repeat step 13 for all samples.

Press Esc to return to the Life Science folder

Press  to display available Options which are described below.



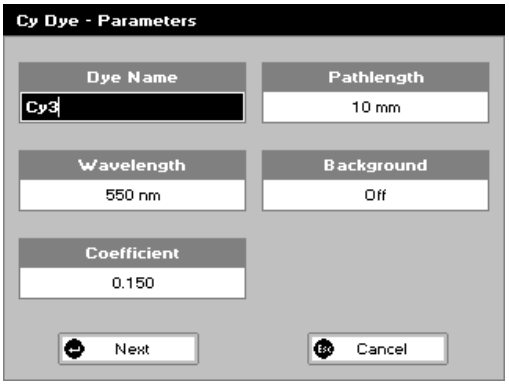
Options (select using key pad numbers)

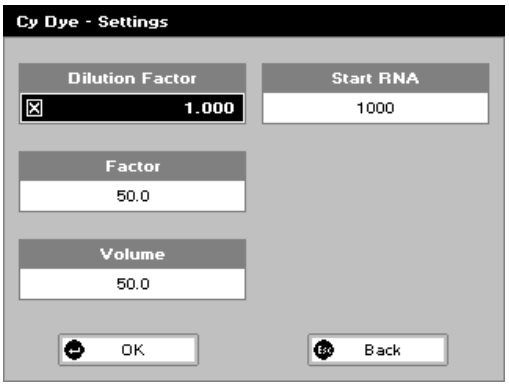
1. Return to parameters screen (step 1 above).
2. Print result via selected method.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save  .
9. Auto-print – toggles auto-print on/off.

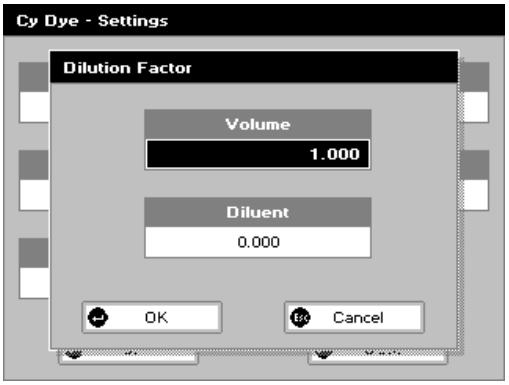
Exit options by pressing Esc, or wait.

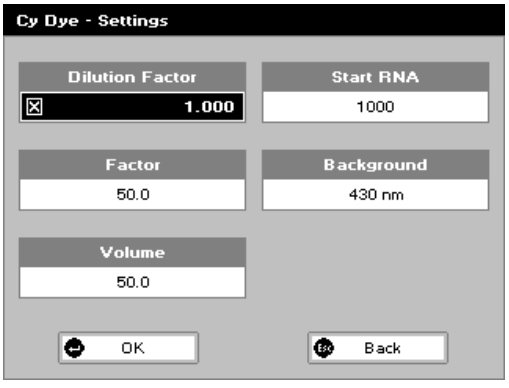
5. Cy dye

Measurement of the labelling efficiency of fluorescently labelled cDNA probes before 2-color microarray hybridization ensures that there is sufficient amount of each probe to give satisfactory hybridization signals. These data also provides an opportunity to balance the relative intensities of each fluorescent dye by adjusting the concentration of each probe before hybridization. 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.









Step 1
Press 5 to enter the Cy dye parameter screen
Step 2
Enter the name of the dye being used, the default is Cy3, but others may be generated using the Clear key C and the alphanumeric keypad
Press the down arrow
Step 3
Enter the wavelength of the dye peak absorbance.
Press the down arrow
Step 4
Enter the dye coefficient of extinction.
Press the down arrow
Step 5
Enter the pathlength of the sample cell: 5 or 10 mm.
Press the down arrow
Step 6
Select whether background correction is on or off.
Step 7

Press Next ↵ to enter the settings screen
OR

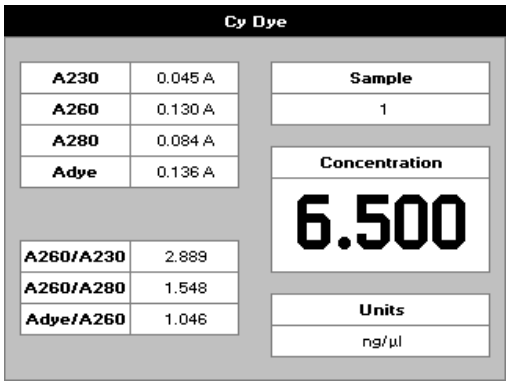
Press Cancel Esc to return to the Life Sciences folder.
Step 8 (dilution factor known)
Enter the dilution factor using the keypad numbers. Range 1.00 to 9999. Use the C button to backspace and clear the last digit entered.
OR
Step 8 (calculate dilution factor)
Press ☰ to enter the dilution factor screen (see second image to the left). Enter the volume of the sample using the keypad numbers. Range 0.01 to 9999. Press the down arrow.
Enter the volume of the diluent using the keypad numbers. Range 0.01 to 9999.

Press OK ↵ to calculate the dilution factor and return to the Parameters screen.

OR Press Esc to cancel the selections and return to the Parameters screen.
Step 9
Enter the conversion factor using the keypad numbers. Press the down arrow
Step 10
Enter the volume of the probe in µl
Press the down arrow
Step 11
Enter the amount of starting RNA in ng. Maximum 99999 ng.
Step 12 (if background correction is on)
Enter the background correction wavelength if required
Step 13

Press OK ↵ to select these settings and start to measure

OR Press Cancel Esc to cancel the settings and return to the parameters screen.



Results Screen
Step 14

Insert the reference sample. Press 0A 100%T. This will be used for all subsequent samples until changed.

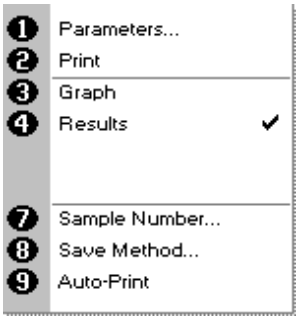
Step 15

Insert sample and press  . The system returns the dye concentration.

Repeat step 15 for all samples.

Press Esc to return to the Life Science folder

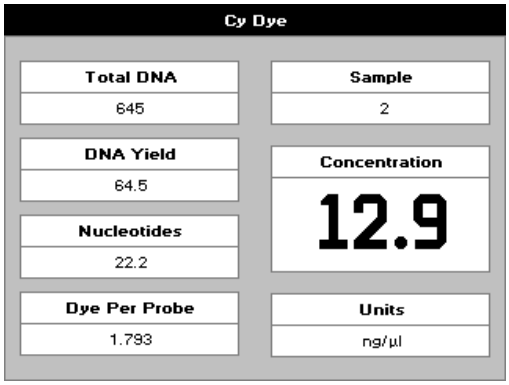
Press  to display available Options which are described below.



Options (select using key pad numbers)

1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. The graph shows the spectral scan of the dye sample over the wavelength range of interest
4. Toggle results on/off. When off the results screen shows the absorbance values and absorbance ratios. When on the results screen shows the total DNA, DNA Yield, nucleotides and number of dyes/probe. This only functions if the graph display is off (see below)
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save  .
9. Auto-print – toggles auto-print on/off.

Exit options by pressing Esc, or wait.

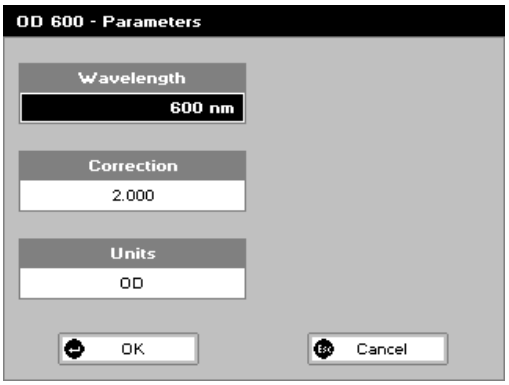


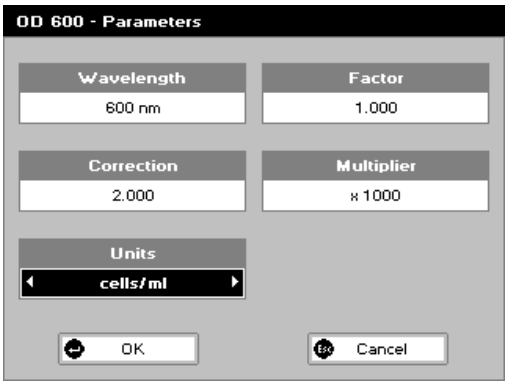
Results screen with option 4 On

6. Bacterial Cell Culture Measurement (OD600)

- Bacterial cell cultures are routinely grown until the absorbance at 600 nm (known as OD600) reaches approximately 0.4 prior to induction or harvesting. A linear relationship exists between cell number (density) and OD 600 up to approx. 0.6.
- It is important to note that for turbid samples such as cell cultures, the absorbance measured is due to light scattering, and **not** the result of molecular absorption. The amount of scatter is affected by the optics of the system (distance between the cell holder and instrument exit slit, geometry of this slit and the monochromator optics). Different spectrophotometer types therefore give different responses for the same turbid sample; to compare results, they must be normalised using calibration curves.
- A calibration curve can be determined by comparing measured OD 600 to expected OD 600. Expected OD 600 is determined by counting cell number using an alternative technique (for example microscope slide method) and converting to OD 600 using the rule of thumb that 1 OD 600 = 8 x 10^8 cells/ml for E. Coli.
- Your GeneQuant instrument has much smaller optics than most conventional spectrophotometers, and more light is transmitted through to the detector resulting in lower than expected OD 600 values. Results obtained by comparing measured OD 600 with expected OD 600 (see above) indicate that a correction factor of 2.0 is required to make the data comparable to larger instruments; this factor is included as a default value in set up.
- The use of 10 mm pathlength disposable cells is recommended for optical density measurements of cell culture solutions; to prevent the suspension settling too quickly and giving an OD that changes with time, glycerol should be added to the sample.

The procedure is as follows



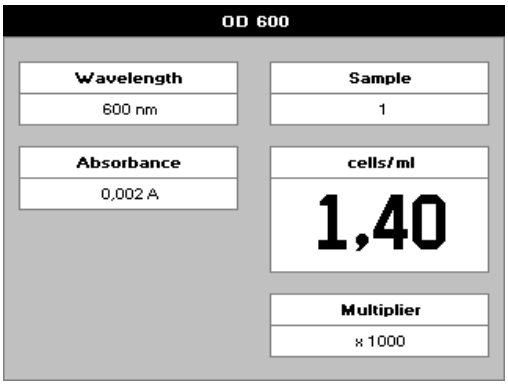


Step 1
Press 6 to select OD600 mode.
Step 2
Select the wavelength using either arrow or alphanumeric keys. Default value is 600 nm.
Press the down arrow.
Step 3
Enter the correction factor to compensate for different optical configurations between this and other instruments. Default value is 2.
Press the down arrow.
Step 4
Select the units. Options are OD or cells/ml. If cells/ml is selected two further parameters are displayed.

Step 5 (if cells/ml selected)
Enter the factor using the keypad numbers. Range 0.00 to 9999. C button backspaces and clears the last digit entered.
Press the down arrow.
Step 6 (if cells/ml selected)
Select the multiplier using the left and right arrows. Options are 1000 or 1,000,000.
Step 7

Press OK ↵ to enter the Results screen
OR

Press Cancel Esc to cancel selections and return to the Life Science folder.



Results Screen
Step 8

Insert the reference and press  . This will be used for all subsequent samples until changed.

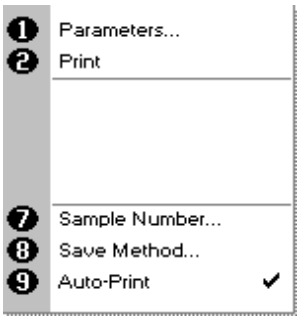
Step 9

Insert the sample and press  .
The wavelength, absorbance and OD600 value is displayed.

Repeat step 9 for all samples.

Press Esc to return to the Life Science folder.

Press  to display available Options which are described below.



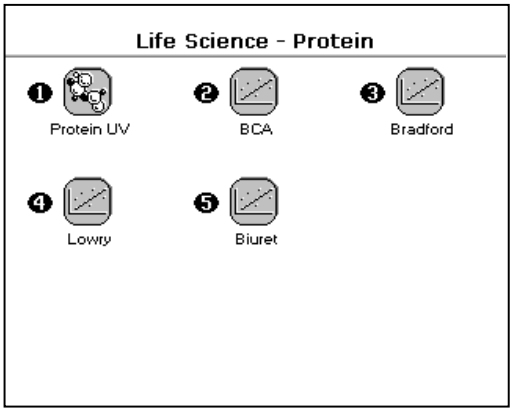
Options (select using key pad numbers)

1. Return to parameters screen (step 1 above).
2. Print result via selected method.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save  .
9. Auto-print – toggles auto-print on/off.

Exit options by pressing Esc, or wait.

7. Protein Determination

The GeneQuant™ 1300 includes five methods for assaying proteins. Press 7 to enter the protein folder.



Protein Determination at 280 nm

Protein can be determined in the near UV at 280 nm due to absorption by tyrosine, tryptophan and phenylalanine amino acids; Abs 280 varies greatly for different proteins due to their amino acid content, and consequently the specific absorption value for a particular protein must be determined.

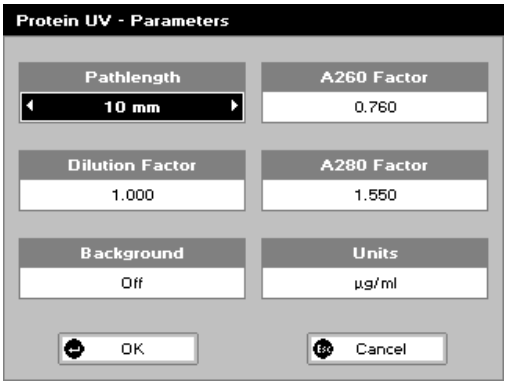
- The presence of nucleic acid in the protein solution can have a significant effect due to strong nucleotide absorbance at 280 nm. This can be compensated by measuring Abs 260, and applying the equation of Christian and Warburg for the protein crystalline yeast enolase (Biochemische Zeitung 310, 384 (1941)):
  Protein (mg/ml) = 1.55 * Abs 280 - 0.76 * Abs 260
  or, Protein conc. = (Factor 1 * Abs 280) - (Factor 2 * Abs 260)
- This equation can be applied to other proteins if the corresponding factors are known. The instrument can determine protein concentration at 280 nm and uses the above equation as default; the factors can be changed, and the use of background correction at 320 nm is optional.
- To customise the equation for a particular protein, the absorbance values 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.
- Set Factor 2 = 0.00 for direct λ280 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.
  Protein (mg/ml) = 1.115 * 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.

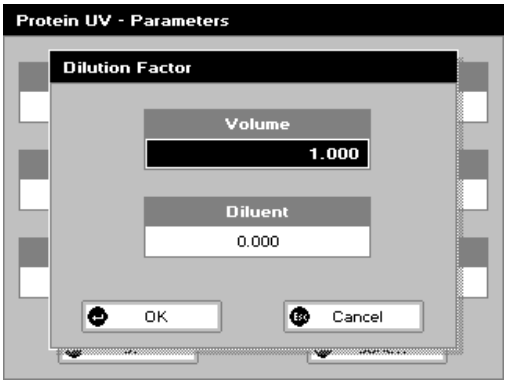
Protein Determination at 595, 546, 562 and 750 nm

- The Bradford method depends on quantitating the binding of a dye, Coomassie Brilliant Blue, to an unknown protein and comparing this binding to that of different, known concentrations of a standard protein at 595 nm; this is usually BSA, bovine serum albumin.
- The Biuret method depends on reaction between Cupric ions and peptide bonds in an alkali solution, resulting in the formation of a complex absorbing at 546 nm.
- The BCA method also depends on reaction between cupric ions and peptide bonds, but in addition combines this reaction with the detection of cuprous ions using bicinchoninic acid (BCA), giving an absorbance maximum at 562 nm. The BCA process is less sensitive to the presence of detergents used to break down cell walls.
- The Lowry method depends on quantifying the color obtained from the reaction of Folin-Ciocalteu phenol reagent with the tylsryl residues of an unknown protein and comparing with those derived from a standard curve of a standard protein at 750 nm; this is usually BSA, bovine serum albumin
- Detailed protocols are supplied with these assay kits, and must be closely followed to ensure accurate results are obtained.
- The use of plastic disposable cells is recommended. To use a zero concentration standard include it in the number of standards to be entered and enter 0.00 for concentration; use this when required to enter standard 1.
- A linear regression analysis of the calibration standard data points is calculated; the result, together with the correlation coefficient, can be printed out. A correlation coefficient of between 0.95 and 1.00 indicates a good straight line.

1: Protein UV

This is the Christian and Warburg assay discussed above. The procedure is as follows:





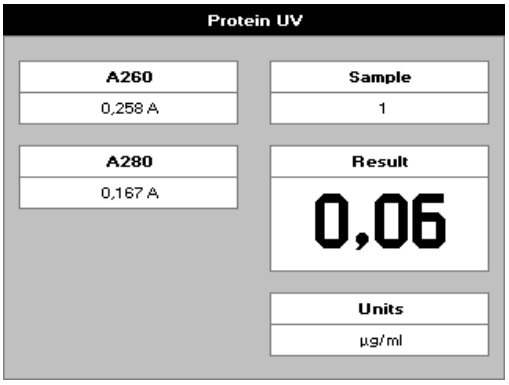
Step 1
Press 1 to select Protein UV mode.
Step 2
Select path length using the left and right arrows. Options are 5 or 10 mm.
Press the down arrow.
Step 3 (dilution factor known)
Enter the dilution factor using the keypad numbers. Range 1.00 to 9999. Use the C button to backspace and clear the last digit entered.
OR
Step 3 (calculate dilution factor)
Press to enter the dilution factor screen, shown to the left.
Enter the volume of the sample using the keypad numbers. Range 0.01 to 9999.
Press the down arrow.
Enter the volume of the diluent using the keypad numbers. Range 0.01 to 9999.

