



BIOWAVE DNA USER MANUAL



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Declaration of Conformity

This is to certify that the Biowave DNA Part number 80-3004-70 manufactured by Biochrom Ltd. conform to the requirements of the following Directives: - 73/23/EEC & 89/336/EEC
Standards to which conformity is declared

EN 61010-1: 2001 Safety requirements for electrical equipment for measurement, control and laboratory use.


EN 61326-2.3: 1998 Electromagnetic compatibility - generic emission standard Electrical equipment for measurement, control and laboratory use.

EN 61000-4-6: 1992 Electromagnetic compatibility - generic immunity standard part 1. Residential, commercial and light industry.

BS EN 591:2001 Instruction for use for in vitro diagnostic instruments for professional use.

BS EN 13612:2002 Performance evaluation of in vitro diagnostic medical devices

2002/96/EC This appliance is marked according to the European directive 2002/96/EC on Waste Electrical and Electronic Equipment (WEEE). By ensuring this product is disposed of correctly, you will help prevent potential negative consequences for the environment and human health, which could otherwise be caused by inappropriate waste handling of this product.

The symbol  on the product, or on the documents accompanying the product, indicates that this appliance may not be treated as household waste. Instead it shall be handed over to the applicable collection point for the recycling of electrical and electronic equipment. Disposal must be carried out in accordance with local environmental regulations for waste disposal.

Signed:

Dated: 19th December 2005



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ESSENTIAL SAFETY NOTES

There are a number of warning labels and symbols on your instrument. These are there to inform you where potential danger exists or particular caution is required. Before commencing installation, please take time to familiarise yourself with these symbols and their meaning.



Caution (refer to accompanying documents).
Background colour yellow, symbol and outline black.

Unpacking, Positioning and Installation

- Check the contents of the pack against the packing list. If any shortages are discovered, inform your supplier immediately.
- 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 35°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).
 - 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 (< 4.5 kg) so that air can circulate freely around the instrument.
- This equipment must be connected to the power supply with the power cord supplied. It 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. 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.

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

INTRODUCTION

Your spectrophotometer

Your spectrophotometer is a simple-to-use UV/Visible instrument with a CCD array detector (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 "Biowave DNA" offering the choice of

DNA	Concentration and purity check for DNA samples
RNA	Concentration and purity check for RNA samples
Oligo	Concentration and purity check for Oligo samples
Absorbance/Concentration	Absorbance at a user defined wavelength or a colorimetric assay at a single wavelength based on a simple factor either entered or calculated from a simple standard
OD600	Bacterial cell culture measurement
Methods	Allows 9 frequently used configured methods to be stored for easy re-use
Protein	This folder contains various protein assay methods
Utilities	Instrument set up (date, time, language, etc)

The instrument can be used for an optional program PVC (Print via Computer) available separately. When used with a USB cable to connect to a PC onto which the software has been installed, it enables the user to "print through" the PC directly to any printer that is connected to that PC. 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 or in native PVC format for later access

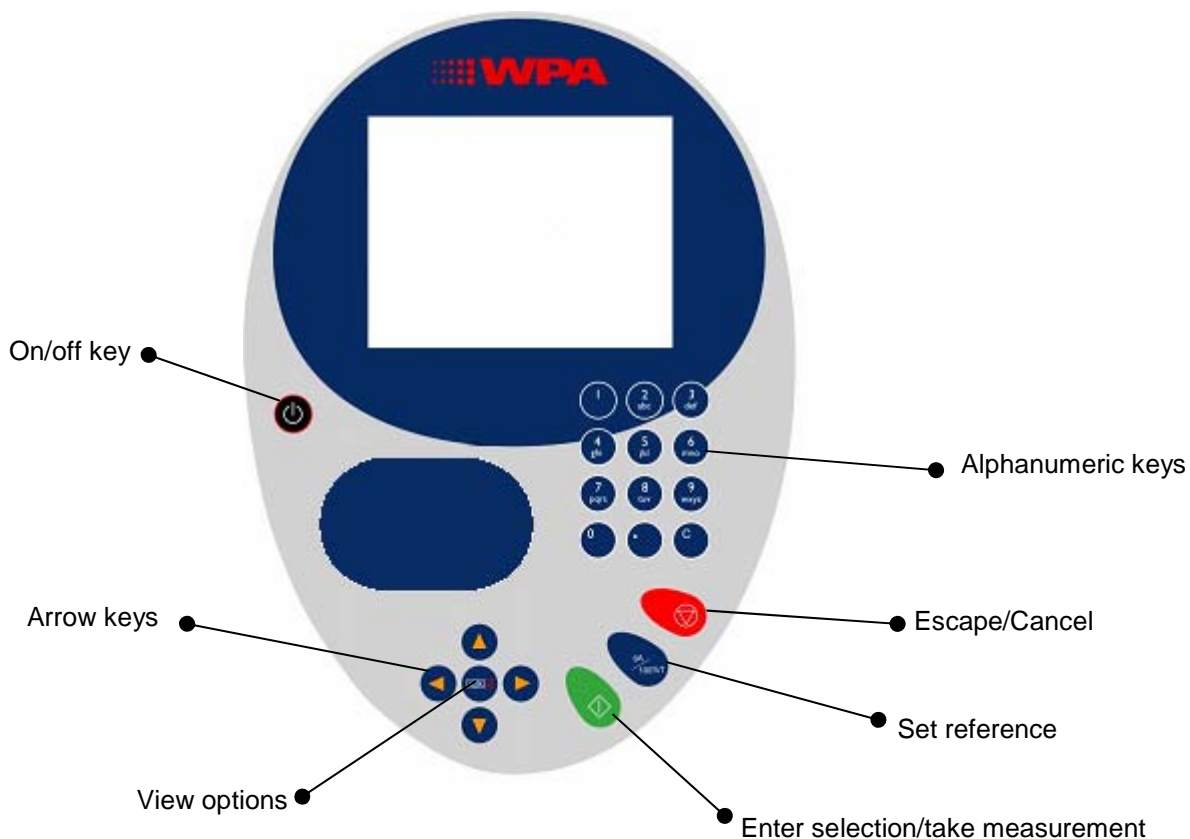
An internal printer is also available for the instrument; this may either be supplied pre-installed or is available as an optional accessory if the need for its use arises after installation of the product.

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 an 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

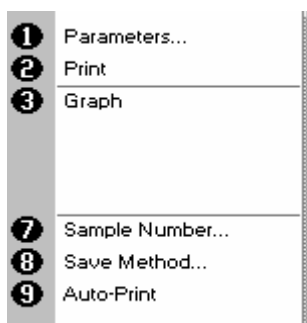
The back-lit liquid crystal display is very easy to navigate around using the alphanumeric entry and navigation arrow keys on the hard wearing, spill proof membrane keypad.



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/Cancel: 🛑	Escape from a selection and return to the previous folder. Stop making measurements.
Set Reference: 0A/100%T	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, do a reference scan.
Enter: ⬇️	Enter, or confirm, a selection. Take a measurement.

Options

Within each application the user is able 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).



Options (select using key pad numbers)

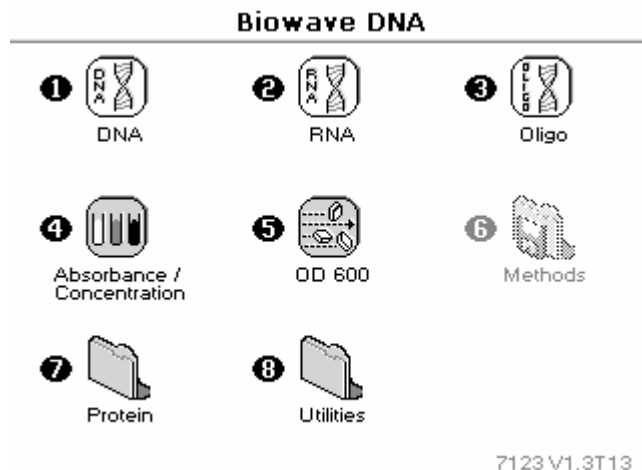
1. View parameters for the experiments
2. Print the results
3. Display a graph of the results
- 4,5,6. Specific to an application
7. Define the sample number you wish to start from
8. Save the parameters as a method in the Methods folder with a defined method name.
9. Toggle auto-print on/off. Default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

Exit options by pressing , 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 either functions or folders of files which are displayed on the home page when the instrument is switched on. Each function or folder is numbered and opened by using the associated number key on the keypad. Functions take you to a parameter screen where you can set the required parameters before using the function to take measurements and folders take you to another screen of further options.