Press OK to calculate the dilution factor and return to the Parameters screen.

OR Press Esc to cancel the selections and return to the Parameters screen.
Step 4
Select whether the background correction at 320 nm is used or not with the left and right arrows.
Press the down arrow.
Step 5
Enter the coefficient value at 260 nm using the keypad numbers (see method described in introduction). Default value is 0.76, range is 0 to 9999.
Press the down arrow.
Step 6
Enter the coefficient value at 280 nm using the keypad numbers (see method described in introduction). Default value is 1.55, range is 1 to 9999.
Press the down arrow.
Step 7
Select the units of measurement using the left and right arrows. Options: µg/ml, ng/µl and µg/µl.
Step 8

Press OK to enter the Results screen
OR

Cancel Esc to return to the Protein folder



Results Screen
Step 9

Insert the reference sample. Press 0A 100%T. This will be used for all subsequent samples until changed.

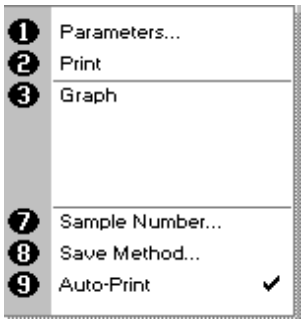
Step 10

Insert a sample and press  . This measures at both 260 and 280 nm wavelengths and displays the result. Protein concentration is calculated (corrected by background wavelength value if selected).

Repeat step 10 for all samples.

Press Esc to return to the Protein folder.

Press  to display available Options which are described below.



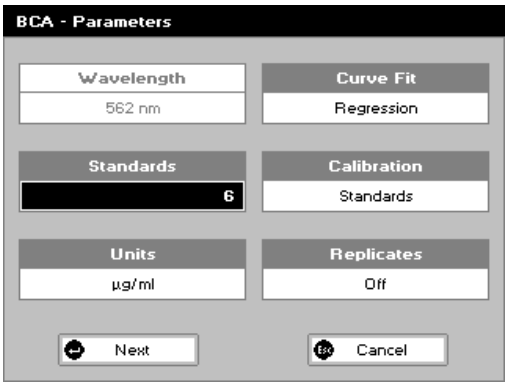
Options (select using key pad numbers)

1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. The graph shows a wavescan plot across the range 220 nm to 330 nm with cursors denoting 230, 260, 280 and (if background correction selected) 320 nm.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save  .
9. Auto-print – toggles auto-print on/off.

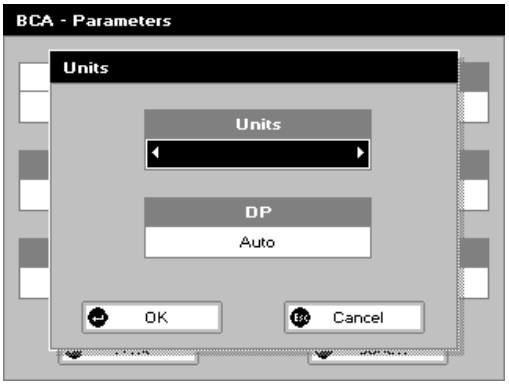
Exit options by pressing Esc, or wait.

2: BCA

The procedure is as follows:



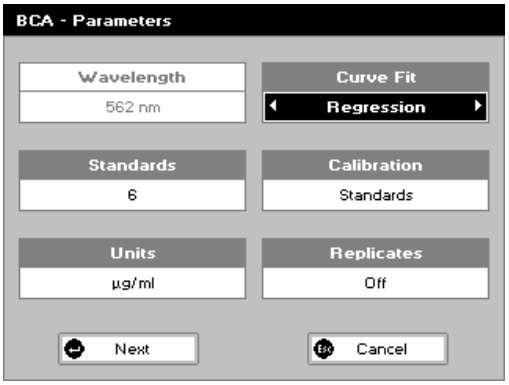
Step 1
Press 2 to select BCA mode.
Step 2
The wavelength for this method is set at 562 nm.
Step 3
Enter the number of standard concentration points (1-9) to be used in the curve using the keypad numbers or left and right arrows.
Press the down arrow.



Step 4
Units: The user can enter a text string up to 8 characters long. To access a list of pre-defined units press the Options key and then use the left/right arrows (µg/ml, µg/µl, pmol/µl, mg/dl, mmol/l, µmol/l, g/l, mg/l, µg/l, U/l, %, ppm, ppb, conc or none). These units can also be edited once OK is pressed.
This screen also allows the number of displayed decimal points (DP) to be selected, from 0 to 2 Note that the result will always be fixed to 5 significant figures regardless of how many decimal points are selected (so 98768.2 will display as 98768 even with 1 decimal point selected).

Press OK to store the chosen parameters or Cancel Esc

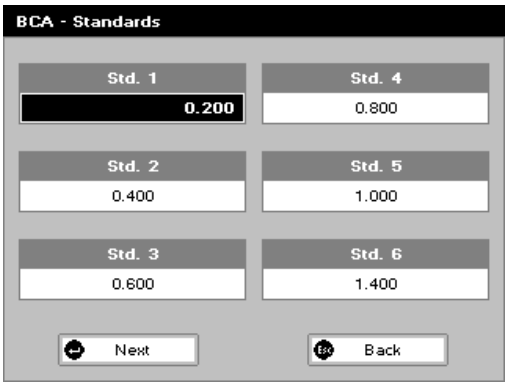
Step 5
Enter the type of curve fit. Options are straight line regression, zero regression (forces the straight line through the origin), interpolated or cubic spline.
Press the down arrow.



Step 6
Select the calibration mode, either standards (measure prepared standards) or manual (keypad data entry, go to step 9)
Step 7 (standards selected)
Select the number of replicates using the left and right arrows. This determines the number of standards to be measured and averaged at each standard concentration point. Can be OFF (1), 2 or 3.
Step 8 (standards selected)

Press Next to enter the Standards screen
OR

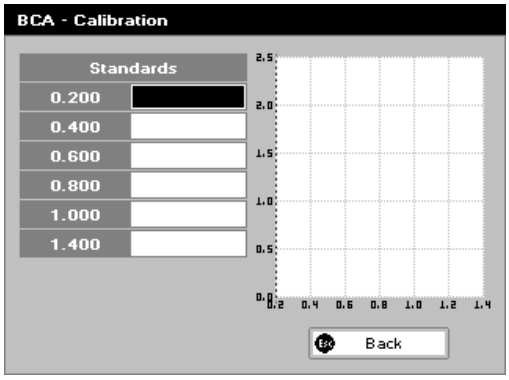
Press Cancel Esc to cancel selections and return to the Protein folder.



Standards Screen
Step 9 (standards/manual selected)
Enter the concentration values by using the keypad numbers and the up and down arrows to move between the different standard boxes. Range 0.001 to 9999. C button backspaces and clears the last digit entered.
Step 10 (standards/manual selected)

Press Next to enter the Calibration screen. If there are duplicate or non-monotonic (increasing) entries the unit will beep and highlight the incorrect entry.

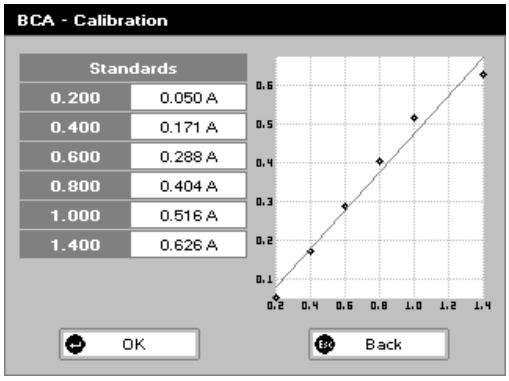
OR Press Back Esc to return to the Parameter screen.



Calibration Screen (replicates off)
This shows the calibration values and allows standards to be measured or entered using the keypad numbers (if calibration mode is manual).
Step 11 (standards selected)

Insert the reference sample. Press 0A/100%T.
This will be used for all subsequent samples until changed.
Step 12 (standards selected)
Insert the standard (use C to clear previously stored results before measuring)

Press  to measure the standard and store the result.

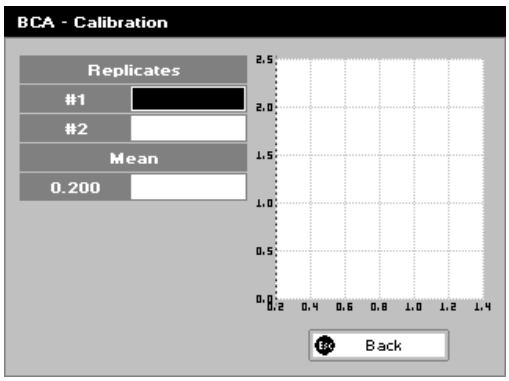


Repeat step 12 for all standards. A graph will display the results and the fitted curve as the measurements are made.

Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.

Step 13 (standards/manual selected)
When all standards are measured the OK box appears. Press OK ↵ to accept the calibration and go to the Results screen (see below)
OR

Press Back Esc to cancel selections and return to the Standards screen.

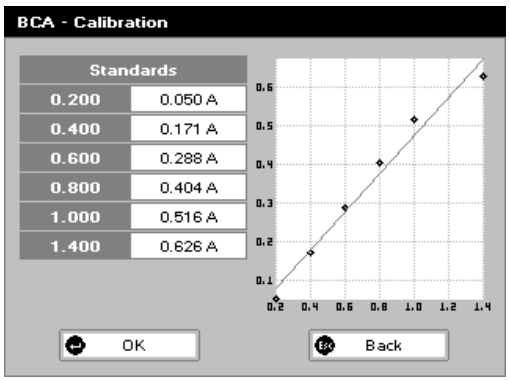


Calibration Screen (replicates on)
This shows the calibration values and allows standards to be measured.
Step 11 (standards selected)

Insert the reference sample. Press 0A/100%T.
This will be used for all subsequent samples until changed.
Step 12 (standards selected)

Press Replicates ↵ to display the replicate entry boxes. Use C to clear previously stored results before measuring.

Insert the standard and press  to measure the standard and store the result.



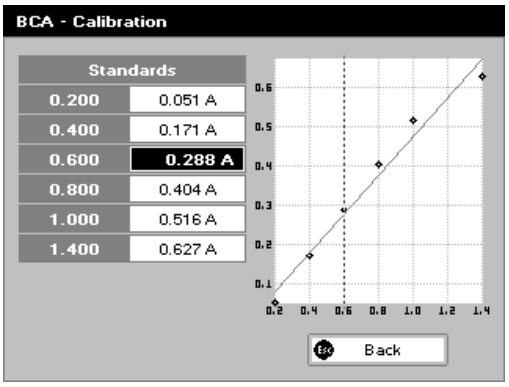
Repeat for all replicates and standards. Use Next ↵ to bring up fields for the next standard.

A graph will display the results and the fitted curve as the measurements are input.
Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.

Step 13 (standards/manual selected)

Press OK ↵ to accept the calibration and go to the Results screen (see below)
OR

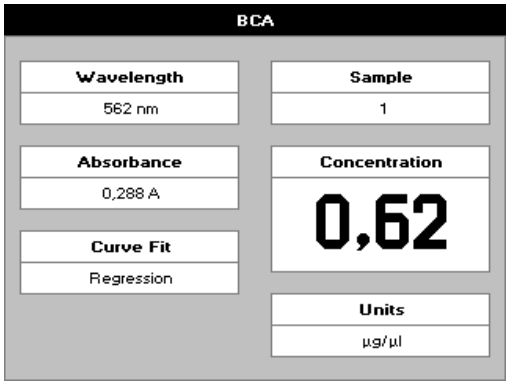
Press Back Esc to return to the Standards screen.



Calibration (Manual entry)
Shows previously entered calibration values and allows values to be entered via the keypad.
The highlighted box can be edited in order to enter an absorbance value corresponding to a given concentration value using the keypad numbers. Range 0.001 to 9999. Use C to backspace and clear the last digit entered and the up and down arrows to move between boxes. Pressing the down arrow from the last standard will bring up the OK box.

Press OK ↵ to accept the calibration and go to the Results screen (see below)
OR

Press Back Esc to return to the Standards screen.



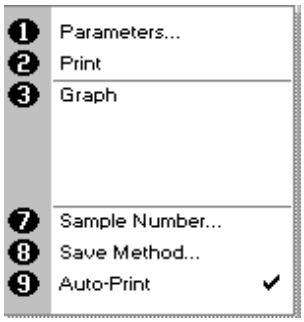
Results screen
Step 14

Insert the reference sample and press 0A 100%T. This will be used for all subsequent samples until changed.
Step 15

Insert the sample and press .
The concentration of the sample is taken and displayed.
Repeat step 15 for all samples.

Press Esc to return to the Protein Folder.

Press to display available Options which are described below.

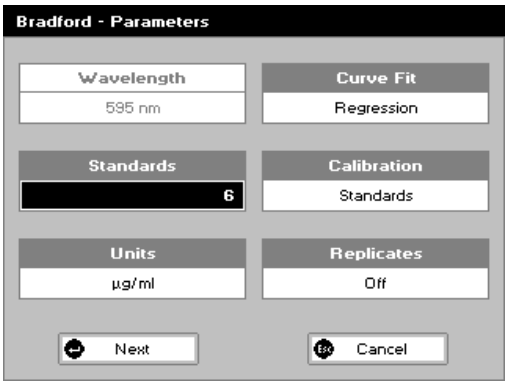


Options (select using key pad numbers)
1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. Displays the calibration graph, cursors give values for last measured sample.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save ↵.
9. Auto-print – toggles auto-print on/off.

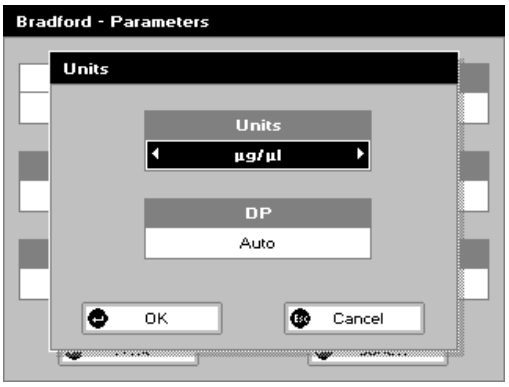
Exit options by pressing Esc, or wait.

3: Bradford

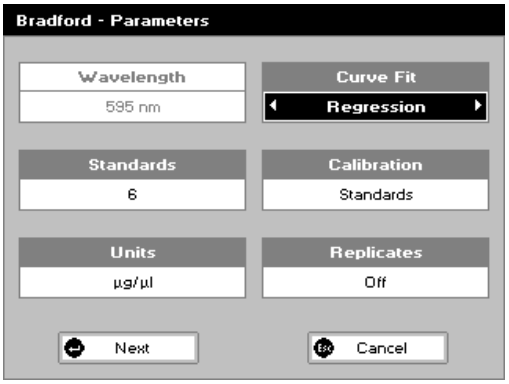
The procedure is as follows:



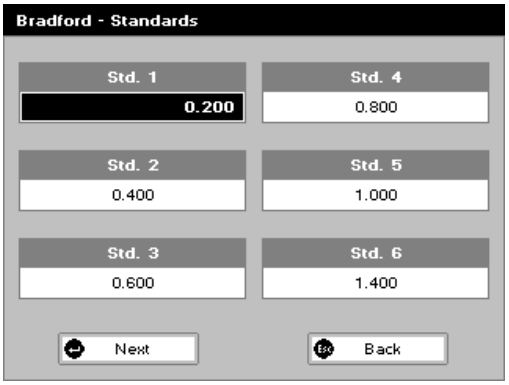
Step 1
Press 3 to select Bradford method.
Step 2
The wavelength for this method is set at 595 nm.
Step 3
Enter the number of standard concentration points (1-9) to be used in the curve using the keypad numbers or left and right arrows.
Press the down arrow.



Step 4
Units: The user can enter a text string up to 8 characters long. To access a list of pre-defined units press the Options key and then use the left/right arrows (µg/ml, µg/µl, pmol/µl, mg/dl, mmol/l, µmol/l, g/l, mg/l, µg/l, U/l, %, ppm, ppb, conc or none). These units can also be edited once OK is pressed.
This screen also allows the number of displayed decimal points (DP) to be selected, from 0 to 2 Note that the result will always be fixed to 5 significant figures regardless of how many decimal points are selected (so 98768.2 will display as 98768 even with 1 decimal point selected).
Press OK to store the chosen parameters or Cancel Esc.



Step 5
Enter the type of curve fit. Options are: straight line regression, zero regression (forces the straight line through the origin), interpolated or cubic spline.
Press the down arrow.
Step 6
Select the calibration mode, either standards (measure prepared standards) or manual (keypad data entry, go to step 9)
Step 7 (standards selected)
Select the number of replicates using the left and right arrows. This determines the number of standards to be measured and averaged at each standard concentration point. Can be OFF (1), 2 or 3.
Step 8 (standards selected)
Press Next to enter the Standards screen
OR Press Cancel Esc to cancel selections and return to the Protein folder.



Standards Screen
Step 9 (standards/manual selected)
Enter the concentration values by using the keypad numbers and the up and down arrows to move between the different standard boxes. Range 0.001 to 9999. C button backspaces and clears the last digit entered.
Step 10 (standards/manual selected)
Press Next to enter the Calibration screen. If there are duplicate or non-monotonic (increasing) entries the unit will beep and highlight the incorrect entry.
OR
Press Back Esc to return to the Parameters screen

Calibration Screen (replicates off)
This shows the calibration values and allows standards to be measured or entered using the keypad numbers (if calibration mode is manual).
Step 11 (standards selected)

Insert the reference sample. Press 

This will be used for all subsequent samples until changed.
Step 12 (standards selected)
Insert the standard (use C to clear previously stored results before measuring)

Press  to measure the standard and store the result.

Repeat step 12 for all standards. A graph will display the results and the fitted curve as the measurements are made.

Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.

Step 13 (standards/manual selected)
When all standards are measured the OK box appears. Press

OK  to accept the calibration and go to the Results screen (see below)

OR Press Back 

to cancel selections and return to the Standards screen.

Calibration Screen (replicates on)
This shows the calibration values and allows standards to be measured.
Step 11 (standards selected)

Insert the reference sample. Press 

This will be used for all subsequent samples until changed.
Step 12 (standards selected)

Press replicates  to display the replicate entry boxes. Use C to clear previously stored results before measuring.

Insert the standard and press  to measure the standard and store the result.

Repeat for all replicates and standards. Press Next  to move from replicates of one standard to replicates of the next standard.

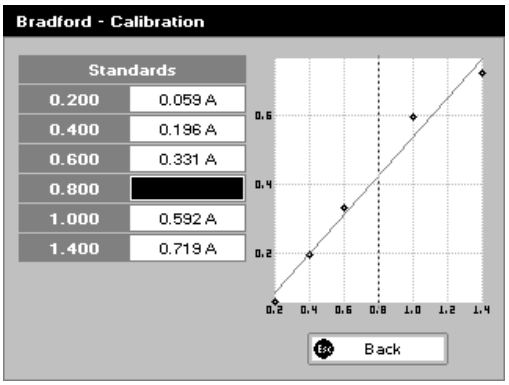
A graph will display the results and the fitted curve as the measurements are input.
Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.

Step 13 (standards/manual selected)

Press OK  to accept the calibration and go to the Results screen (see below)
OR

Press Back 

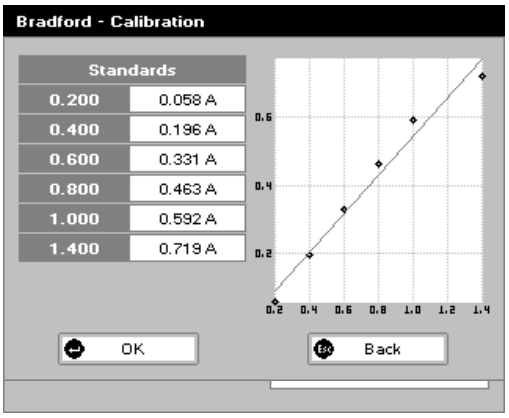
to return to the Standards screen.



Calibration (Manual entry)
Shows previously entered calibration values and allows values to be entered via the keypad.
The highlighted box can be edited in order to enter an absorbance value corresponding to a given concentration value using the keypad numbers. Range 0.001 to 9999. Use C to backspace and clear the last digit entered and the up and down arrows to move between boxes. Pressing the down arrow from the last standard will bring up the OK box.

Press OK ↵ to accept the calibration and go to the Results screen (see below)
OR

Press Back Esc to return to the Standards screen.

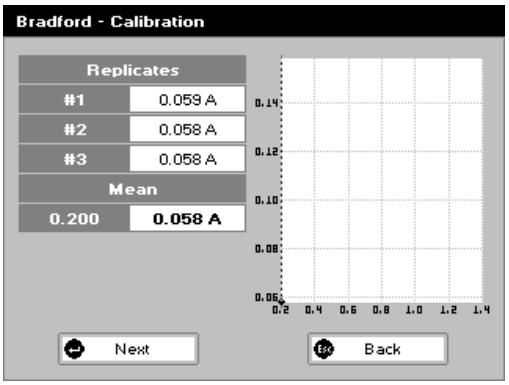


Results screen
Step 14

Insert the reference sample and press 0A 100%T. This will be used for all subsequent samples until changed.
Step 15

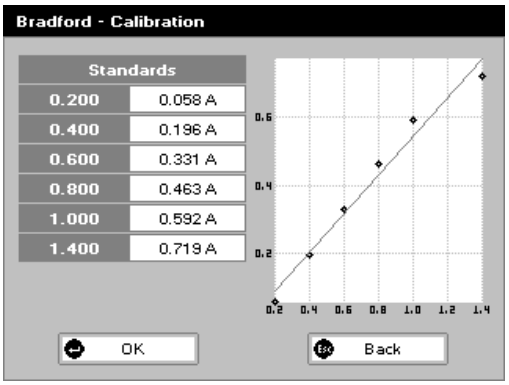
Insert the sample and press .
The concentration of the sample is taken and displayed.
Repeat step 15 for all samples.
Press Esc to return to the Protein Folder.

Press ☰ to display available Options which are described below.



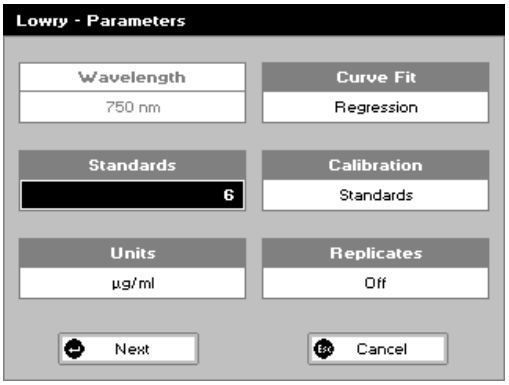
Options (select using key pad numbers)
1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3 Toggle graph on/off. Displays the calibration graph, cursors give values for last measured sample.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save ↵.
9. Auto-print – toggles auto-print on/off.

Exit options by pressing Esc, or wait.

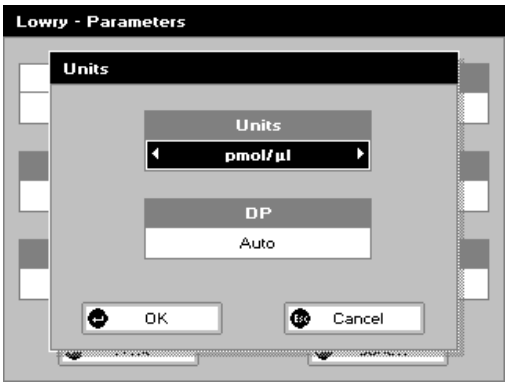


4: Lowry

The procedure is as follows:

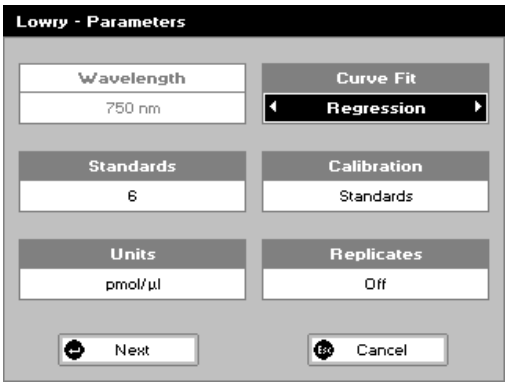


Step 1
Press 4 to select Lowry method.
Step 2
The wavelength for this method is set at 750 nm.
Step 3
Enter the number of standard concentration points (1-9) to be used in the curve using the keypad numbers or left and right arrows.
Press the down arrow.



Step 4
Units: The user can enter a text string up to 8 characters long. To access a list of pre-defined units press the Options key and then use the left/right arrows (µg/ml, µg/µl, pmol/µl, mg/dl, mmol/l, µmol/l, g/l, mg/l, µg/l, U/l, %, ppm, ppb, conc or none). These units can also be edited once OK is pressed.
This screen also allows the number of displayed decimal points (DP) to be selected, from 0 to 2 Note that the result will always be fixed to 5 significant figures regardless of how many decimal points are selected (so 98768.2 will display as 98768 even with 1 decimal point selected).

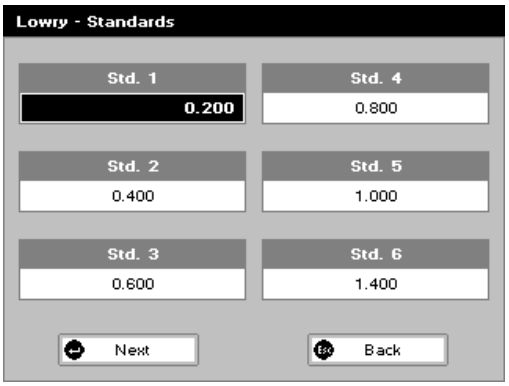
Press OK ↵ to store the chosen parameters or Cancel Esc.



Step 5
Enter the type of curve fit. Options are straight line regression, zero regression (forces the straight line through the origin), interpolated or cubic spline.
Press the down arrow.
Step 6
Select the calibration mode, either standards (measure prepared standards) or manual (keypad data entry, go to step 9)
Step 7 (standards selected)
Select the number of replicates using the left and right arrows. This determines the number of standards to be measured and averaged at each standard concentration point. Can be OFF (1), 2 or 3.
Step 8 (standards selected)

Press Next ↵ to enter the Standards screen

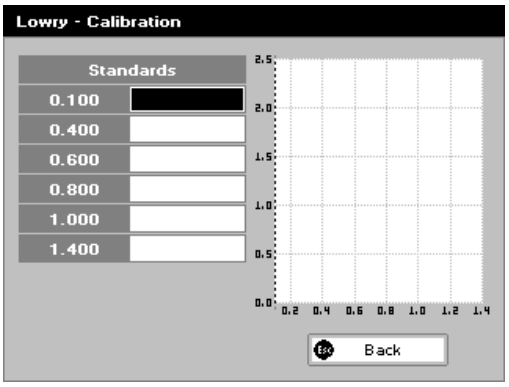
OR Press Cancel Esc to cancel selections and return to the Protein folder.



Standards Screen
Step 9 (standards/manual selected)
Enter the concentration values by using the keypad numbers and the up and down arrows to move between the different standard boxes. Range 0.001 to 9999. C button backspaces and clears the last digit entered.
Step 10 (standards/manual selected)

Press Next ↵ to enter the Calibration screen. If there are duplicate or non-monotonic (increasing) entries the unit will beep and highlight the incorrect entry.

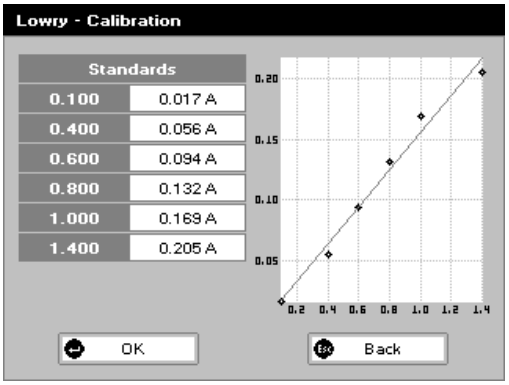
OR Press Back Esc to return to the Parameters screen



Calibration Screen (replicates off)
This shows the calibration values and allows standards to be measured or entered using the keypad numbers (if calibration mode is manual).
Step 11 (standards selected)

Insert the reference sample. Press 0A 100%T.
This will be used for all subsequent samples until changed.
Step 12 (standards selected)
Insert the standard (use C to clear previously stored results before measuring)

Press  to measure the standard and store the result.

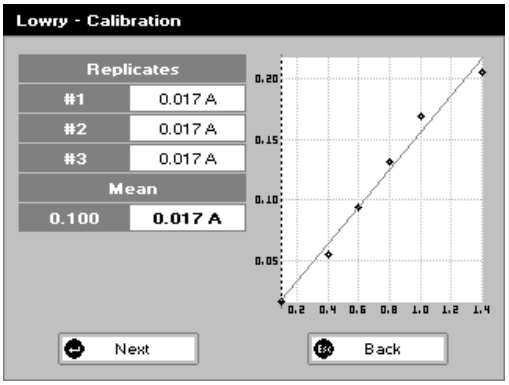


Repeat step 12 for all standards. A graph will display the results and the fitted curve as the measurements are made.

Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.

Step 13 (standards/manual selected)
When all standards are measured the OK box appears. Press OK  to accept the calibration and go to the Results screen (see below)

OR Press Back Esc to cancel selections and return to the Standards screen.



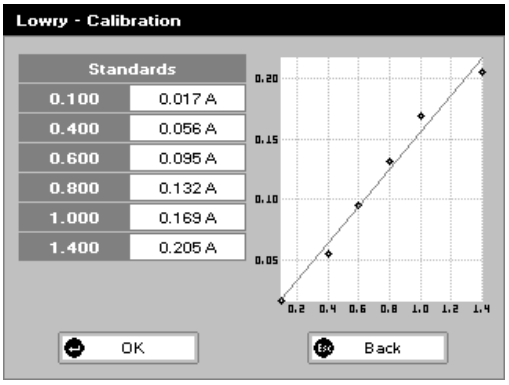
Calibration Screen (replicates on)
This shows the calibration values and allows standards to be measured.
Step 11 (standards selected)

Insert the reference sample. Press 0A 100%T.
This will be used for all subsequent samples until changed.
Step 12 (standards selected)

Press replicates  to display the replicate entry boxes. Use C to clear previously stored results before measuring.

Insert the standard and press  to measure the standard and store the result. Repeat for all replicates and standards.

Press Next  to move from replicates of one standard to replicates of the next standard.

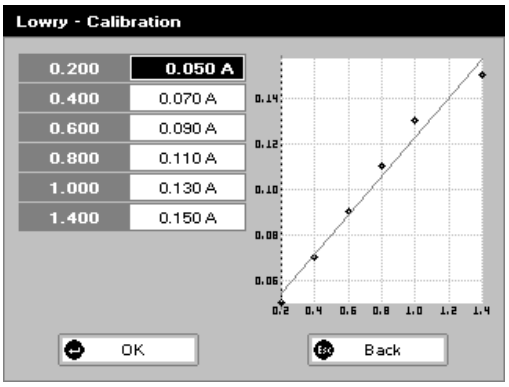


A graph will display the results and the fitted curve as the measurements are input.
Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.

Step 13 (standards/manual selected)

Press Next  to accept the calibration and go to the Results screen (see below)

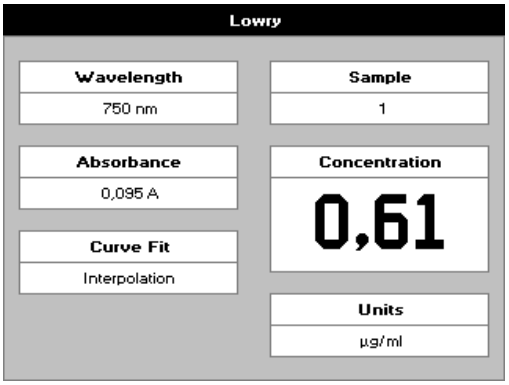
OR Press Back Esc to return to the Standards screen.



Calibration (Manual entry)
Shows previously entered calibration values and allows values to be entered via the keypad.
The highlighted box can be edited in order to enter an absorbance value corresponding to a given concentration value using the keypad numbers. Range 0.001 to 9999. Use C to backspace and clear the last digit entered and the up and down arrows to move between boxes.

Press OK ↵ to accept the calibration and go to the Results screen (see below)

OR Press Back Esc to return to the Standards screen.



Results screen
Step 14

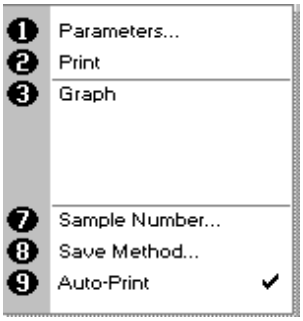
Insert the reference sample and press 0A 100%T. This will be used for all subsequent samples until changed.

Step 15

Insert the sample and press  .
The concentration of the sample is taken and displayed.
Repeat step 15 for all samples.

Press Esc to return to the Protein Folder.

Press  to display available Options which are described below.



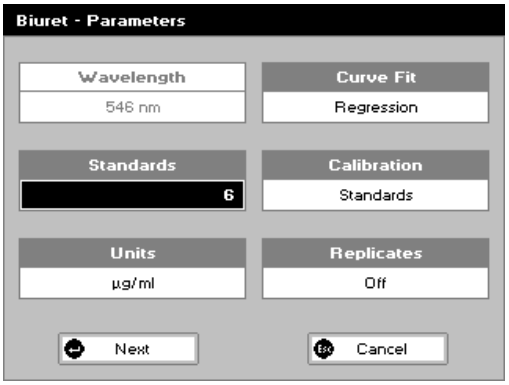
Options (select using key pad numbers)

1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. Displays the calibration graph, cursors give values for last measured sample.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save ↵.
9. Auto-print – toggles auto-print on/off.

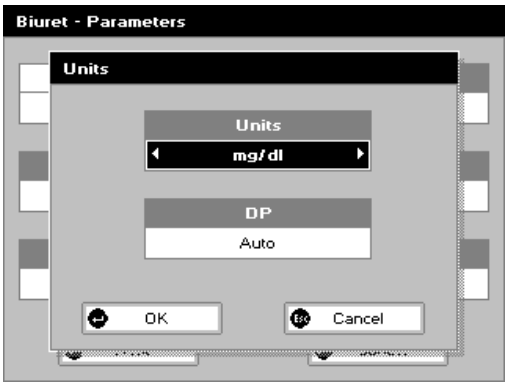
Exit options by pressing Esc, or wait.

5: Biuret

The procedure is as follows:

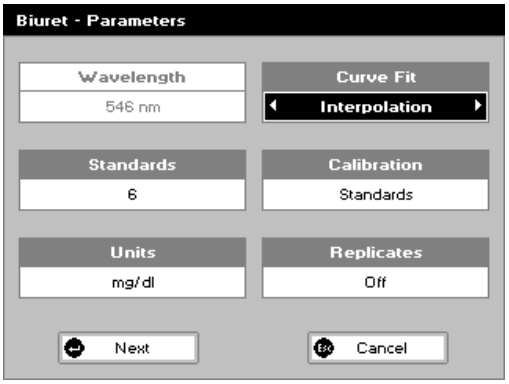


Step 1
Press 5 to select Biuret method.
Step 2
The wavelength for this method is set at 546 nm.
Step 3
Enter the number of standard concentration points (1-9) to be used in the curve using the keypad numbers or left and right arrows.
Press the down arrow.