Summary:

Function	Key pad number	Description
 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
 Absorbance / Concentration	4	Absorbance at a user defined wavelength or a colorimetric assay at a single wavelength based on a simple factor either entered or calculated from a simple standard
 OD 600	5	Measurement of cell cultures
 Methods	6	Allows 9 frequently used methods to be stored for easy re-use
 Protein	7	This folder contains various protein assay methods
 Utilities	8	Instrument set up (date, time, language, etc)

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.

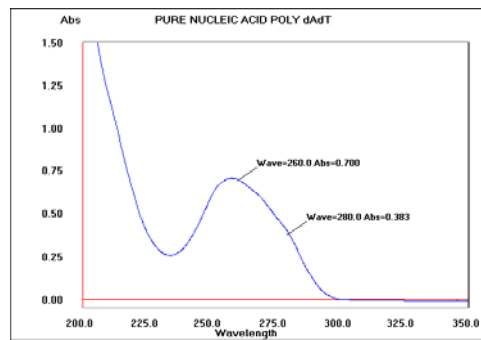
Nucleic Acid Purity Checks

- 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 ≥ 1.8 and ≥ 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:

DNA - Parameters	
Pathlength	Units
10 mm	µg/ml
Dilution Factor	Factor
1,00	50,0
Background	
Off	
OK	Cancel

DNA - Parameters	
Dilution Factor	
Volume	1.000
Diluent	0.000
OK	Cancel

DNA		
A230	0,089 A	Sample
A260	0,258 A	1
A280	0,167 A	Concentration
A260/A280		12,9
1,54		Units
A260/A230		µg/ml
2,90		

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 $\boxtimes\boxtimes\boxtimes$ 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 \blacklozenge to calculate the dilution factor and return to the Parameters screen.

OR Press \blacktriangledown 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 \blacklozenge to enter the Results screen.

OR

Cancel \blacktriangledown to return to the initial display screen.

Results Screen

Step 8

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

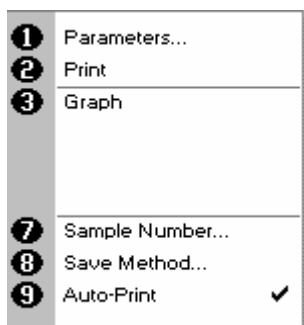
Step 9

Insert sample and press \blacklozenge . 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 \blacktriangledown to return to the initial display screen.

Press $\boxtimes\boxtimes\boxtimes$ 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 wvescan 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.

2: RNA

The procedure is as follows:

RNA - Parameters	
Pathlength	Units
10 mm	µg/ml
Dilution Factor	Factor
1.00	40.0
Background	
Off	
OK	Cancel

Dilution Factor	
Volume	1.000
Diluent	0.000
OK	Cancel

RNA		
A230	0.089 A	Sample
A260	0.258 A	
A280	0.166 A	
A260/A280		Concentration
1.55		
A260/A230		Units
2.90		
		10.3
		µg/ml

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 $\square\square\square$ 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 \blacklozenge to calculate the dilution factor and return to the Parameters screen.

OR Press C 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: $\mu\text{g/ml}$, $\text{ng}/\mu\text{l}$, $\mu\text{g}/\mu\text{l}$.

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 \blacklozenge to enter the Results screen

OR

Cancel C to return to the initial display screen.

Results Screen

Step 8

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

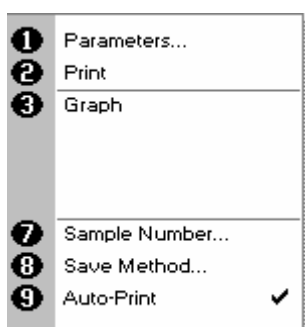
Step 9

Insert sample and press \blacklozenge . 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 C to return to the initial display screen.

Press $\square\square\square$ to display available Options which are described below.



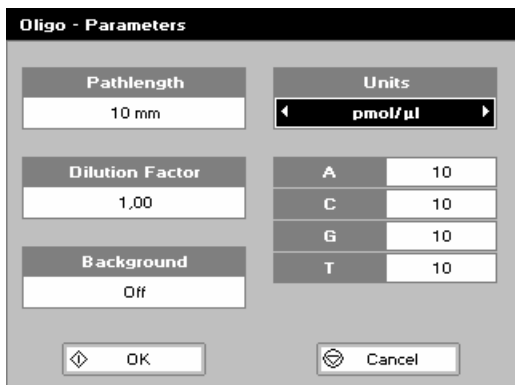
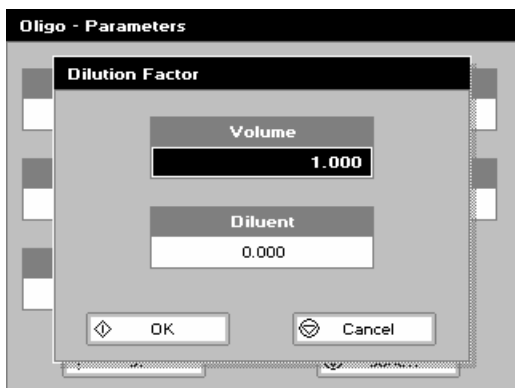
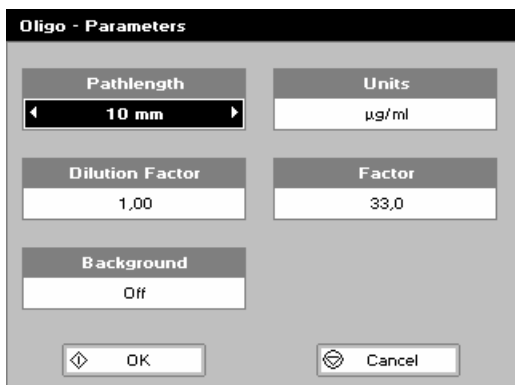
Options (select using key pad numbers)

1. Return to parameters screen (step 1 above).
2. Print result via selected method.
3. Toggle graph on/off. The graph shows a wvescan 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.
9. Save method – use the alpha-numeric keys to enter a name for the method and press Save .
10. Auto-print – toggles auto-print on/off.

Exit options by pressing , 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 $\square\square\square$ 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 \blacklozenge to calculate the dilution factor and return to the Parameters screen.

OR Press \blacktriangledown 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: $\mu\text{g/ml}$, $\text{ng}/\mu\text{l}$, $\mu\text{g}/\mu\text{l}$ and $\text{pmol}/\mu\text{l}$. If $\text{pmol}/\mu\text{l}$ 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 $\text{pmol}/\mu\text{l}$)

Enter the factor using the keypad numbers. Default value is 33, range is 0.01 to 9999.

OR

Step 6 (units $\text{pmol}/\mu\text{l}$)

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 \blacklozenge to enter the Results screen

OR

Cancel \blacktriangledown to return to the initial display screen.

Oligo		
A230	0,089 A	Sample 1
A260	0,258 A	
A280	0,167 A	
A260/A280		Concentration 0,60
1,54		
A260/A230		Units pmol/μl
2,90		

Results Screen

Step 8

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

Step 9

Insert sample and press \diamond . 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 initial display screen

Press $\boxtimes\boxtimes\boxtimes$ to display available Options which are described below.

1	Parameters...
2	Print
3	Graph
7	Sample Number...
8	Save Method...
9	Auto-Print <input checked="" type="checkbox"/>

Options (select using key pad numbers)

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

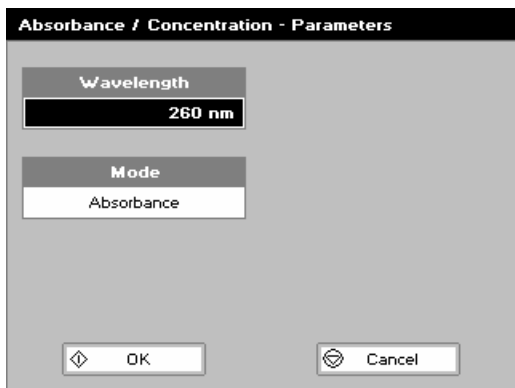
Exit options by pressing ∇ , or wait.

MEASURING ABSORBANCE/CONCENTRATION

This application has two functions:

1. makes simple absorbance (A) measurements on samples, measuring the amount of light that has passed through a sample relative to a reference (this can be air).
2. 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

Press 4 to select Absorbance/concentration mode.

Step 2

Set wavelength by using keypad numbers or left and right arrows.

Press the down arrow key.

Step 3

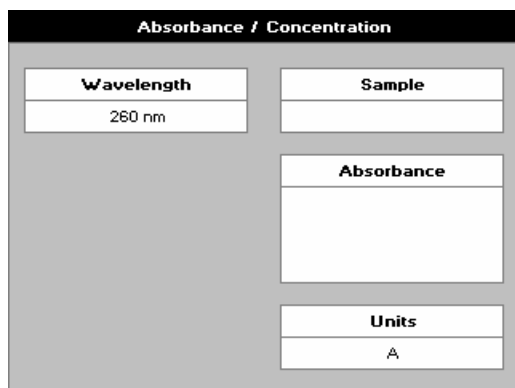
Select the mode: Absorbance, Factor or Standard, using the left and right arrows.

Step 4 (Absorbance selected)

To enter the results screen with the selected parameters press OK

OR

Cancel the selections and return to the initial display screen by pressing Cancel



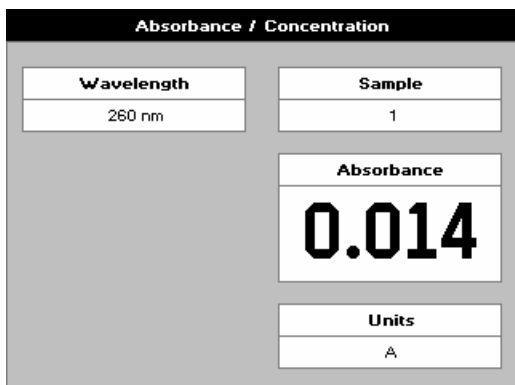
Step 5 (Absorbance selected)

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

Step 6 (Absorbance selected)

Insert sample and press .

Repeat step 6 for all samples.

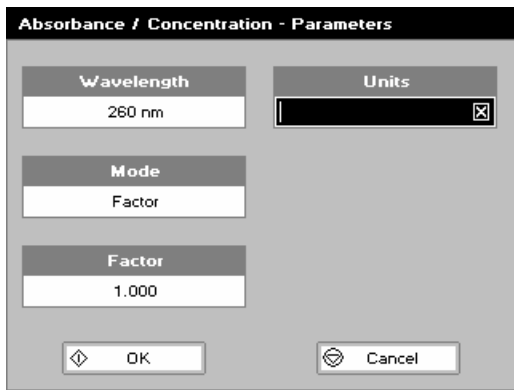


Results (Absorbance selected)

The result at the selected wavelength is displayed on screen.

Press Cancel to return to the initial display screen.

Press to display available Options which are described below.



Step 4 (Factor selected)

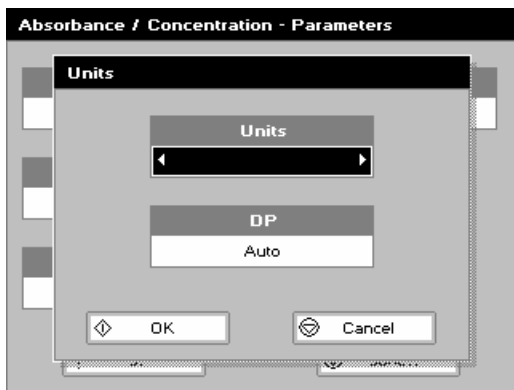
Enter the Factor using the keypad numbers. Range 0.001 to 9999. Use the C button to delete the last digit entered.

Press the down arrow key.

Step 4 (Standard selected)

Enter the concentration using keypad numbers. Range 0.01-9999. Use the C button to delete the last digit entered.

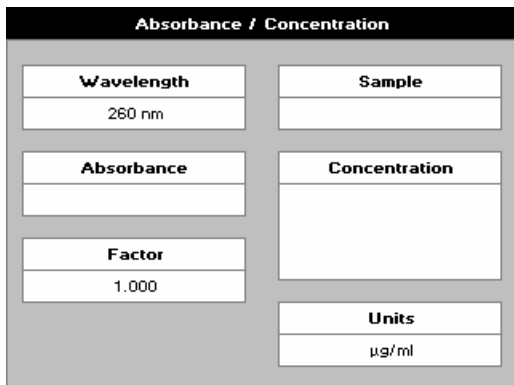
Press the down arrow key.



Step 5 (Factor/Standard selected)

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 [X][X][X] 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 [D] to store the chosen parameters or Cancel [V].



Step 6 (Factor/Standard selected)

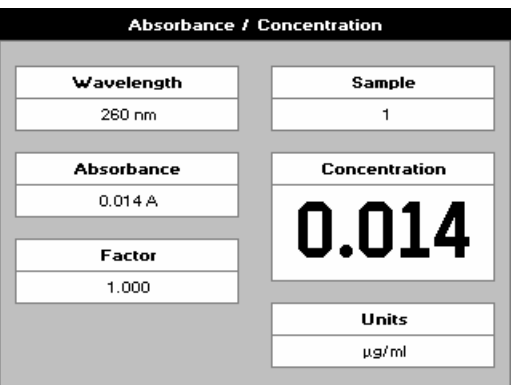
To enter the results screen with the selected parameters press OK [D]

OR

Cancel the selections and return to the initial display screen by pressing Cancel [V].

Step 7 (Factor selected)

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



Step 8 (Factor selected)

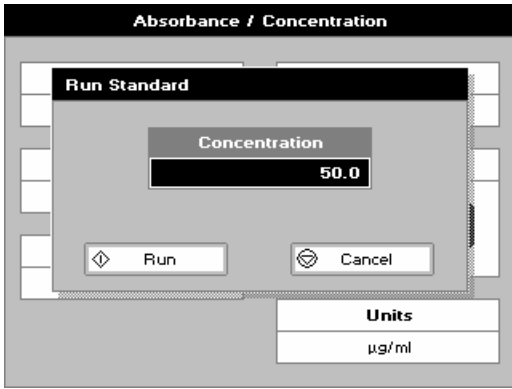
Insert sample and press [D].

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

Repeat step 7 for all samples.

Press [V] to return to the initial display screen

Press [X][X][X] to display available Options which are described below.



Step 7 (Standard selected)

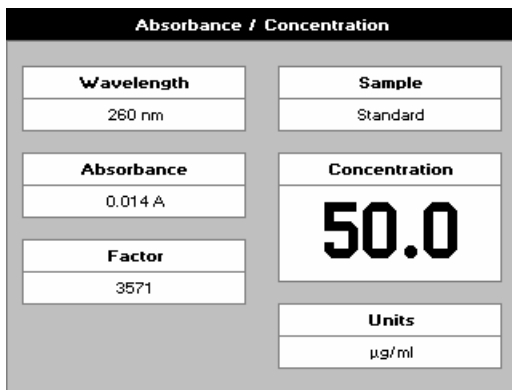
Insert the reference sample. Press 0A/100% key. This will be used for all subsequent samples until changed.