Step 4
Units: The user can enter a text string up to 8 characters long. To access a list of pre-defined units press the Options key and then use the left/right arrows (µg/ml, µg/µl, pmol/µl, mg/dl, mmol/l, µmol/l, g/l, mg/l, µg/l, U/l, %, ppm, ppb, conc or none). These units can also be edited once OK is pressed.
This screen also allows the number of displayed decimal points (DP) to be selected, from 0 to 2 Note that the result will always be fixed to 5 significant figures regardless of how many decimal points are selected (so 98768.2 will display as 98768 even with 1 decimal point selected).

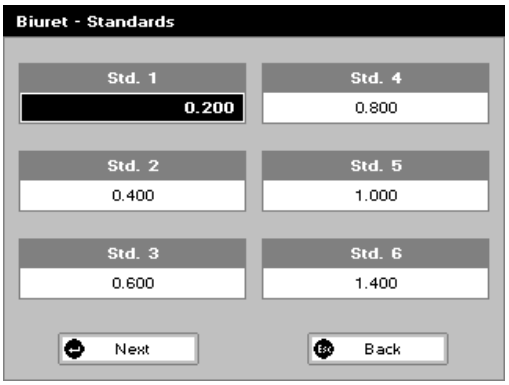
Press OK ↵ to store the chosen parameters or Cancel Esc.



Step 5
Enter the type of curve fit. Options are straight line regression, zero regression (forces the straight line through the origin), interpolated or cubic spline.
Press the down arrow.
Step 6
Select the calibration mode, either standards (measure prepared standards) or manual (keypad data entry, go to step 9)
Step 7 (standards selected)
Select the number of replicates using the left and right arrows. This determines the number of standards to be measured and averaged at each standard concentration point. Can be OFF (1), 2 or 3.
Step 8 (standards selected)

Press Next ↵ to enter the Standards screen
OR

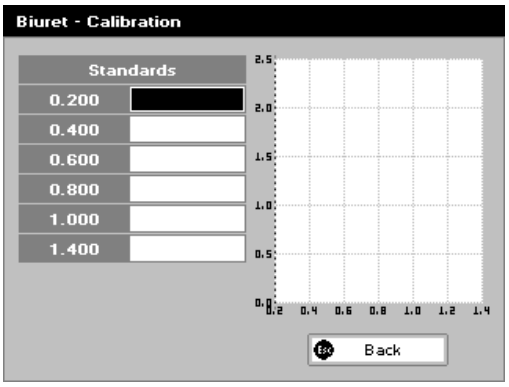
Press Cancel Esc to cancel selections and return to the Protein folder.



Standards Screen
Step 9 (standards/manual selected)
Enter the concentration values by using the keypad numbers and the up and down arrows to move between the different standard boxes. Range 0.001 to 9999. C button backspaces and clears the last digit entered.
Step 10 (standards/manual selected)

Press Next ↵ to enter the Calibration screen

OR Press Back Esc to return to the Parameters screen

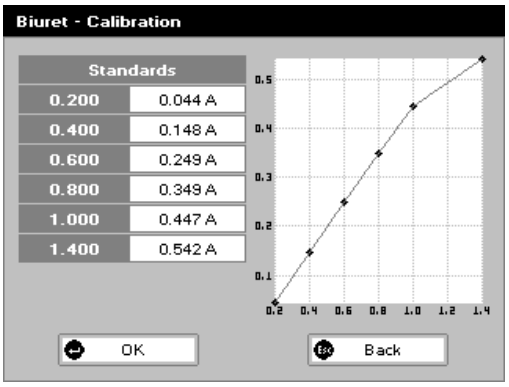


Calibration Screen (replicates off)
This shows the calibration values and allows standards to be measured or entered using the keypad numbers (if calibration mode is manual).
Step 11 (standards selected)

Insert the reference sample. Press 0A/100%T.
This will be used for all subsequent samples until changed.
Step 12 (standards selected)
Insert the standard (use C to clear previously stored results before measuring)

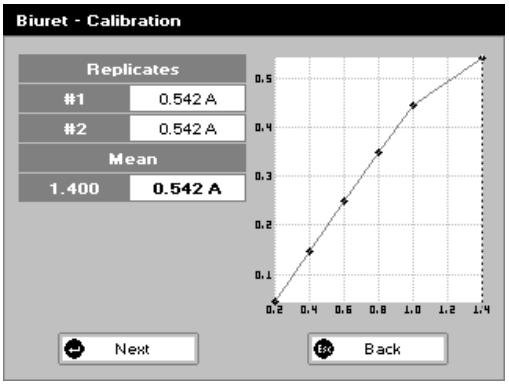
Press  to measure the standard and store the result.
Repeat step 12 for all standards. A graph will display the results and the fitted curve as the measurements are made.

Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.



Step 13 (standards./manual selected)
When all standards are measured the OK box appears. Press OK ↵ to accept the calibration and go to the Results screen (see below)

OR Press Back Esc to cancel selections and return to the Standards screen.



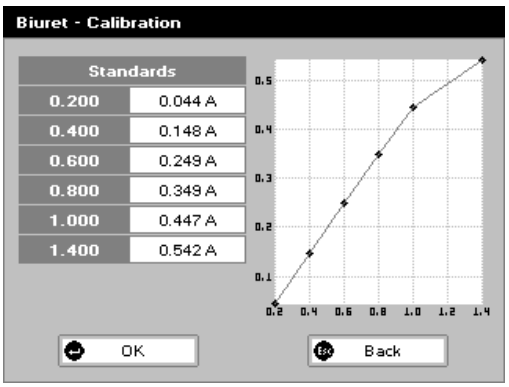
Calibration Screen (replicates on)
This shows the calibration values and allows standards to be measured.
Step 11 (standards selected)

Insert the reference sample. Press 0A/100%T
This will be used for all subsequent samples until changed.
Step 12 (standards selected)

Press replicates ↵ to display the replicate entry boxes. Use C to clear previously stored results before measuring.

Insert the standard and press  to measure the standard and store the result.

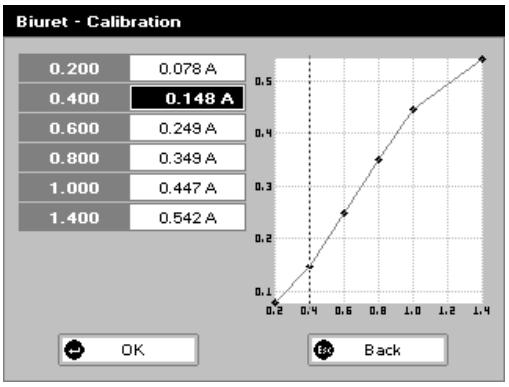
Repeat for all replicates and standards. Press Next ↵ to move from replicates of one standard to replicates of the next standard.
A graph will display the results and the fitted curve as the measurements are input.
Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.



Step 13 (standards/manual selected)

Press OK ↵ to accept the calibration and go to the Results screen (see below)

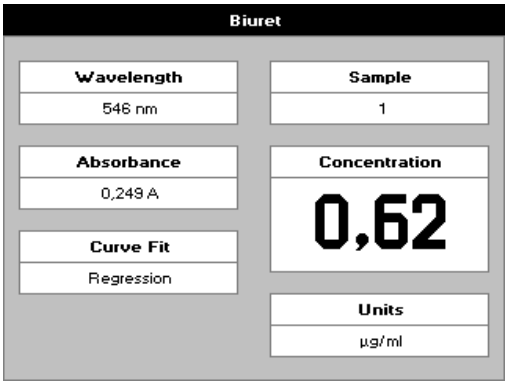
OR Press Back Esc to return to the Standards screen.



Calibration (Manual entry)
Shows previously entered calibration values and allows values to be entered via the keypad.
The highlighted box can be edited in order to enter an absorbance value corresponding to a given concentration value using the keypad numbers. Range 0.001 to 9999. Use C to backspace and clear the last digit entered and the up and down arrows to move between boxes.

Press OK ↵ to accept the calibration and go to the Results screen (see below)
OR

Press Back Esc to return to the Standards screen.



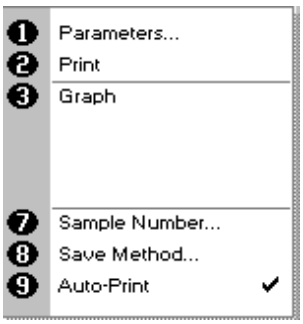
Results screen
Step 14

Insert the reference sample and press 0A 100%T. This will be used for all subsequent samples until changed.
Step 15

Insert the sample and press  .
The concentration of the sample is taken and displayed.
Repeat step 15 for all samples.

Press Esc to return to the Protein Folder.

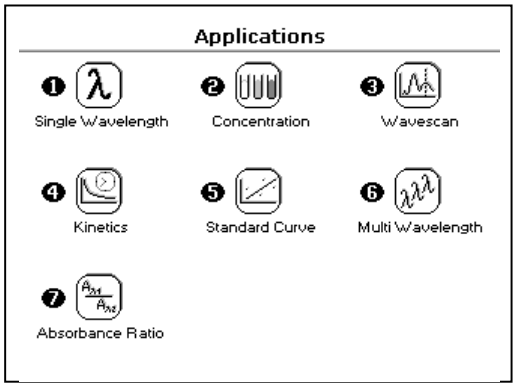
Press  to display available Options which are described



Options (select using key pad numbers)
1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. Displays the calibration graph, cursors give values for last measured sample.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the alpha-numeric keys to enter a name for the method and press Save ↵.
9. Auto-print – toggles auto-print on/off.

Exit options by pressing Esc, or wait.

THE APPLICATIONS FOLDER



SUMMARY:

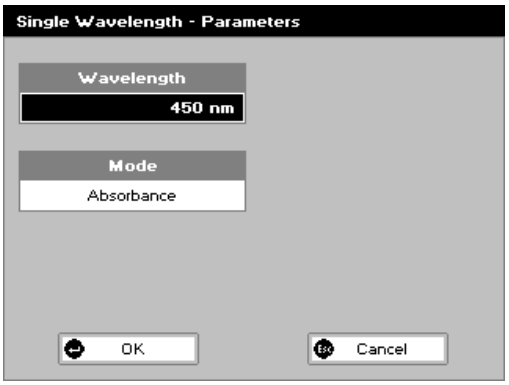
Function	Key pad number	Description
❶ Single Wavelength	1	Absorbance or %T (transmission) at a single user defined wavelength.
❷ Concentration	2	Concentration measurement at a single wavelength based on a simple Factor entered or calculated from a single standard.
❸ Wavescan	3	Wavelength scan between two user defined wavelengths. Range 200-900 nm, with user configurable peak finding function.
❹ Kinetics	4	Absorbance versus time measurements either rate or end value based.
❺ Standard Curve	5	Generation of calibration curve by measuring standards at a single wavelength.
❻ Multi Wavelength	6	Absorbance or %T (transmission) at up to 5 user defined wavelengths.
❼ Absorbance Ratio	7	Ratio of absorbance values at two user specified wavelengths.

OPTIONS

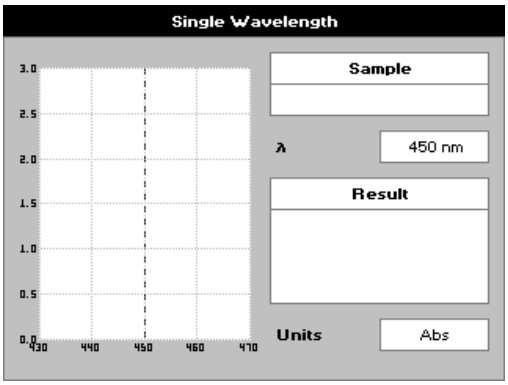
Within each application the user has the possibility to select various options that define the way results are treated. If not using a stored method, it is advisable to check that these Options have been appropriately set for your experiment when coming to the instrument. Note that setting the “History” parameter to on (see Preferences later) will cause the instrument to store its last settings. If the “History” parameter is turned off, all parameters and options will return to their default settings when you leave that application. (Unless it has been saved as a method).

1: Single Wavelength – Abs and %T

This makes simple absorbance (A) and % transmission (%T) measurements on samples, measuring the amount of light that has passed through a sample relative to a reference (this can be air).
The procedure is as follows:



Step 1
Set wavelength by using keypad numbers or left and right arrows. Range 190 – 1100 nm.
Press the down arrow key.
Step 2
Select the mode, *Absorbance* or *%T*, using the left and right arrows.
Step 3
To enter the results screen with the selected parameters press OK .
OR
Cancel the selections and return to the Applications Folder by pressing Cancel Esc.

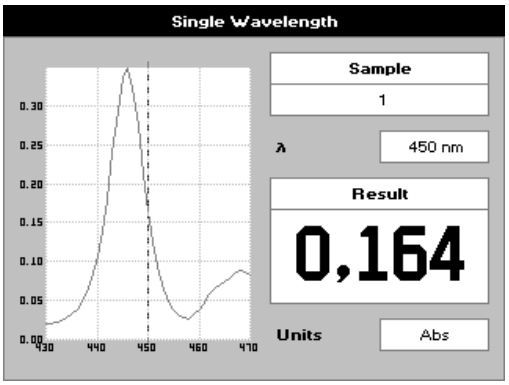


Step 4

Insert the reference. Press 0A 100%T. This will be used for all subsequent samples until changed.
Step 5

Insert sample and press .

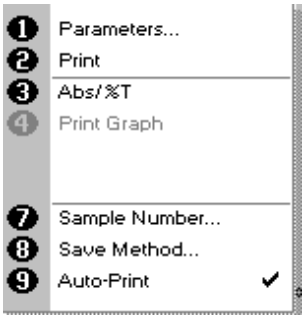
Repeat step 5 for all samples.



Results
The result at the selected wavelength is displayed on screen. Use the left and right arrows to move the cursor and display the value at the cursor position (+/- 15nm from set wavelength).

Press Cancel Esc to return to the Applications Folder.

Press to display available Options which are described below.



Options (select using key pad numbers)

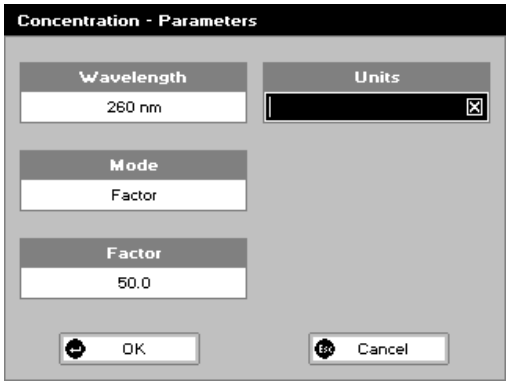
1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle between Absorbance and %T mode.
4. Print graph – greyed out if no data are available.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the left and right arrows to select a folder to store in (Favorites/Methods 1-9), press the down arrow and enter name.
9. Auto-print – toggles auto-print on/off.

Exit options by pressing Esc, or wait.

2: Concentration

This makes simple concentration measurements on samples, by measuring the amount of light that has passed through a sample relative to a reference (this can be air). Concentration is obtained by multiplying the measured absorbance at a specific wavelength by a factor. The factor may be known in advance, or may be calculated by the instrument by measuring a standard of known concentration.

The procedure is as follows:

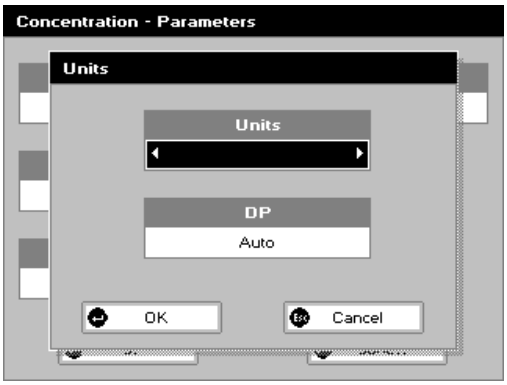


Step 1
Set wavelength by using keypad numbers or left and right arrows. Range 190-1100 nm.
Press the down arrow key.

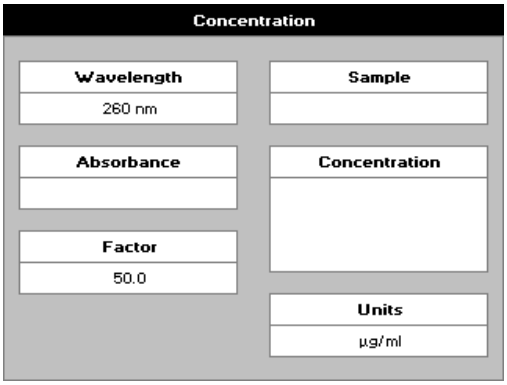
Step 2
Select the mode, Factor (user entered) or Standard (factor is calculated from a calibration sample), using the left and right arrows.
Press the down arrow key.

Step 3 (if Factor is selected)
Enter the Factor using the keypad numbers. Range 0.001 to 9999. Use the C button to delete the last digit entered.
Press the down arrow key.

Step 3 (if Standard is selected)
Enter the concentration using keypad numbers. Range 0.01-9999. Use the C button to delete the last digit entered.
Press the down arrow key.

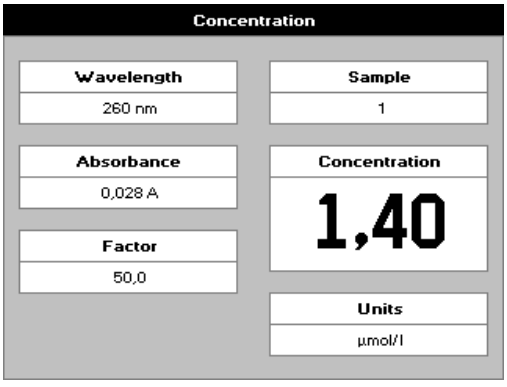


Step 4
Units: The user can enter a text string up to 8 characters long. To access a list of pre-defined units press the Options key and then use the left/right arrows (µg/ml, µg/µl, pmol/µl, mg/dl, mmol/l, µmol/l, g/l, mg/l, µg/l, U/l, %, ppm, ppb, conc or none). These units can also be edited once OK is pressed.
This screen also allows the number of displayed decimal points (DP) to be selected, from 0 to 2 Note that the result will always be fixed to 5 significant figures regardless of how many decimal points are selected (so 98768.2 will display as 98768 even with 1 decimal point selected). Press OK to store the chosen parameters or Cancel Esc.