Press \blacklozenge to display the Run Standard screen.

Run the standard by pressing Run, \blacklozenge

OR

Press cancel \blacklozenge to return to the measure screen.



Step 8 (Standard selected)

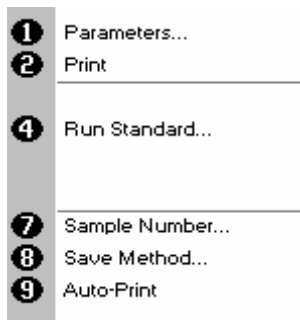
Insert the sample and press \blacklozenge .

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

Repeat step 8 for all samples.

Press \blacklozenge to return to the initial display screen.

Press $\boxtimes\boxtimes\boxtimes$ 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. Return to Run Standard screen (only available if Standard is the selected mode).
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 \blacklozenge .
9. Auto-print – toggles auto-print on/off.

Exit options by pressing \blacklozenge , or wait.

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 = 8×10^8 cells/ml for E. Coli.
- Your Biowave 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

OD 600 - Parameters

Wavelength
600 nm
Correction
2.00
Units
OD

OK Cancel

Step 1

Select the wavelength. Default value is 600 nm.
Press the down arrow.

Step 2

Enter the factor to compensate for different optical configurations between this and other instruments. Default value is 2.
Press the down arrow.

Step 3

Select the units. Options are OD or cells/ml. If cells/ml is selected two further parameters are displayed.

OD 600 - Parameters

Wavelength	Factor
600 nm	1.00
Correction	Multiplier
2	x 1000
Units	
cells/ml	

OK Cancel


Step 4 (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.

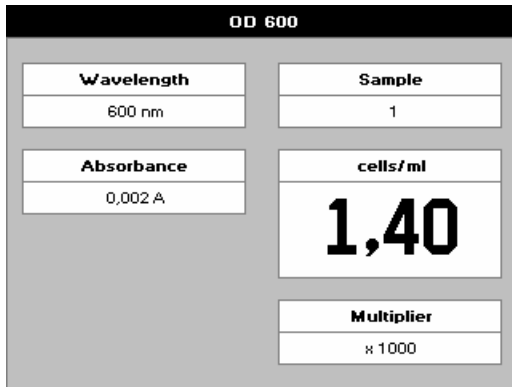
Step 5 (if cells/ml selected)

Select the multiplier using the left and right arrows. Options are 1000 or 1,000,000.

Step 6

Press OK  to enter the Results screen
OR

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



Results Screen

Step 8


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


Step 9

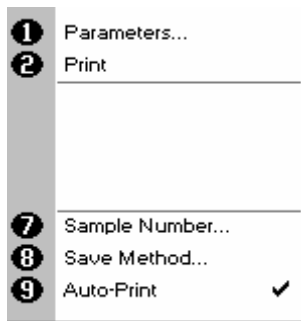
Insert the sample and press .

The wavelength, absorbance and OD600 value is displayed.

Repeat step 9 for all samples.

Press  to return to the Life Science Folder.

Press  to display available Options which are described below.



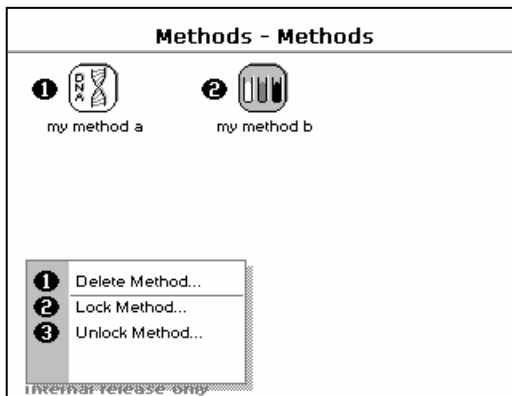
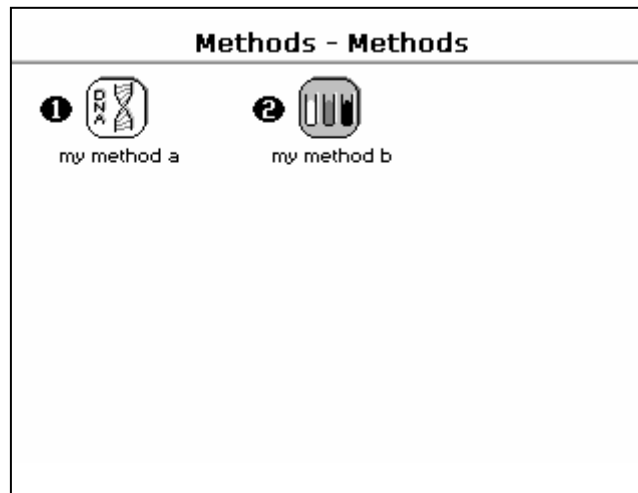
Options (select using key pad numbers)

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 left and right arrows to select a folder to store in (Favourites/Methods 1-9), press the down arrow and enter name.
9. Auto-print – toggles auto-print on/off.

Exit options by pressing , or wait.

STORING METHODS

This folder allows you to store up to 9 user modified methods than can be saved using the Options menu. To retrieve previously stored methods press 5 on the keypad to enter the methods 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 $\boxtimes\boxtimes\boxtimes$ key.

Delete Method

Press 1 to select delete method.

Select the method to be deleted using the left and right arrows.

Press \blacktriangleleft to delete the method

OR \blacktriangledown cancel to return to the 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 \blacktriangleleft to lock the method

OR \blacktriangledown cancel to return to the 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.

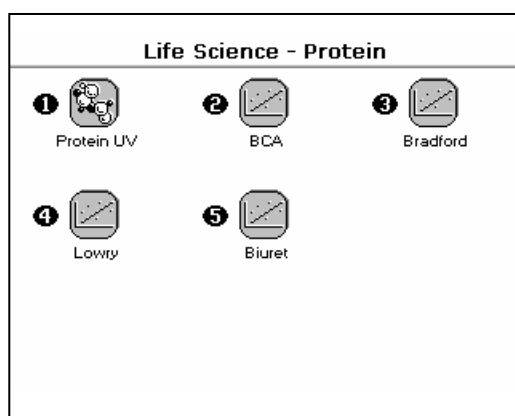
Enter the pass code using the keypad numbers or left and right arrows.

Press \blacktriangleleft to unlock the method

OR \blacktriangledown cancel to return to the Methods folder.

PROTEIN DETERMINATION

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

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 λ 280 UV protein measurement; Factor 1 is based on the extinction coefficient of the protein. If BSA (bovine serum albumin) is an acceptable standard, setting Factor 1 = 1.115 will give linear results from 0 to 0.8 mg/ml protein.
$$\text{Protein (mg/ml)} = 1.115 * \text{Abs 280}$$
- Rapid measurements such as this at Abs 280 are particularly useful after isolation of proteins and peptides from mixtures using spin and HiTrap columns by centrifuge and gravity, respectively.

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 colour obtained from the reaction of Folin-Ciocalteu phenol reagent with the tyrosyl 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:

Protein UV - Parameters

Pathlength	Coeff. 1
10 mm	1.55
Dilution Factor	Coeff. 2
1.00	0.76
Background	Units
Off	µg/ml

OK Cancel

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 $\boxtimes\boxtimes\boxtimes$ 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 \blacklozenge to calculate the dilution factor and return to the Parameters screen.

OR Press \blacktriangledown 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 co-efficient 1 (280 nm) using the keypad numbers. Default value is 1.55, range is 1.00 to 9999.

Press the down arrow.