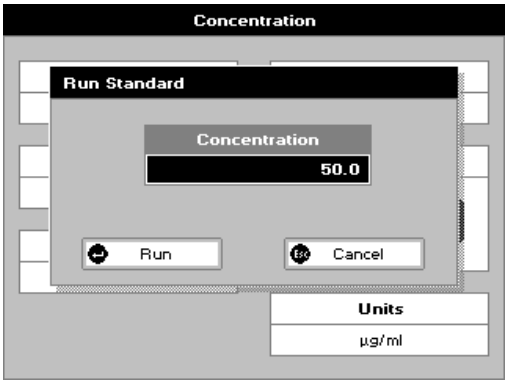


Step 5
To enter the results screen with the selected parameters press OK
OR
Cancel the selections and return to the Applications Folder by pressing Cancel Esc.

Step 6 (if using a Factor)
Insert the reference. Press 0A 100%T. This will be used for all subsequent samples until changed.



Step 7
Insert sample and press .
The concentration of the sample is displayed. Results shown as ---- indicate the concentration is out of range.
Repeat step 7 for all samples.
Press Esc to return to the Applications folder.
Press to display available Options which are described below.



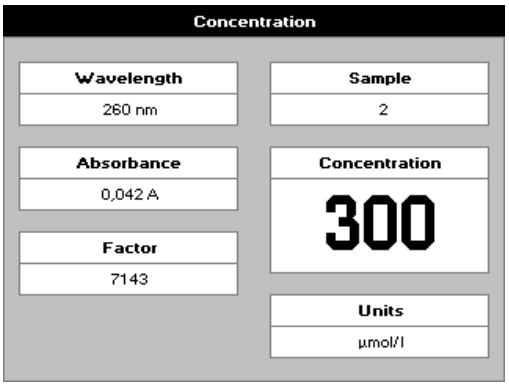
Step 6 (if using standard mode)

Insert the reference. Press 0A 100%T. This will be used for all subsequent samples until changed.

Press  to display the Run Standard screen.

Run the standard by pressing ↵
OR

Press cancel Esc to return to the measure screen.



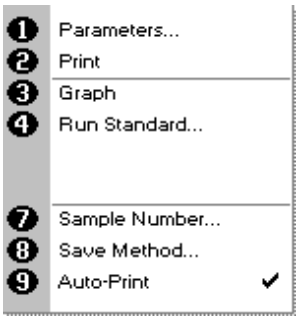
Step 7

Insert the sample and press  .
The concentration of the sample is displayed. Results shown as ---- indicate the concentration is out of range.

Repeat step 7 for all samples.

Press Esc to return to the Applications Folder.

Press  to display available Options which are described below.



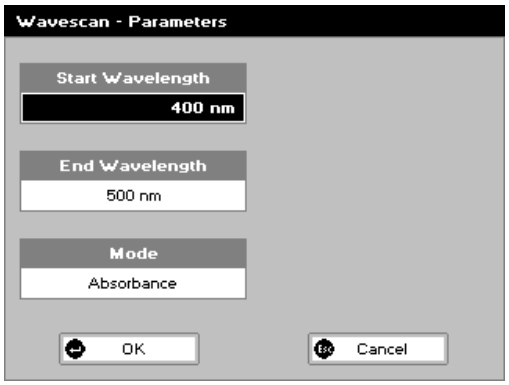
Options (select using key pad numbers)

1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggles on/off, displaying a graph of wavescan +/- 20 nm from selected wavelength.
4. Return to Run Standard screen.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the left and right arrows to select a folder to store in (Favorites/Methods 1-9), press the down arrow and enter name.
9. Auto-print – toggles auto-print on/off.

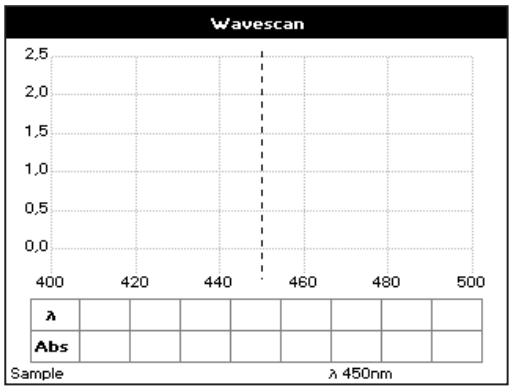
Exit options by pressing Esc, or wait.

3: Wavescan

An absorption spectrum can be obtained from your instrument, enabling simple identification of peak height and position. The procedure is as follows:



Step 1
Set start wavelength by using keypad numbers or left and right arrows. Range 200 – 890 nm.
Press the down arrow key.
Step 2
Set end wavelength by using keypad numbers or left and right arrows. Range 210 – 900 nm.
Press the down arrow key.
Step 3
Select the mode, *Absorbance* or *%T*, using the left and right arrows.
Step 4
To enter the measurements screen with the selected parameters press OK
OR
Cancel the selections and return to the Applications Folder by pressing Cancel Esc.

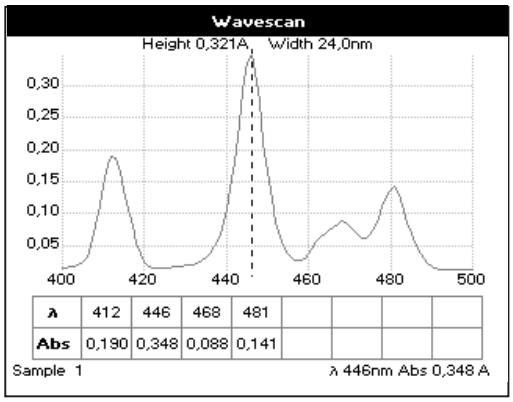


Step 5

Insert the reference. Press 0A 100%T. This will be used for all subsequent samples until changed.
Step 6

Insert sample and press .

Repeat step 6 for all samples.



Results
A graph of the wavescan is displayed, along with a table of Absorbance/%T at each peak. Use the left and right arrows to move the cursor along the graph. When it reaches a peak the peak height and width of the peak is displayed at the top of the screen.
To zoom in on the wavelength scale, use the up arrow. This auto-scales on the Absorbance/%T scale (dependent on the Graph Scale option) and this is retained for subsequent measurements.
To zoom out again, use the down arrow.

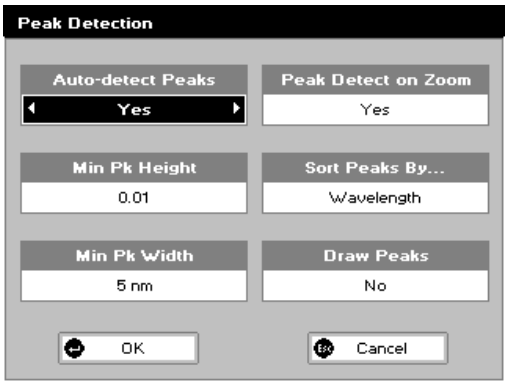
Press Esc to return to the Applications Folder.

Press to display available Options which are described next.

Options (select using key pad numbers)

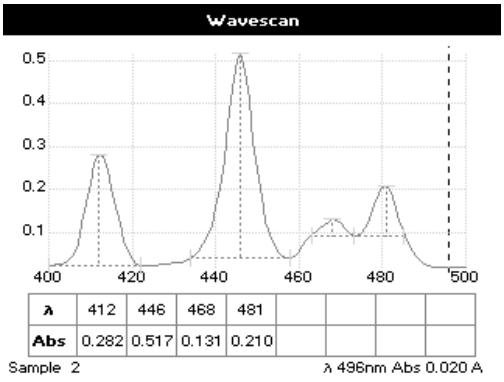
1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle between Absorbance and %T mode.
4. Displays Peak Detection Parameter Screen. See description below.
5. Manually adds a peak position to the peak table in the results screen at the position set by the cursor. If the cursor is returned to this position the legend “User Defined Peak” is displayed at the top of the scan and this option changes to Delete Peak.
6. Displays Graph Scale Parameter Screen. See description below.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the left and right arrows to select a folder to store in (Favorites/Methods 1-9), press the down arrow and enter name.
9. Auto-print – toggles auto-print on/off.

Exit options by pressing Esc, or wait.



Peak Detection (Shortcut button 4)

AutoDetect Peaks: Turns on and off the automatic peak detection. The following options determine how peaks are detected:
Minimum peak height: Minimum height the peak has to be above the higher of the two adjacent minima for the peak to be detected
Minimum peak width: Minimum width of the peak as determined by the difference in wavelength between the higher of the two adjacent minima and the opposing intersection of that higher minimum level and the peak profile. (See the screen displayed below).

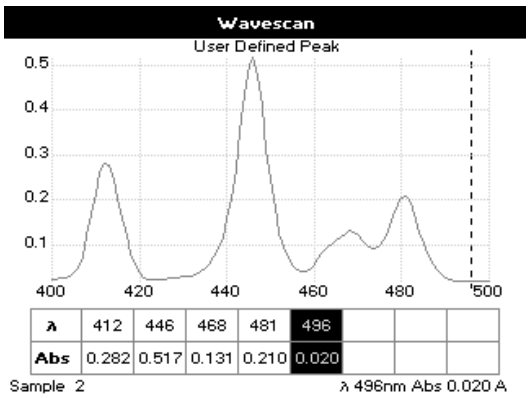


Peak Detect on Zoom: Determines whether peaks are re-assessed and tabulated when the user zooms into a region of the wavescan. If off leaves the peak detection as determined on the un-zoomed display

Sort peaks by…: Determines the sequence that peaks are reported by. Can be wavelength, peak height or peak width.

Draw Peaks: Switches display of peak cursors on and off. These show vertical dashed lines displaying the measured peak height and horizontal dashed lines showing the peak width

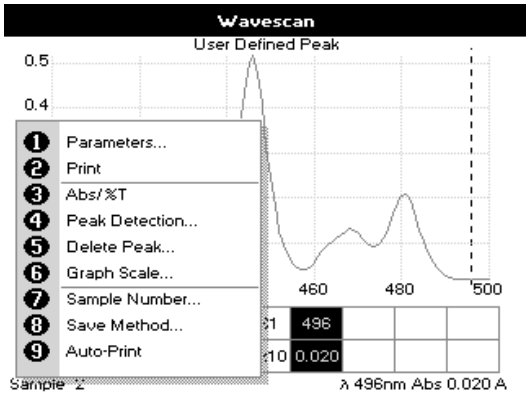
Pressing Cancel Esc ignores the selection, pressing OK ↵ accepts them.

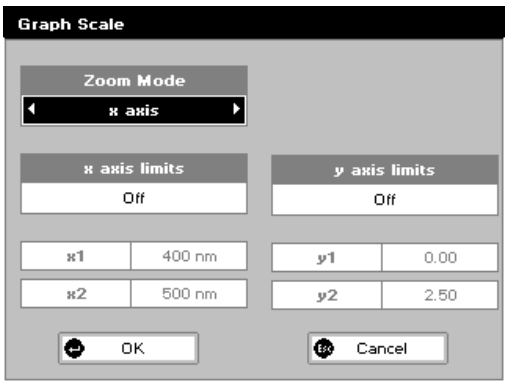


Add Peak... (Shortcut button 5)

Adds a used defined peak at the current cursor position. The entry is then displayed in inverse coloring to discriminate between user defined peaks and auto-detect peaks. When the cursor is positioned over the user defined peak a legend “User Defined Peak” appears at the top of the graph. The option then changes to Delete Peak to enable the user to remove the peak.

Note Storing a method at this stage will save these user defined wavelengths, each time method is run Absorbance value at these wavelengths is reported





Graph Scale… (Shortcut button 6)
This enables the user to set up a defined graph by defining the limits in either or both of the x and y axes.

Zoom mode:
This sets up the operation of the Zoom keys (up and down arrows). “x & y axes” expands the display around the cursor measurement point, whilst the other options select the absorbance or wavelength axes respectively. With x or y axis limits set to on, zooming out will only be permitted to the set limits.

x/y axis limits:
Setting “x (or y) axis limits” to “On” activates the start and finish points of the desired graph to user defined specific wavelengths and/or absorbance values.

Pressing Cancel Esc ignores the selection; pressing OK ↵ accepts them and displays the required graph.

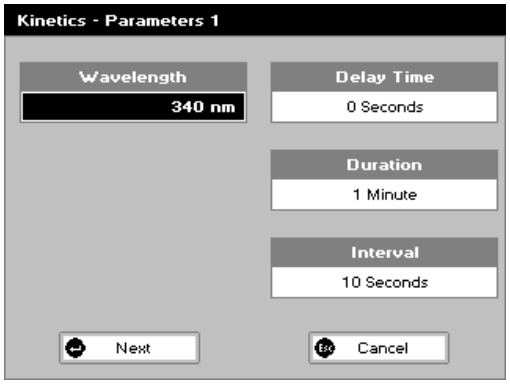
4: Simple Kinetics

Kinetics studies, where the change in absorbance needs to be followed as a function of time at a fixed wavelength, can be readily performed.

Reagent test kits are routinely used for the enzymatic determination of compounds in food, beverage and clinical laboratories by measuring NAD / NADH conversion at 340 nm. The change in absorbance over a specified time period can be used to provide useful information when an appropriate factor, defined in the reagent kit protocol, is applied. Reaction rate and enzyme activity can be calculated if the factor used takes account of the absorbance difference per unit time, as opposed to the absorbance difference *per se*.

For this reason, the change in absorbance per minute **(ΔA/min**), concentration (**ΔA/min x factor**) and **correlation coefficient** (calculated from a best fit of the data points) are displayed. They may not be relevant for simple kinetics experiments.

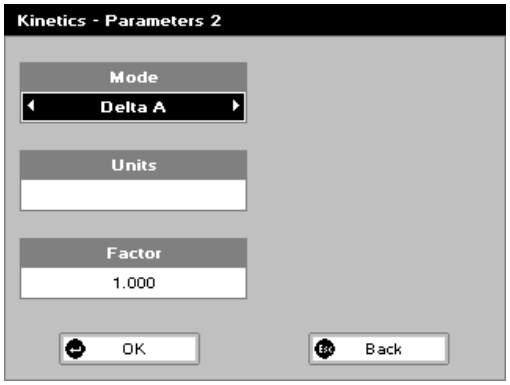
The procedure to define a new method is as follows:



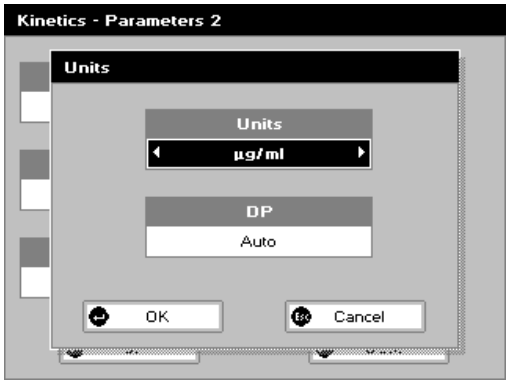
Kinetics Parameter 1 Screen
Step 1 (Wavelength)
Enter the wavelength using the keypad numbers or the left and right arrows. Range 190-1100 nm.
Press the down arrow
Step 2 (Delay time)
Enter the delay time in seconds before measurements are taken. This can be a maximum of 600 seconds (10 minutes).
Press the down arrow.
Step 3 (Duration)
Enter the time in minutes over which measurements are taken. This can be a maximum of 60 minutes.
Press the down arrow.
Step 4 (Interval)
Enter the interval time in seconds between measurements using the left and right arrows. Options are: 1, 5, 10, 20, 30 or 60 seconds.
Press the down arrow.
Step 5

Press Next ↵ to go to the next parameters screen
OR

Press Cancel Esc to return to the Applications Folder.

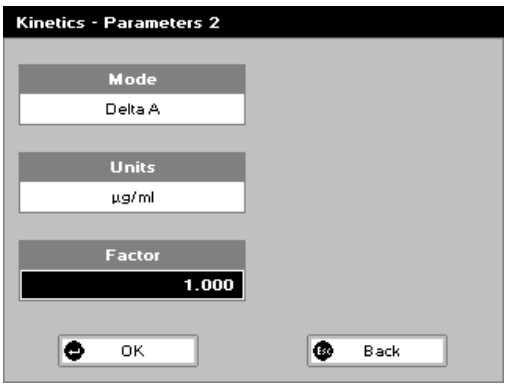


Kinetics Parameters 2 Screen
Step 6
Select the measurement mode using the left and right arrows.
Delta A: change in absorbance over the measurement duration (or selected period).
Final A: absorbance at the end of the measurement duration (or selected time).
Slope: rate of change of absorbance over the measurement duration or selected period.



Step 7

Units: The user can enter a text string up to 8 characters long. To access a list of pre-defined units press the Options key ☒☒☑ and then use the left/right arrows (µg/ml, µg/µl, pmol/µl, mg/dl, mmol/l, µmol/l, g/l, mg/l, µg/l, U/l, %, ppm, ppb, conc or none). These units can also be edited once OK is pressed.
This screen also allows the number of displayed decimal points (DP) to be selected, from 0 to 2 Note that the result will always be fixed to 5 significant figures regardless of how many decimal points are selected (so 98768.2 will display as 98768 even with 1 decimal point selected). Press OK ↵ to store the chosen parameters or Cancel Esc.

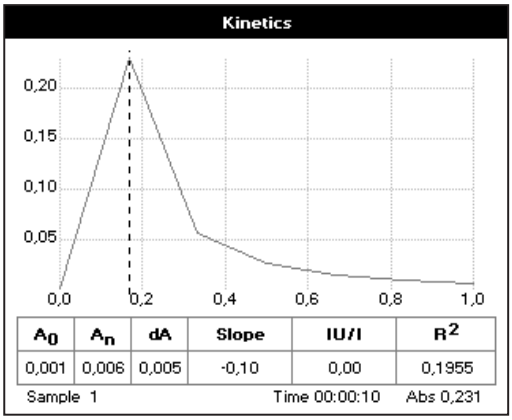


Step 8

Set the Factor by which the result is multiplied to give the amount in the chosen range using the left and right arrows. Range of 0.01 to 9999.