Step 6

Enter co-efficient 2 (260 nm) using the keypad numbers. Default value is 0.76. Range is 0 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 \blacklozenge to enter the Results screen

OR

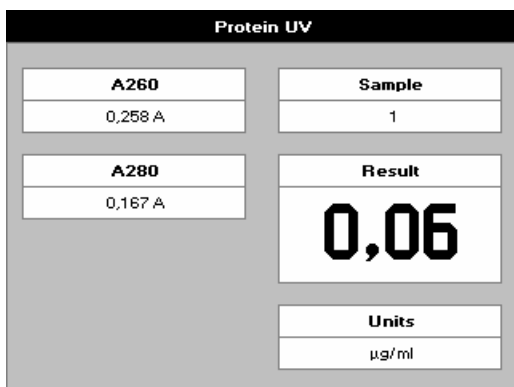
Cancel \blacktriangledown to return to the Protein folder

Protein UV - Parameters

Dilution Factor

Volume
1.000
Diluent
0.000

OK Cancel



Results Screen

Step 9

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

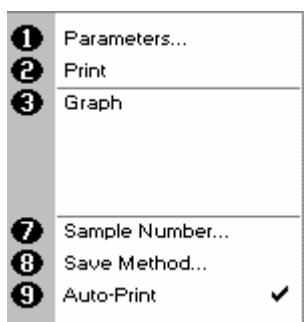
Step 10

Insert sample and press \diamond . 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 ∇ to return to the Protein folder.

Press $\boxtimes\boxtimes\boxtimes$ 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 wvescan plot across the range 250 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 \diamond .
9. Auto-print – toggles auto-print on/off.

Exit options by pressing ∇ , or wait.

2: BCA

The procedure is as follows:

BCA - Parameters

Wavelength	562 nm	Curve Fit	Regression
Standards	6	Calibration	Standards
Units	µg/ml	Replicates	Off

Next Cancel

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 $\boxtimes\boxtimes\boxtimes$ and then use the left/right arrows ($\mu\text{g}/\text{ml}$, $\mu\text{g}/\mu\text{l}$, $\text{pmol}/\mu\text{l}$, mg/dl , mmol/l , $\mu\text{mol}/\text{l}$, g/l , mg/l , $\mu\text{g}/\text{l}$, 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 \blacklozenge to store the chosen parameters or Cancel ⓧ .

Units

Units

DP

Auto

OK Cancel

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 \blacklozenge to enter the Standards screen

OR

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

BCA - Parameters

Wavelength	562 nm	Curve Fit	Regression
Standards	6	Calibration	Standards
Units	µg/ml	Replicates	Off

Next Cancel

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

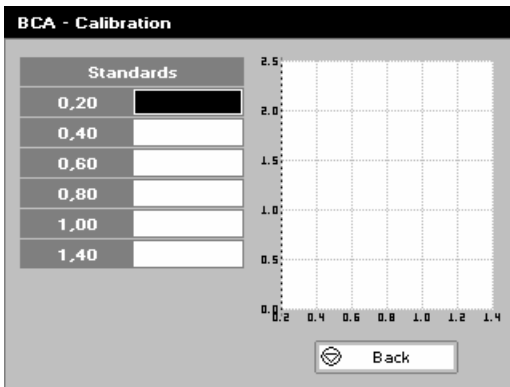
OR

Press Back ⓧ to return to the Parameter screen.

BCA - Standards

Std. 1	0,20	Std. 4	0,80
Std. 2	0,40	Std. 5	1,00
Std. 3	0,60	Std. 6	1,40

Next Back



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% key.

This will be used for all subsequent samples until changed.

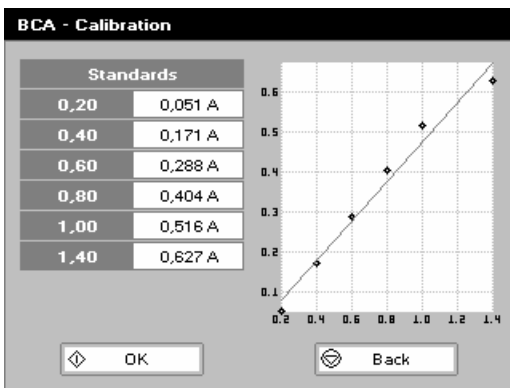
Step 12 (standards selected)

Insert the standard (use C to clear previously stored results before measuring)

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

OR

Press Back \blacktriangledown 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% key.

This will be used for all subsequent samples until changed.

Step 12 (standards selected)

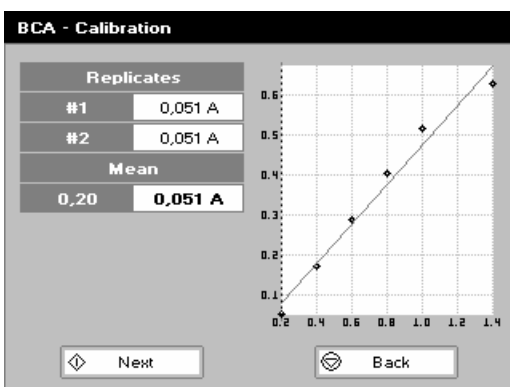
Press \blacklozenge to display the replicate entry boxes. Use C to clear previously stored results before measuring.

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

Repeat for all replicates and 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.

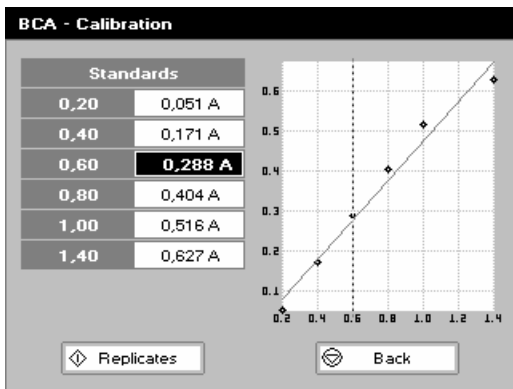


Step 13 (standards/manual selected)

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

OR

Press Back \blacktriangledown to return to the Standards screen.



Calibration (Manual entry)

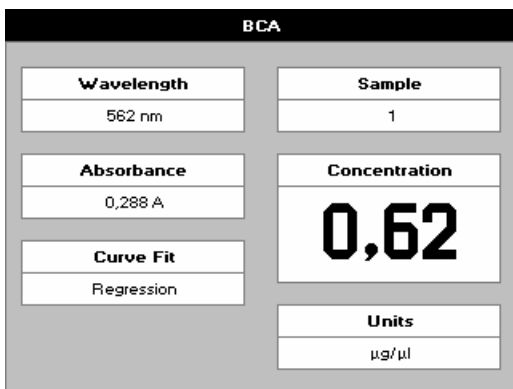
Shows previously entered calibration values and allows values to be entered via the keypad.

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

OR

Press Back \blacktriangleleft to return to the Standards screen.



Results screen

Step 14

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

Step 15

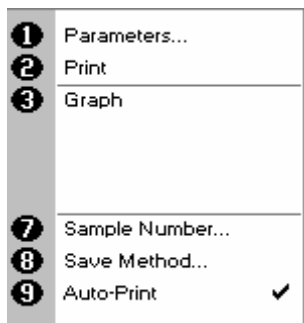
Insert the sample and press \blacklozenge .

The concentration of the sample is taken and displayed.

Repeat step 15 for all samples.

Press \blacktriangleleft to return to the Protein Folder.

Press $\boxtimes\boxtimes\boxtimes$ 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 \blacklozenge .
9. Auto-print – toggles auto-print on/off.

Exit options by pressing \blacktriangleleft , or wait.

3: Bradford

The procedure is as follows:

Wavelength	595 nm	Curve Fit	Regression
Standards	6	Calibration	Standards
Units	µg/ml	Replicates	Off
Next		Cancel	

Units	DP		
Auto	Auto		
OK		Cancel	

Wavelength	595 nm	Curve Fit	Regression
Standards	6	Calibration	Standards
Units	µg/ml	Replicates	Off
Next		Cancel	

Std. 1	0.20 µg/ml	Std. 4	0.80 µg/ml
Std. 2	0.40 µg/ml	Std. 5	1.00 µg/ml
Std. 3	0.60 µg/ml	Std. 6	1.40 µg/ml
Next		Back	

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 $\boxtimes\boxtimes\boxtimes$ 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 \blacklozenge to store the chosen parameters or Cancel \textcircled{V} .