Step 9

Press OK ↵ to enter the Results screen
OR
Press Back Esc to return to the Parameters 1 screen.



Results

Insert the reference and press 0A 100%T.

Insert the sample and press ↓ to start the run.

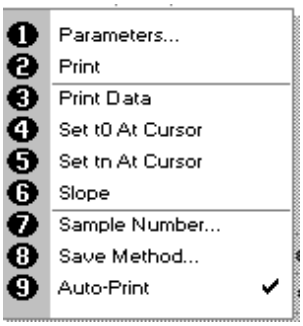
Time (min) is displayed at the bottom of the screen, and absorbance data are plotted on the graph as testing proceeds. The table below the graph gives: absorbance values at T_0 (start of calculation), T_n (finish of calculation, change in absorbance, slope, regression parameter (R^2) of the calculated slope and the result calculated from the selected parameter (dA, final A or slope).

Use the left and right arrows to move the cursor and display the time and absorbance value at measured data points.

Use the up and down arrows to zoom in or out.

Press Cancel Esc to return to the Applications Folder.

Press ☰ to display available Options which are described below.



Options (select using key pad numbers)

1. Return to parameter 1 screen (step 1 above).
2. Print data on the results screen via selected method.
3. Print all the data.
4. Set the t_0 position (starting point for the slope and dA calculation) at the current cursor position. Value is retained for subsequent samples.
5. Set the t_n position (finishing point for the slope and dA calculation) at the current cursor position. Value is retained for subsequent samples.
6. Toggle the calculated slope line on and off.
   Note: if any data points enclosed by t_0 and t_n are beyond the range of the instrument (>2.5A or <-0.3A) then this option is greyed out.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the left and right arrows to select a folder to store in (Favorites/Methods 1-9), press the down arrow and enter name.
9. Auto-print – toggles auto-print on/off.

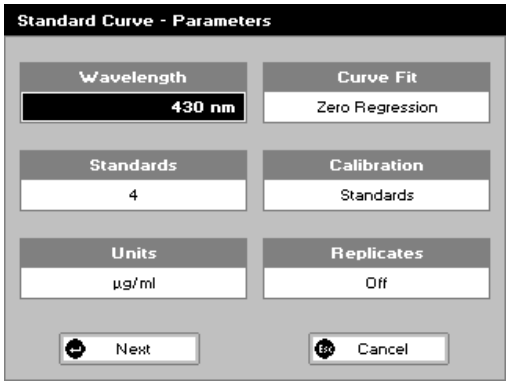
Exit options by pressing Esc, or wait.

5: Standard Curve

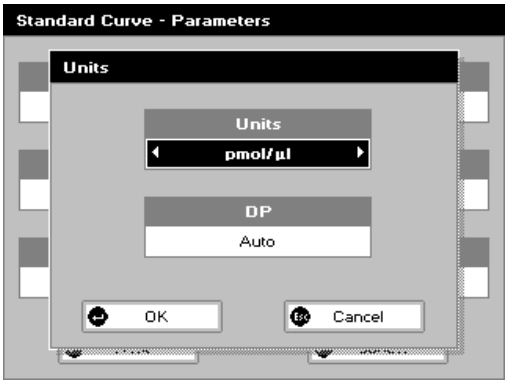
The construction of a multi-point calibration curve from standards of known concentration to quantify unknown samples is a fundamental use of a spectrophotometer; this instrument has the advantage of being able to store this curve as a method, using up to 9 standards.

To include a zero concentration standard, include this in the number of standards to be entered and enter 0.00 for concentration; use a reagent blank when required to enter the zero standard.

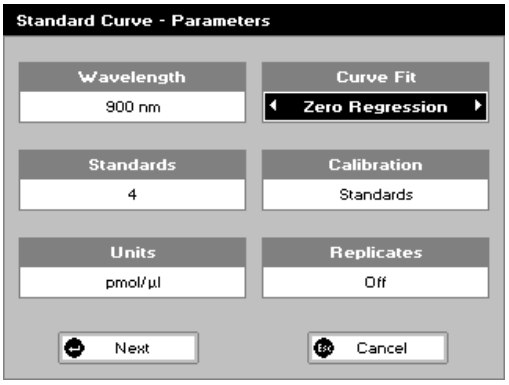
The procedure is as follows:



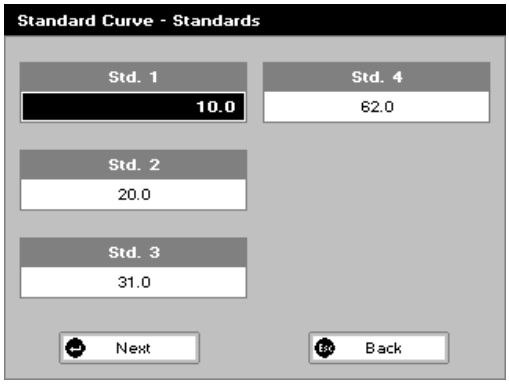
Step 1
Select the wavelength using the keypad numbers or left and right arrows. Range 190-1100 nm.
Press the down arrow.
Step 2
Enter the number of standard concentration points to be used in the curve (1-9).
Press the down arrow.



Step 3
Units: The user can enter a text string up to 8 characters long. To access a list of pre-defined units press the Options key and then use the left/right arrows (µg/ml, µg/µl, pmol/µl, mg/dl, mmol/l, µmol/l, g/l, mg/l, µg/l, U/l, %, ppm, ppb, conc or none). These units can also be edited once OK is pressed.
This screen also allows the number of displayed decimal points (DP) to be selected, from 0 to 2 Note that the result will always be fixed to 5 significant figures regardless of how many decimal points are selected (so 98768.2 will display as 98768 even with 1 decimal point selected). Press OK to store the chosen parameters or Cancel Esc.



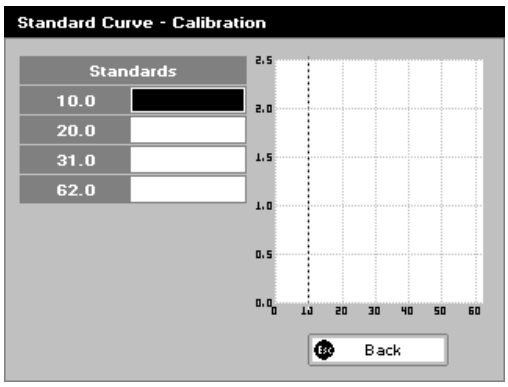
Step 4
Select the type of curve fit using the left and right arrows. Options: straight line regression, a zero regression (this forces the straight line through the origin), interpolated or cubic spline.
Step 5
Select the calibration mode: either Standards (measure prepared standards) or Manual (keypad data entry).
Press the down arrow.
Step 6 (if standards has been selected in step 5)
Select the number of standards to be measured and averaged at each standard concentration point. Can be OFF (1), 2 or 3.
Step 7
Press Next to enter the Standards screen
OR
Press Cancel Esc to cancel selections and return to the Applications Folder.



Standards screen
Step 8
Enter the concentration values by using the keypad numbers and the up and down arrows to move between the different standard boxes. Range 0.001 to 9999.
Step 9

Press Next ↵ to enter the Calibration screen. If any duplicate or non-monotonic (increasing entries) are present the unit will beep and highlight the incorrect entry
OR

Press Back Esc to return to the Parameter screen.

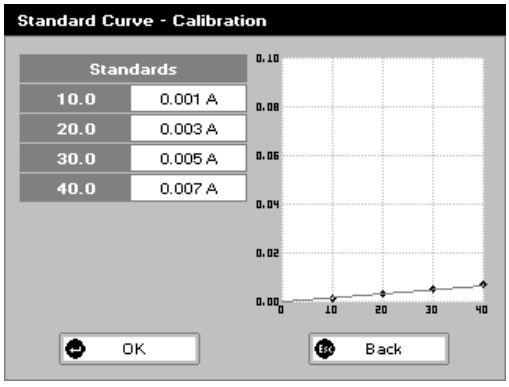


Calibration Screen (replicates off)
This shows the calibration values and allows standards to be measured.
Step 10

Insert the reference. Press 0A 100%T.
This will be used for all subsequent samples until changed.
Step 11
Insert the standard (use C to clear previously stored results before measuring).

Press  to measure the standard and store the result.

Repeat for all standards.



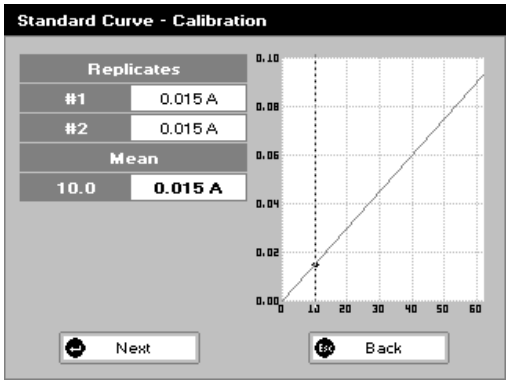
A graph will display the results and the fitted curve as the measurements are input.

Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.

Step 12

Press OK ↵ to accept the calibration and go to the Results screen (see below)
OR

Press Back Esc to return to the Standards screen.



Calibration Screen (replicates on)
This shows the calibration values and allows standards to be measured.

Step 10

Insert the reference. Press 0A 100%T.
This will be used for all subsequent samples until changed.

Step 11

Press replicates ↵ to display the replicate entry boxes. Use C to clear previously stored results before measuring.

Insert the standard and press ← to measure the standard and store the result.

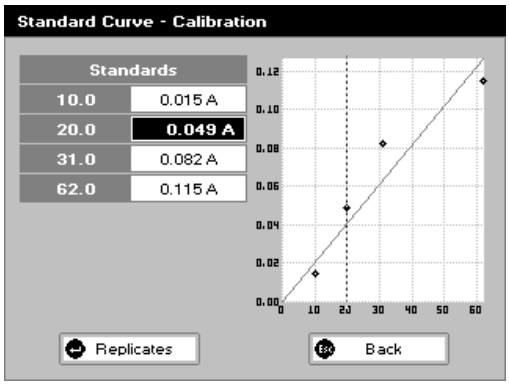
Repeat for all replicates. Press Next ↵ to measure the next standard and repeat the process for all standards.

A graph will display the results and the fitted curve as the measurements are input.
Use the up and down arrows to select a standard to be repeated if a poor reading has been obtained. Use C to clear the previous reading.

Step 12

Press Next ↵ to accept the calibration and go to the Results screen (see below)
OR

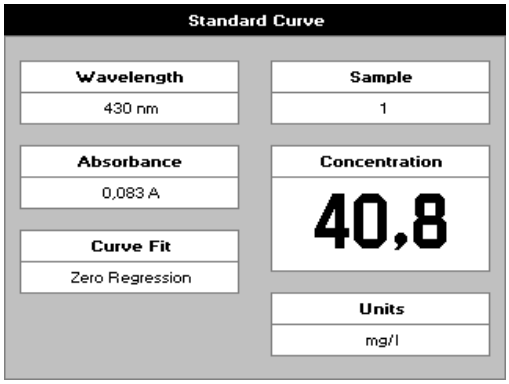
Press Back Esc to return to the Standards screen.



Calibration (Manual entry)
Shows previously entered calibration values and allows values to be entered via the keypad.
The highlighted box can be edited in order to enter an absorbance value corresponding to a given concentration value using the keypad numbers. Range 0.001 to 9999. Use C to backspace and clear the last digit entered and the up and down arrows to move between boxes.

Press OK ↵ to accept the calibration and go to the Results screen (see below)
OR

Press Back Esc to return to the Standards screen.



Results screen

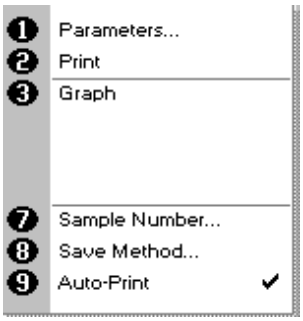
Step 13

Insert the reference and press the 0A 100%T. This will be used for all subsequent samples until changed.

Step 14

Insert the sample and press ←.
The concentration of the sample is taken and displayed.
Repeat step 14 for all samples.

Press Esc to return to the Applications Folder.



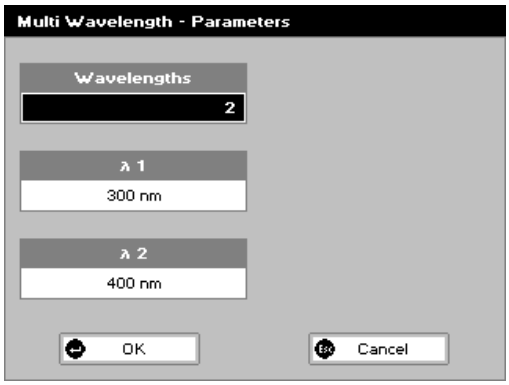
Options (select using key pad numbers)

1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. Displays calibration graph, cursors give values for last measured sample.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the left and right arrows to select a folder to store in (Favorites/Methods 1-9), press the down arrow and enter name.
9. Auto-print – toggles auto-print on/off.

Exit options by pressing Esc, or wait.

6: Multiple Wavelength

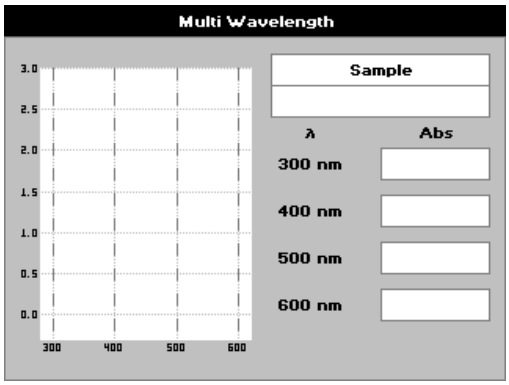
This makes up to 5 absorbance measurements on the same sample. The procedure is as follows:



Step 1
Select the number of wavelengths. Range 2-5.
Press the down arrow.
Step 2
Enter the first wavelength using either the number keys or the left and right arrows.
Press the down arrow.
Enter the second wavelength as above and repeat for the number of wavelengths selected (up to 5).
Step 3

Press OK  to enter the results screen
OR

Press Cancel  to return to the Applications Folder.

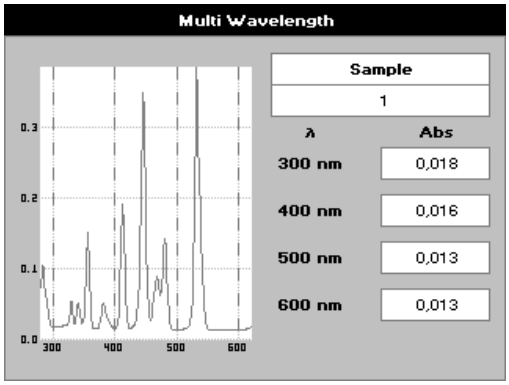


Step 4

Insert the reference. Press  . This will be used for all subsequent samples until changed.
Step 5

Insert sample and press  .

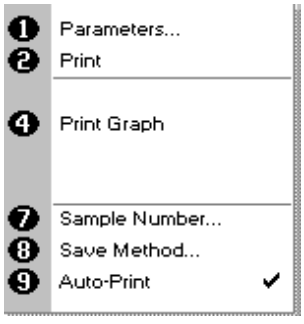
Repeat step 5 for all samples.



Results
A scan plot covering the range of wavelengths selected (with cursors at the relevant wavelengths) and a table of values is displayed.

Press  to return to the Applications Folder.

Press  to display available Options which are described below.



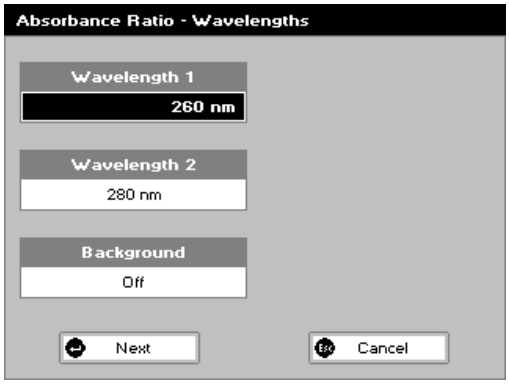
Options (select using key pad numbers)

1. Return to parameters screen (step 1 above).
2. Print result via selected method.
4. Print graph using selected method. Grayed out if no data are available.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the left and right arrows to select a folder to store in (Favorites/Methods 1-9), press the down arrow and enter name.
9. Auto-print – toggles auto-print on/off.

Exit options by pressing  , or wait.

7: Absorbance Ratio

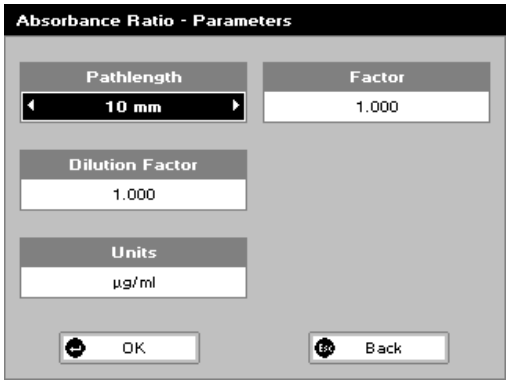
This makes simple absorbance ratio measurements on samples, measuring the amount of light that has passed through a sample relative to a blank (this can be air) at two wavelengths. The procedure is as follows:



Step 1
Enter the first wavelength by using the keypad numbers or the left and right arrows.
Press the down arrow.
Step 2
Enter the second wavelength as above.
Press the down arrow.
Step 3
Select whether a background correction is applied to both wavelengths 1 and 2 using the left and right arrows.
Step 4 (If background correction is On)
Enter the third wavelength, from which the background correction will be obtained).
Step 5

Press Next ↵ to enter the Parameters screen
OR

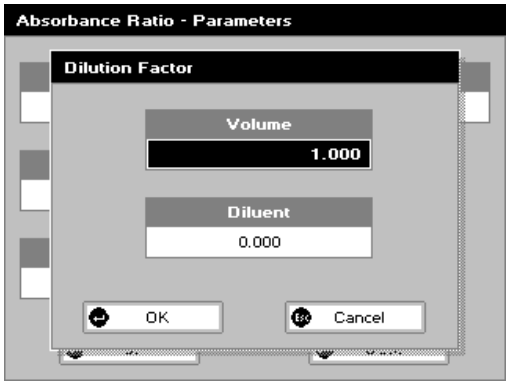
Press Cancel Esc to return to the Applications Folder.