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 \blacklozenge to enter the Standards screen

OR

Press Cancel \textcircled{V} to cancel selections and return to the Protein folder.

Standards Screen

Step 9 (standards/manual selected)

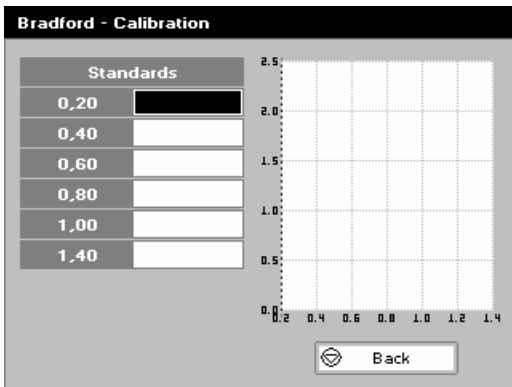
Enter the concentration values by using the keypad numbers and the up and down arrows to move between the different standard boxes. Range 0.001 to 9999. C button backspaces and clears the last digit entered.

Step 10 (standards/manual selected)

Press Next \blacklozenge 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 \textcircled{V} 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% key.

This will be used for all subsequent samples until changed.

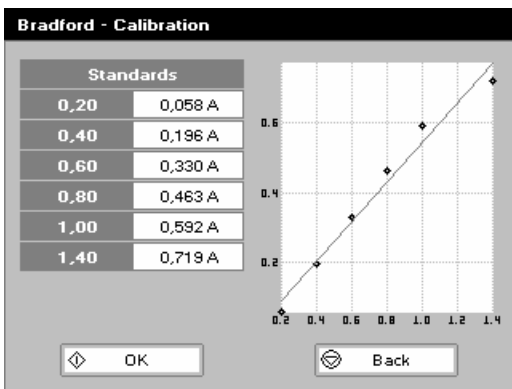
Step 12 (standards selected)

Insert the standard (use C to clear previously stored results before measuring)

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

OR

Press Back \blacktriangledown 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% key.

This will be used for all subsequent samples until changed.

Step 12 (standards selected)

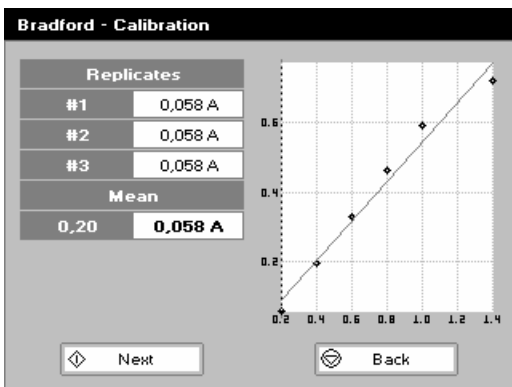
Press \blacklozenge to display the replicate entry boxes. Use C to clear previously stored results before measuring.

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

Repeat for all replicates and 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.

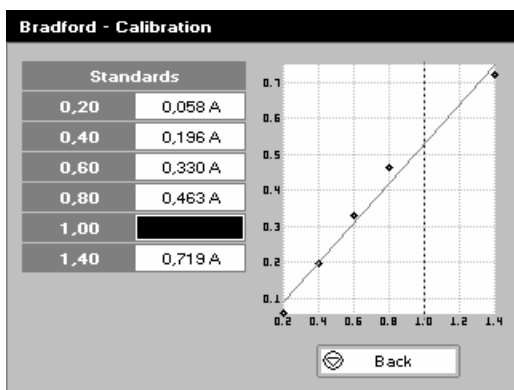


Step 13 (standards/manual selected)

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

OR

Press Back \blacktriangledown to return to the Standards screen.



Calibration (Manual entry)

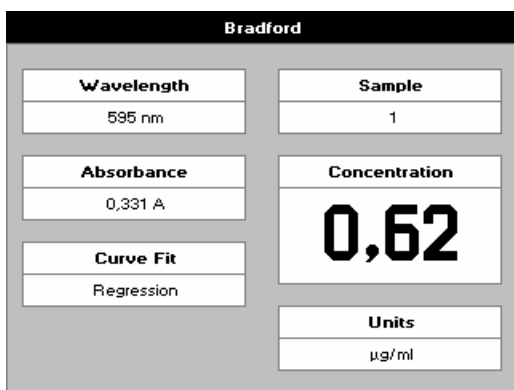
Shows previously entered calibration values and allows values to be entered via the keypad.

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

OR

Press Back \blacktriangleleft to return to the Standards screen.



Results screen

Step 14

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

Step 15

Insert the sample and press \blacklozenge .

The concentration of the sample is taken and displayed.

Repeat step 15 for all samples.

Press \blacktriangleleft to return to the Protein Folder.

Press $\boxtimes\boxtimes\boxtimes$ to display available Options which are described below.

- 1 Parameters...
- 2 Print
- 3 Graph

- 7 Sample Number...
- 8 Save Method...
- 9 Auto-Print

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 \blacklozenge .
9. Auto-print – toggles auto-print on/off.

Exit options by pressing \blacktriangleleft , or wait.

4: Lowry

The procedure is as follows:

Wavelength	750 nm	Curve Fit	Regression
Standards	6	Calibration	Standards
Units	µg/ml	Replicates	Off

Next Cancel

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.

Units

Units

DP

Auto

OK Cancel

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 $\square\square\square$ and then use the left/right arrows ($\mu\text{g}/\text{ml}$, $\mu\text{g}/\mu\text{l}$, $\text{pmol}/\mu\text{l}$, mg/dl , mmol/l , $\mu\text{mol}/\text{l}$, g/l , mg/l , $\mu\text{g}/\text{l}$, 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 \blacklozenge to store the chosen parameters or Cancel V .

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 \blacklozenge to enter the Standards screen

OR

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

Wavelength	750 nm	Curve Fit	Regression
Standards	6	Calibration	Standards
Units	µg/ml	Replicates	Off

Next Cancel

Std. 1	0.20 µg/ml	Std. 4	0.80 µg/ml
Std. 2	0.40 µg/ml	Std. 5	1.00 µg/ml
Std. 3	0.60 µg/ml	Std. 6	1.40 µg/ml

Next Back

Standards Screen

Step 9 (standards/manual selected)

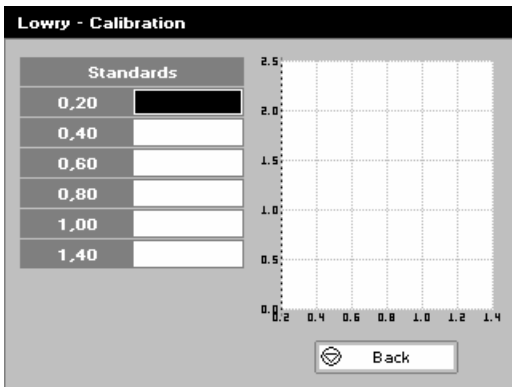
Enter the concentration values by using the keypad numbers and the up and down arrows to move between the different standard boxes. Range 0.001 to 9999. C button backspaces and clears the last digit entered.

Step 10 (standards/manual selected)

Press Next \blacklozenge 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 V 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% key.

This will be used for all subsequent samples until changed.

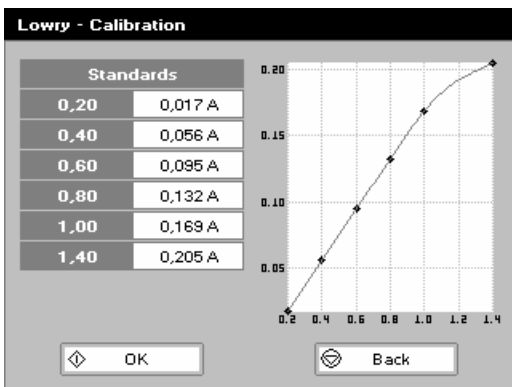
Step 12 (standards selected)

Insert the standard (use C to clear previously stored results before measuring)

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

OR

Press Back \blacktriangledown 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% key.

This will be used for all subsequent samples until changed.

Step 12 (standards selected)

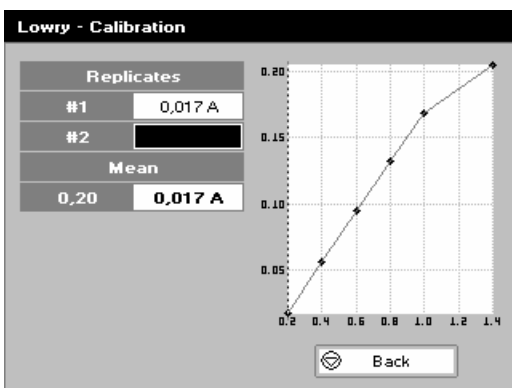
Press \blacklozenge to display the replicate entry boxes. Use C to clear previously stored results before measuring.

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

Repeat for all replicates and 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.

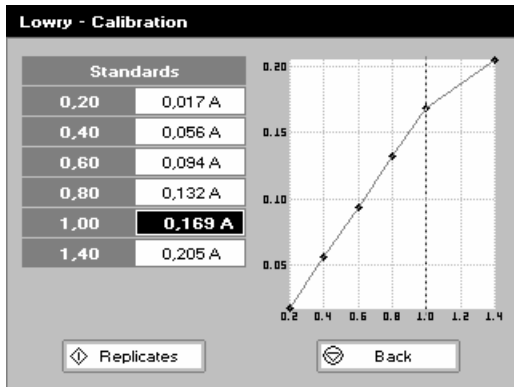


Step 13 (standards/manual selected)

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

OR

Press Back \blacktriangledown to return to the Standards screen.



Calibration (Manual entry)

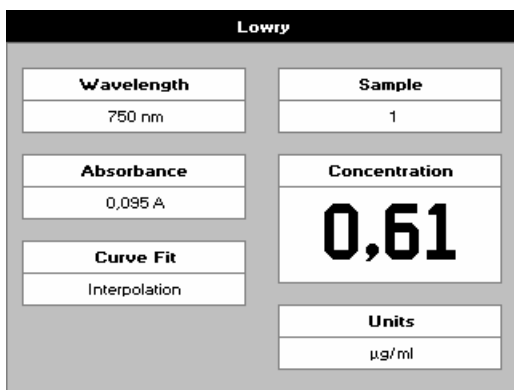
Shows previously entered calibration values and allows values to be entered via the keypad.

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 to return to the Standards screen.



Results screen

Step 14

Insert the reference sample and press the 0A/100%T key. 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 to return to the Protein Folder.

Press to display available Options which are described below.

- Parameters...
- Print
- Graph
- Sample Number...
- Save Method...
- Auto-Print

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 , or wait.

5: Biuret

The procedure is as follows:

Wavelength	546 nm	Curve Fit	Regression
Standards	6	Calibration	Standards
Units	µg/ml	Replicates	Off
Next		Cancel	

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.

Units	DP		
Auto	Auto		
OK		Cancel	

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 $\square\square\square$ and then use the left/right arrows ($\mu\text{g}/\text{ml}$, $\mu\text{g}/\mu\text{l}$, $\text{pmol}/\mu\text{l}$, mg/dl , mmol/l , $\mu\text{mol}/\text{l}$, g/l , mg/l , $\mu\text{g}/\text{l}$, 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 \blacklozenge to store the chosen parameters or Cancel \blacklozenge .

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 \blacklozenge to enter the Standards screen
OR

Press Cancel \blacklozenge to cancel selections and return to the Protein folder.

Wavelength	546 nm	Curve Fit	Regression
Standards	6	Calibration	Standards
Units	µg/ml	Replicates	Off
Next		Cancel	

Std. 1	Std. 4		
0,20 mg/dl	0,80 mg/dl		
Std. 2	Std. 5		
0,40 mg/dl	1,00 mg/dl		
Std. 3	Std. 6		
0,60 mg/dl	1,40 mg/dl		
Next		Back	

Standards Screen

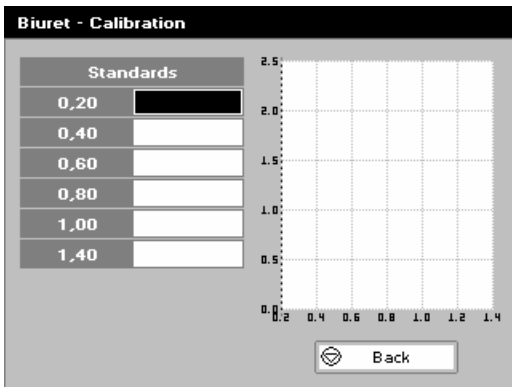
Step 9 (standards/manual selected)

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 \blacklozenge to enter the Calibration screen
OR

Press Back \blacklozenge 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% key.

This will be used for all subsequent samples until changed.

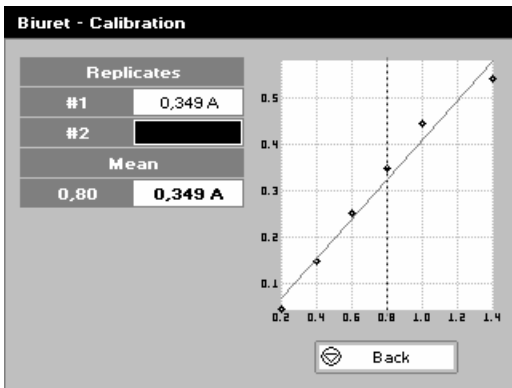
Step 12 (standards selected)

Insert the standard (use C to clear previously stored results before measuring)

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

OR

Press Back \blacktriangledown 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% key.

This will be used for all subsequent samples until changed.

Step 12 (standards selected)

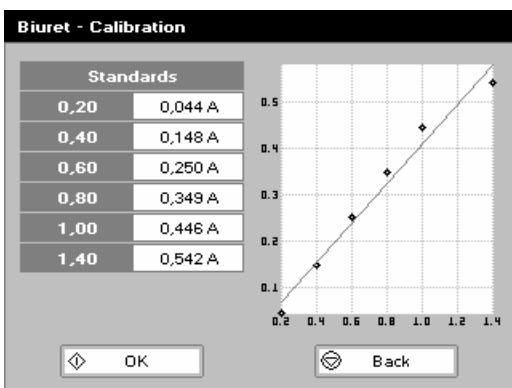
Press \blacklozenge to display the replicate entry boxes. Use C to clear previously stored results before measuring.

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

Repeat for all replicates and 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.

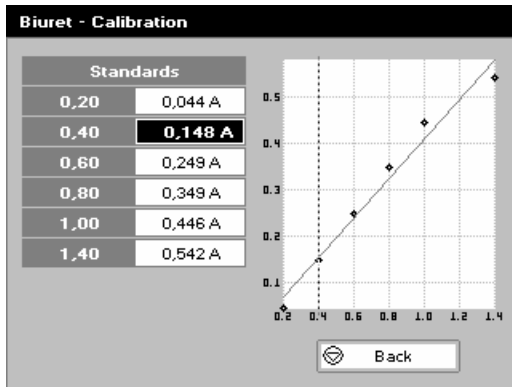


Step 13 (standards/manual selected)

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

OR

Press Back \blacktriangledown to return to the Standards screen.



Calibration (Manual entry)

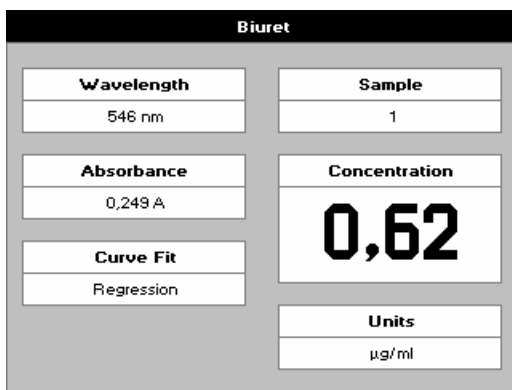
Shows previously entered calibration values and allows values to be entered via the keypad.