Absorbance Ratio – Parameters Screen
Step 6
Select the pathlength (5 or 10 mm) using the left and right arrows.
Press the down arrow.
Step 7 (Dilution Factor known)
Enter a dilution factor by using the keypad numbers within the range 1.00 – 9999.
OR
Step 7 (Calculate Dilution Factor)

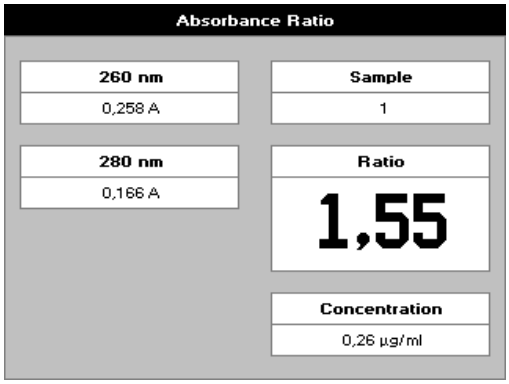
Press the options key: ☰
Enter the volume of the sample (range 0.01 – 9999), using the keypad numbers.
Press the down arrow.
Enter the volume of diluent (range 0.01-9999) by using the keypad numbers.

Press OK ↵ to calculate the dilution factor and return to the Parameters screen (or press Cancel, Esc to cancel selections).



Step 8
Select units of measurement, using left and right arrows. Options are: µg/ml, ng/µl, µg/µl.
Press the down arrow.
Step 9
Enter the factor using the keypad numbers (Range 0.001 to 9999).

Press OK ↵ to enter the results screen or Cancel Esc to return to the Applications Folder.



Results Screen
Step 10

Insert the reference. Press 

. This will be used for all subsequent samples until changed.

Step 11

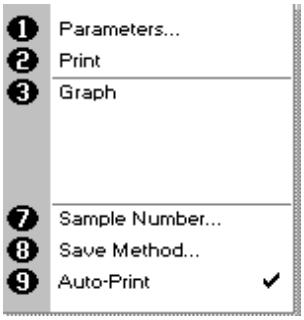
Insert sample and press  .

Repeat step 11 for all samples.
The absorbance at selected wavelengths is measured and the ratio between wavelengths 1 and 2 is calculated (both corrected by the background wavelength value if this was selected).

Press 

to return to the Applications Folder.

Press  to display available Options which are described below.



Options (select using key pad numbers)

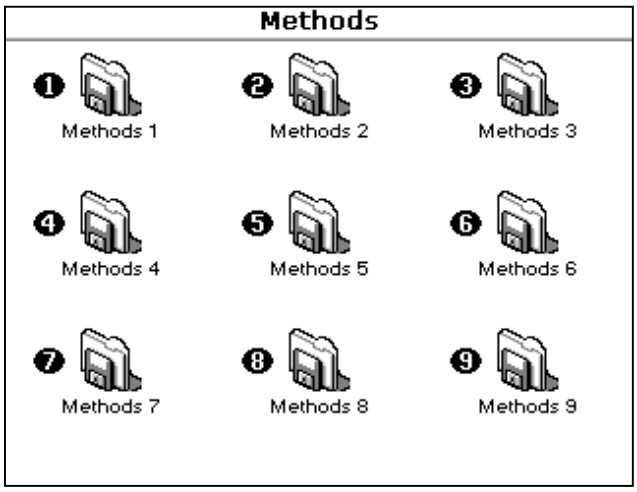
1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. Graph shows a wavescan plot across the selected wavelengths in place of the individual wavelength.
7. Sample number – add a prefix to the sample number and reset the incrementing number to the desired value.
8. Save method – use the left and right arrows to select a folder to store in (Favorites/Methods 1-9), press the down arrow and enter name.
9. Auto-print – toggles auto-print on/off.

Exit options by pressing , or wait.

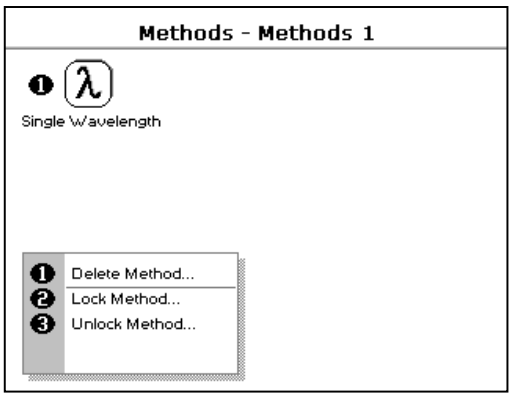
FAVORITES AND METHODS FOLDERS

These folders are the storage locations for any user modified Applications (Methods) that are saved in the Options menu. Both are accessible from the home folders page.

Favorites:
This folder enables the user to quickly select any frequently used Methods. Up to 9 Methods may be stored in the folder.



Methods:
These are further storage folders enclosed in the top level Methods folder. Up to 9 Methods may be stored in each folder.
Operation is identical to the Favorites Folder.
Saved methods can be locked, unlocked and deleted using the Options menu. Select the method by pressing the relevant key pad number and then press the key.

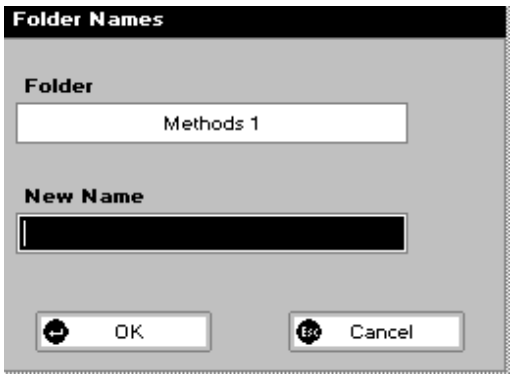


Delete Method
Press 1 to select delete method.
Select the method to be deleted using the left and right arrows.
Press ↵ to delete the method OR cancel Esc to return to Favorites/Methods folder.

Lock Method
Press 2 to select lock method.
Select the method to be locked using the left and right arrows.
Press the down arrow.
Select a pass code using the keypad numbers or left and right arrows.
Press ↵ to lock the method OR cancel Esc to return to the Favorites/Methods folder.

Unlock Method
Press 3 to select unlock method.
Select the method to be unlocked using the left and right arrows.
Press the down arrow.
Enter the pass code using the keypad numbers or left and right arrows.
Press ↵ to unlock the method OR cancel Esc to return to the Favorites/Methods folder.

Folder Names



Select the folder you wish to rename using the left and right arrows.
Press the down arrow.
Input the new name for the folder.

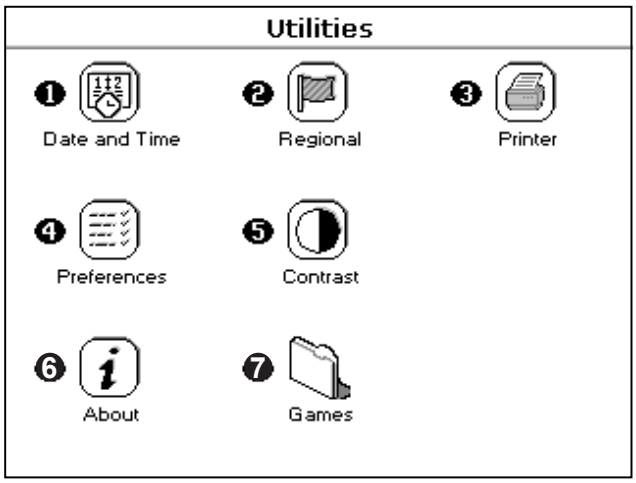
Press OK ↵ to store the settings and return to the Utilities folder
OR

Press Cancel Esc to return to the Utilities folder without storing the settings.

This folder allows you to rename the method or favorite folders

UTILITIES FOLDER

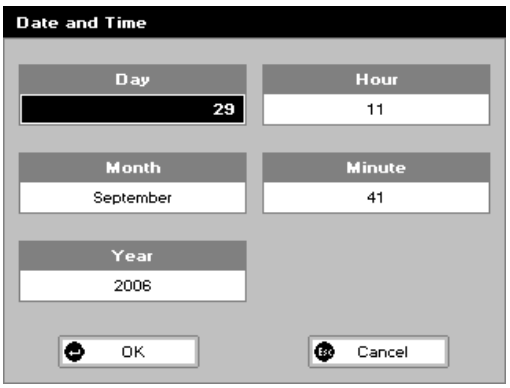
Press 5 to enter the utilities folder.



Summary Function	Keypad number	Description
1 Date and Time	1	Set correct time and date
2 Regional	2	Select preferred language and number format
3 Printer	3	Printer/output options
4 Preferences	4	Select screen layout (themes) and history
5 Contrast	5	Adjust screen contrast & brightness
6 About	6	Serial number and software version
7 Games	7	Spectro Blocks/Sudoku

1: Date and Time

The procedure is as follows:



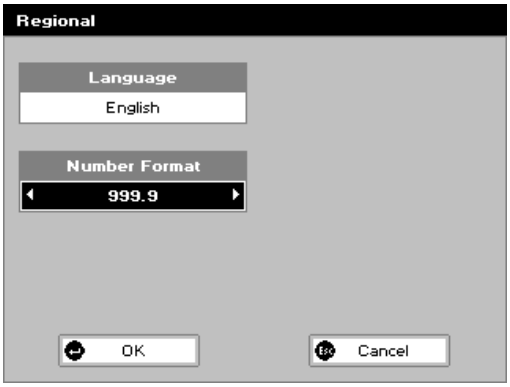
Enter the day using the keypad numbers or left and right arrows.
Press the down arrow.
Enter the month as above.
Press the down arrow.
Enter the year.
Press the down arrow.
Enter the hour.
Press the down arrow
Enter the minute. Seconds are zeroed when OK is pressed.

Press OK ↵ to store the settings and return to the Utilities folder
OR

Press Cancel Esc to return to the Utilities folder without storing the time.

2: Regional

Sets Language and Number Format
The procedure is as follows:



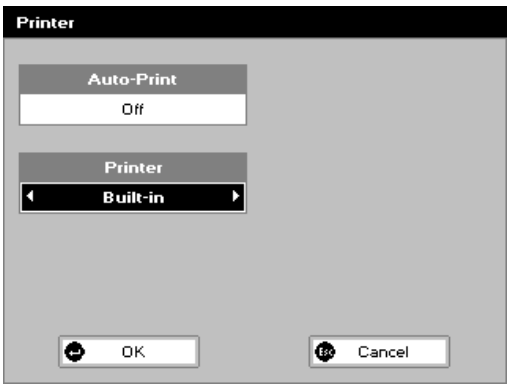
Select a language. Options are English, French, Spanish, Italian or Japanese.
Press the down arrow.
Set the decimal point style. Options are "," or ".".

Press OK ↵ to store the settings and return to the Utilities folder
OR

Press Cancel Esc to return to the Utilities folder without storing the settings.

3: Printer

Sets up printing options
The procedure is as follows:



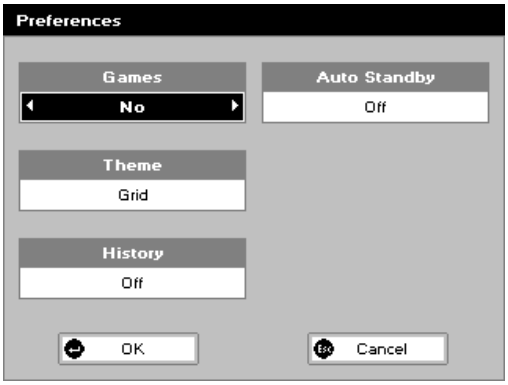
Select whether auto-print is on or off using the left and right arrows. When auto-print is on, the results are automatically printed after a measurement is taken. When it is off, printing has to be initiated manually. This can also be set using the Options key (☰) in each application or method. The default is OFF.
Press the down arrow.
Select how the data are sent. Options are Built in (internal printer), or to a computer via USB port or Bluetooth.

Press OK ↵ to store the settings and return to the Utilities folder
OR

Press Cancel Esc to return to the Utilities folder without storing the settings.

4: Preferences

Sets user preferences
The procedure is as follows:

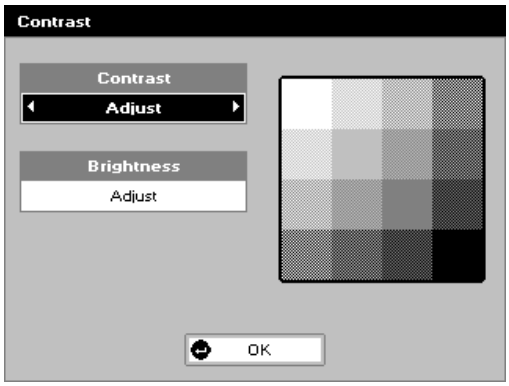


Select games function. This determines whether the games folder is displayed or not. Options are yes or no.
Press the down arrow.
Define the screen layout of folders. Options are either a grid format (default) or a list.
Press the down arrow.
Select whether to use previously entered parameters when the instrument is switched on or to use defaults.
Press the down arrow.
Select whether to use a standby mode after defined periods. Options are 1 hour, 2 hours, at night or off.

Press OK ↵ to store the settings and return to the Utilities folder
OR

Press Cancel Esc to return to the Utilities folder without storing the settings.

5: Contrast



Adjust the contrast using the left and right arrows.
Press the down arrow.
Adjust the brightness using the left and right arrows.
Press the down arrow.

Press OK ↵ to store the settings and return to the Utilities folder

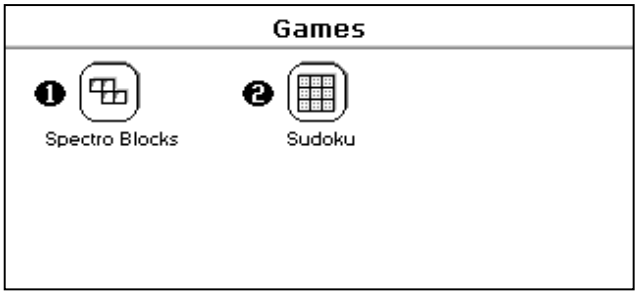
Ambient temperature can affect the display. This function can optimise the display for local conditions
The procedure is as follows:

6: About

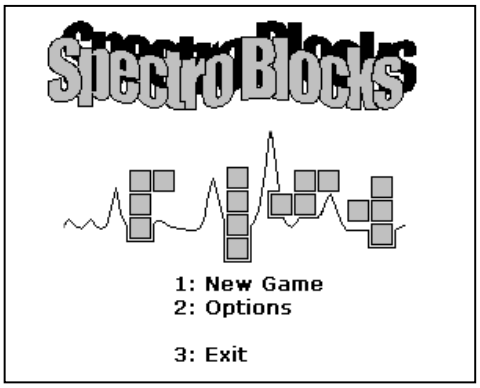
Displays the instrument serial number and software version.

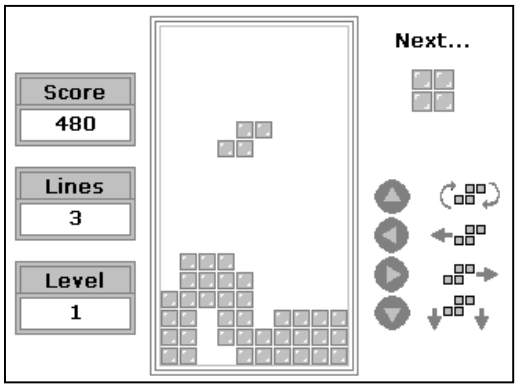
Press OK ↵ to close the window and return to the Utilities folder

7: Games



1: Spectroblocks





Classic block dropping game. Follow the instructions!

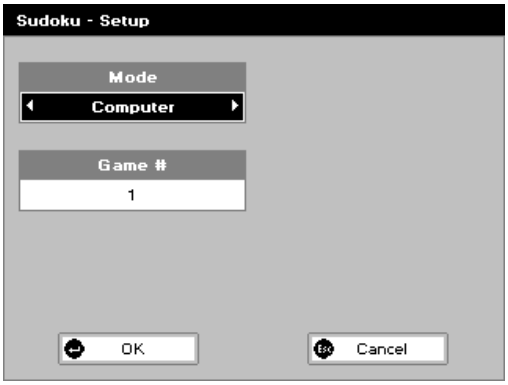
Press Cancel Esc to return to the Utilities folder without storing the settings.

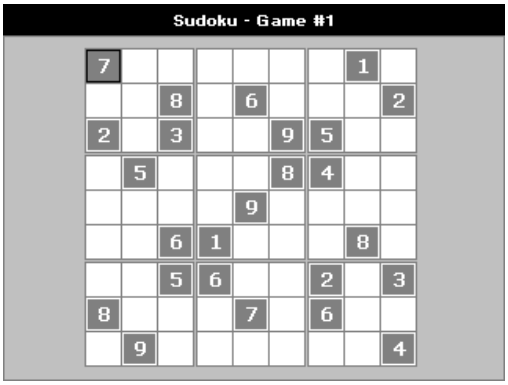
2: Su Doku

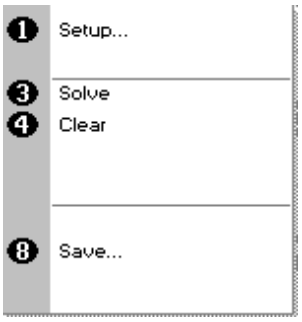
Can be set up as Computer mode (50 preset games) or User mode (enter your own pattern)

Use the cursors to select the square and the key pad to enter a number. Invalid numbers cannot be entered. Cells can be locked (or unlocked) by using the decimal point. Unlocked cells can be cleared using the C key (see also option key below)

The user mode starts with a blank grid.







Options
Press to display the options menu

1. Return to the set-up screen.
3. The instrument solves the game for you!
4. Clear all entries.
8. Save the game. Use the left and right arrows to select a folder to store the game in (Favorites, Methods 1-9), press the down arrow and enter name.

Press Cancel Esc to return to the Utilities folder.

ACCESSORIES INSTALLATION

Printer installation

Figure. 1

Step 1.

Remove the power cable and turn the instrument over onto a soft surface, taking care not to damage the sampling head.

Release the outermost screws using the Allen key provided.

Figure. 2

Step 2.
Turn the instrument back over and remove the accessory covers.

Figure. 3

Step 3.

Attach the printer cable.

Figure. 4

Step 4.

Lower the printer onto the locating bosses.

Figure. 5

Step 5.

Replace the top cover plate, invert the instrument and replace the cap head screws.

Figure. 6

Step 6.

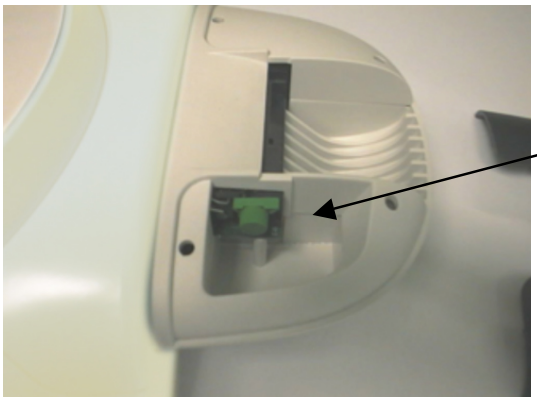
Switch the instrument on and go to utilities/instrument/preferences and select the Built-in printer.

Loading / Changing the printer paper

Step 1.

Lift off the paper cover.

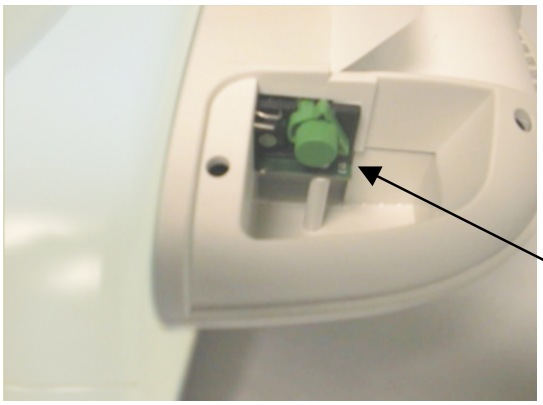
Figure. 7



Step 2

Lock the platen and turn the knob to feed the paper

Figure. 8



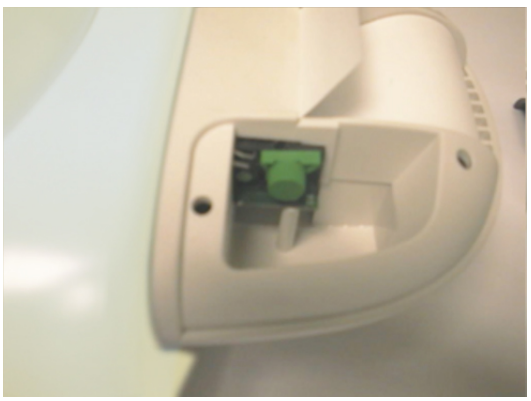
Step 3

Feed in the paper.

Step 4

Sometimes it helps if the platen lock is released.

Figure. 9



Step 5

Paper gripped.

Step 6

Replace cover

Figure. 10

Bluetooth Accessory Installation

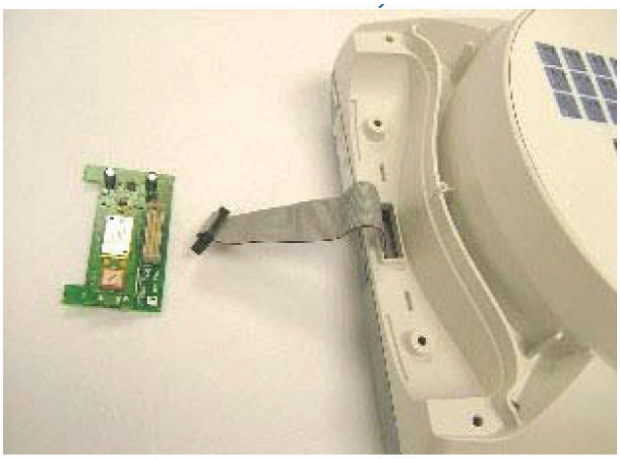
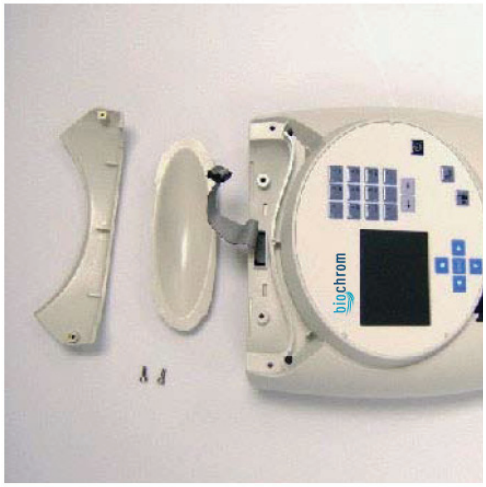


Figure: 18

Step 1.
Remove the power cable and turn the instrument over onto a soft surface, taking care not to damage the sampling head. Release the outer-most screws using the Allen key provided.



Step 2.
Turn the instrument back over and lift the accessory cover vertically upwards to remove. Remove the tie-wrap from the cable.

Figure: 19

Step 3.
Plug the accessory cable into the Bluetooth module.

Step 4.
Note the slots in the base of the case. The two lugs on the Bluetooth module plug into these.

Figure: 20

Step 5.
Load the rear cover into the two slots provided, note the large flage faces upwards.

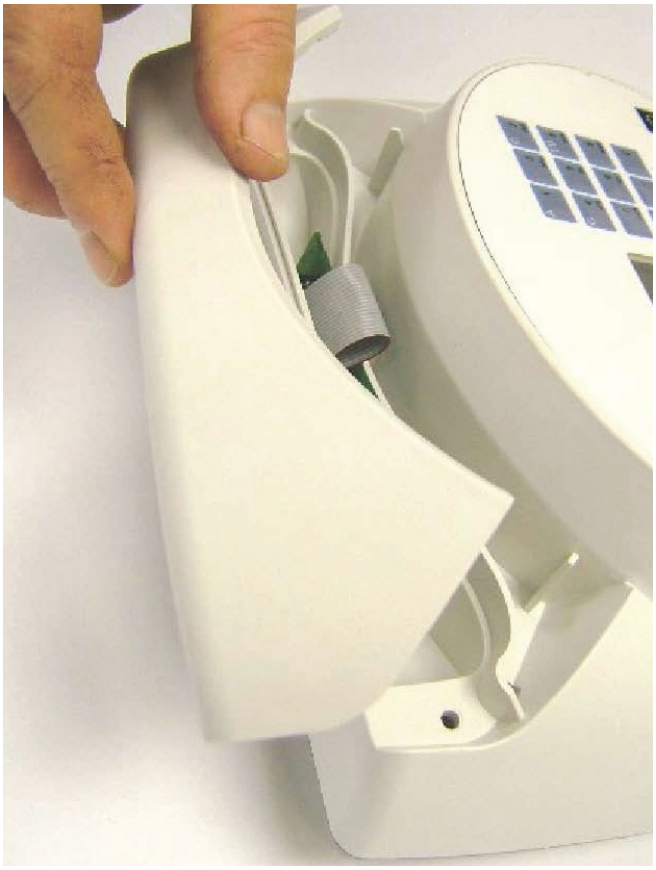


Figure: 21

Step 6.
Re-fit the top plate.

Step 7.
Invert the instrument carefully and replace the cap head screws.

Step 8.
Switch the instrument on and go to the preferences page under utilities/ instrument and select the Bluetooth option.

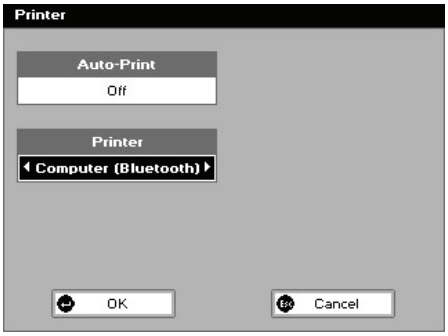


Figure: 22

Step 9.
Attach the license label as shown.

PRINT VIA COMPUTER

- PVC (Print Via Computer) is a small application running under Windows 2000™ to enable a GeneQuant™ 1300 to transfer data into a PC environment. From there the user has a selection of choices, the data can be both printed or saved (in a variety of formats). PVC is capable of supporting several instruments simultaneously, limited only by hardware and the speed of the host system.
- PVC can operate via USB and Bluetooth simultaneously
- PVC can store data either to a common directory or be configured to save to independent directories by both file format and connection.
- PVC can save data in graphics format, text format or as an Excel™ file

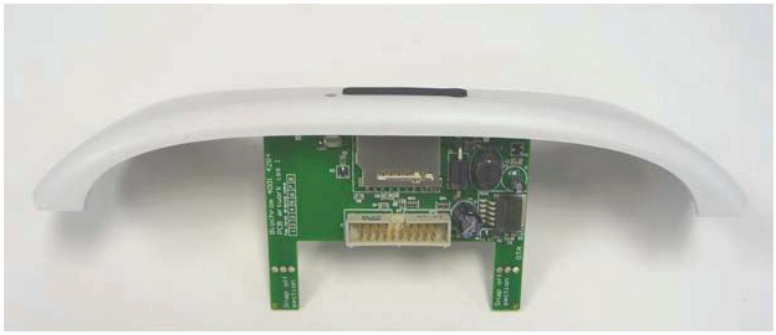
Installation

See the manual included on the PVC CDROM for installation and operating instructions.

FITTING SD MEMORY CARD ACCESSORY

Step 1.
Remove the power cable from the instrument. Turn the instrument over and place onto a soft surface, for example a folded up towel.
Release the outermost cap head screws using the Allen key provided.

Step 2.
Turn the instrument back over and lift the accessory cover vertically upwards to remove. Remove the tie-wrap from the cable.



Step 3.
Plug the accessory cable into the SD memory card PCB module.

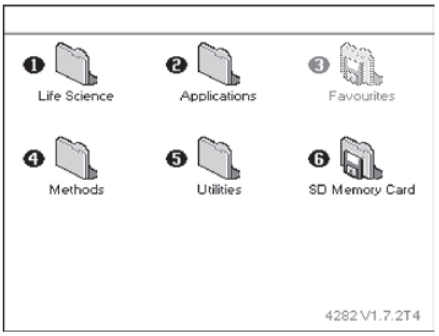
Step 4.
Note the slots in the base of the case. The two lugs on the SD memory card PCB module plug into these. Take care not to damage or snap off the lugs. Lower the PCB card module into place ensuring the lugs fit into these slots.

Step 5.
Fit the oval back cover ensuring it is fitted the correct way up – the continuous lip goes at the top.

Step 6.
Invert the instrument holding the SD Card accessory in position and replace the 2 cap head screws using the Allen key provided.

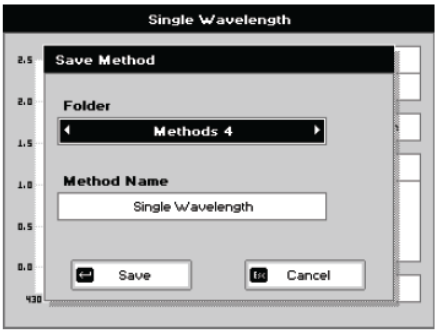
The accessory is now ready for use.

OPERATION
SD memory cards are inserted into the accessory with the contacts facing towards the user and the cut out corner on the right hand side (i.e. downwards). When a compatible SD card is inserted into the accessory, the red light flashes momentarily and the SD memory card icon appears on the instrument home page;



Saving methods to SD memory card
When an SD memory card is inserted into the accessory it is possible to save methods directly to the card.

Methods are stored on the card in a directory named \Instrument Type\Methods (instrument type will be NanoVue Plus); this directory structure is evident when the SD card is inserted into a PC.



To save a method to the SD memory card, the instructions for the relevant application from the instrument user manual must be followed. Typically:
• Press the Options button (or relevant numerical short cut)
• Press Save Method
• Use the right and left arrows to select the folder on the SD memory card to which you wish to save the method
• Change the filename if required
• Press Save.

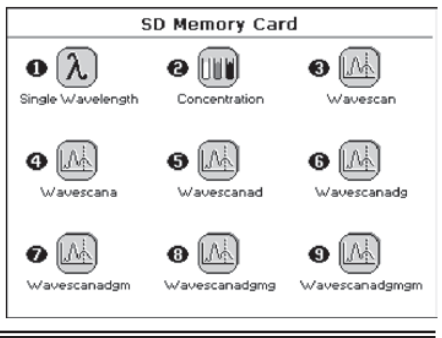
NOTE: A maximum of 9 methods can be stored in the SD memory card folder and in the \Instrument Type\Methods directory.

These stored methods can also be opened on different instruments and then stored into other method folders if required.

When a method is being stored the LED next to the card will light up, the card MUST NOT be removed whilst the light is on otherwise the stored method will be corrupted.

Loading methods from SD memory card
Selecting the SD memory card by pressing the relevant number on the home page shows the methods stored on the card.

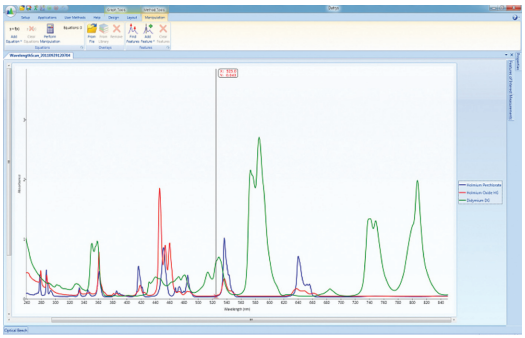
The required method can be loaded by pressing the relevant number on the keyboard and run in the same way as methods stored in any of the method folders on the instrument.



ACCESSORIES

USB cable	source locally
Built-in printer accessory	80-3003-84
Bluetooth accessor	80-3003-96

DATRYS SYSTEM CONTROL SOFTWARE



Datrys software wavelength scan

For many users, having an external PC to control their spectrophotometer and manipulate data, gives them the ultimate in flexibility and control. Whether looking for small differences in multiple spectral overlays, carrying out post-run manipulations on large numbers of samples, Datrys software has the flexibility to work in the way you want.

Datrys software is supplied in different modules to meet the application requirements of different customer groups and offers a simple upgrade path should your requirements change.

Choose:
- Datrys Lite for Quick Read and fast scanning only
- Datrys for all routine measurements
- Datrys Life Science for nucleic acids, proteins and c ell density measurements
- Datrys CFR supports 21 CFR part 11 compatibility

Offering the familiar look and feel of Microsoft™ Office 2007, Datrys software is compatible with Windows™ XP, Windows Vista™, Windows 7, and Windows 8, 8.1 operating systems. Data export options include Microsoft Word™ and Excel plus Adobe™ PDF formats.

Modules	Datrys Lite	Datrys	Datrys Life Science	Datrys CFR
Quick Read and QuickScan	●	●	●	●
Wavelength Scanning, Kinetics, Quantitative Calibration Curves		●	●	●
Validation	●	●	●	●
Method Developer		●	●	●
Life Science Methods			●	●
Accessory Control		●	●	●

Lamp Replacement

The xenon lamp should not need replacement until after several years of use. In the unlikely event that it does need replacing, this should be undertaken by a service engineer from your supplier.

Cleaning and general care of the instrument

External cleaning
Switch off the instrument and disconnect the power cord.
Use a soft damp cloth.
Clean all external surfaces.
A mild liquid detergent may be used to remove stubborn marks.

Changing cell holder or removal for cleaning
This can be removed by undoing the appropriate screws on the bottom of the instrument.

SPECIFICATION AND WARRANTY

Wavelength range	190 - 1100 nm
Monochromator	Flat grating
Wavelength calibration	Automatic upon switch on
Spectral bandwidth	5 nm
Wavelength accuracy	±2 nm
Wavelength reproducibility	±1 nm
Light sources	Pulsed xenon lamp
Detector	1024 element CCD array
Photometric range	- 0.300 to 2.500A, 0 to 199%T
Photometric linearity	±0.005 Abs or 1% of the reading, whichever is the greater @ 546 nm
Photometric reproducibility	±0.003 Abs (0 to 0.5 Abs), ±0.007 Abs (0.5-1.0 Abs)
Stray light	<0.5% at 220 nm and 340 nm using $NaNO_2$
Zero stability	±0.01 Abs/hour after 20 min warm up @ 340 nm
Noise	0.005 pk to pk 0.002 pms
Digital output	USB port standard, Bluetooth option
Dimensions	260 x 390 x 100 mm
Weight	<3.5 kg
Power input	18Vdc from a 100-240 V, 50/60 Hz, Max 30 VA mains power pack

Specifications are measured after the instrument has warmed up at a constant ambient temperature and are typical of a production unit. As part of our policy of continuous development, we reserve the right to alter specifications without notice.

Warranty

- Biochrom guarantees that the product supplied has been thoroughly tested to ensure that it meets its published specification. The warranty included in the conditions of supply is valid for 12 months only if the product has been used according to the instructions supplied. Biochrom can accept no liability for loss or damage, however caused, arising from the faulty or incorrect use of this product.