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

OR

Press Back \blacktriangleleft to return to the Standards screen.



Results screen

Step 14

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

Step 15

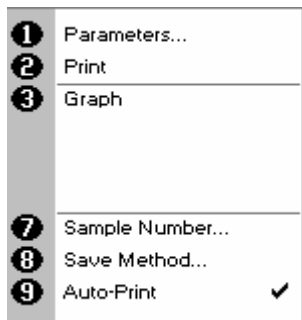
Insert the sample and press \blacklozenge .

The concentration of the sample is taken and displayed.

Repeat step 15 for all samples.

Press \blacktriangleleft to return to the Protein Folder.

Press $\boxtimes\boxtimes\boxtimes$ to display available Options which are described below.



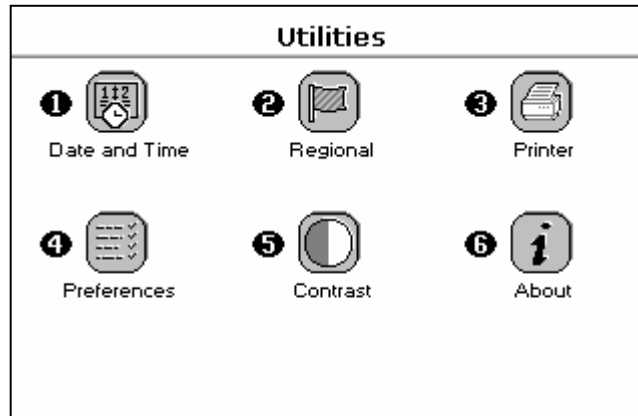
Options (select using key pad numbers)

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 \blacklozenge .
9. Auto-print – toggles auto-print on/off.

Exit options by pressing \blacktriangleleft , or wait.

UTILITIES FOLDER







This folder lets you alter the instrument set up. Press 7 to enter the utilities folder.



Summary Function

Keypad number



Description

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

1: Date and Time

The procedure is as follows:

The screenshot shows the 'Date and Time' settings screen. It has a title bar 'Date and Time'. There are four input fields: 'Day' with '20', 'Hour' with '21', 'Month' with 'March', and 'Minute' with '0'. Below these is a 'Year' field with '2006'. At the bottom are 'OK' and 'Cancel' buttons.



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  to return to the Utilities folder without storing the time.

2: Regional

Sets Language and Number Format

The procedure is as follows:

The screenshot shows the 'Regional' settings screen. It has a title bar 'Regional'. There are two input fields: 'Language' with 'English' and 'Number Format' with '999,9'. At the bottom are 'OK' and 'Cancel' buttons.




Select a language. Options are German, French, English, Spanish or Italian.
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  to return to the Utilities folder without storing the settings.

3: Printer

Sets up printing options

The procedure is as follows:

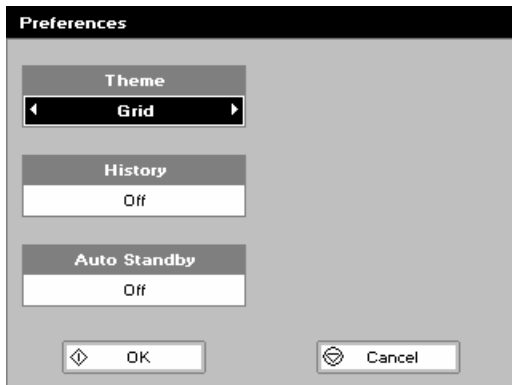
The screenshot shows the 'Printer' settings screen. It has a title bar 'Printer'. There are two input fields: 'Auto-Print' with 'On' and 'Printer' with 'Built-in'. At the bottom are 'OK' and 'Cancel' buttons.

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  to return to the Utilities folder without storing the settings.

4: Preferences

Sets user preferences

The procedure is as follows:



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.

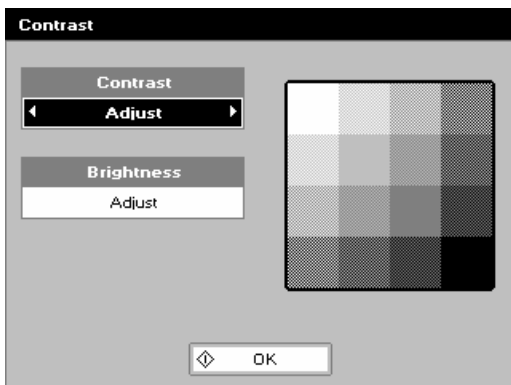
Select whether to use a standby mode after defined periods.

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  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.

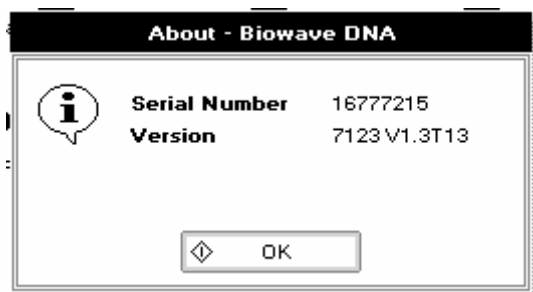
Adjust the brightness using the left and right arrows.

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:

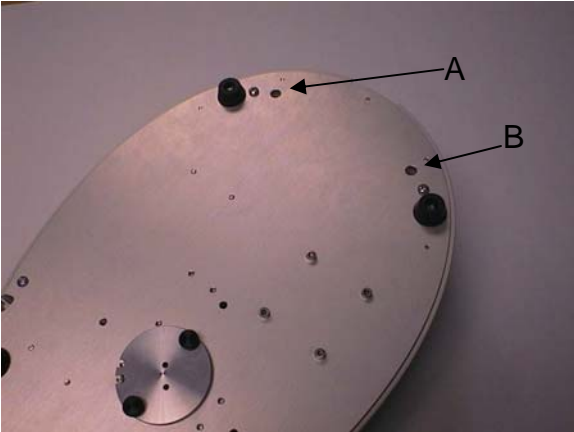
6: About



Displays the instrument serial number and software version.

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

ACCESSORIES: PRINTER INSTALLATION



1. Turn the instrument over and remove cap head screws from positions A and B using the Allen key provided.



2. Turn the instrument back over and lift the accessory cover vertically upwards to remove. Remove the tie-wrap from the cable.
3. Invert the instrument and replace the cap head screws at A and B.

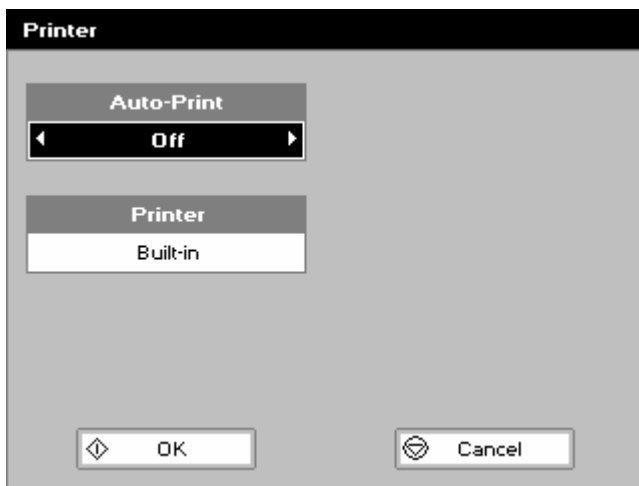


4. Plug the accessory cable into the printer.



5. Lower the printer onto the locating bosses and push down firmly.

Printer configuration



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

Loading / changing the printer paper



1. Lift off the paper cover.

Lock the platen and turn the knob to feed the paper



2. Feed in the paper.

Sometimes it helps if the platen lock is released.



3. Paper gripped.



4. Cover replaced.

PRINT VIA COMPUTER

- PVC (Print Via Computer) is an optional small application running under Windows 2000™ or Windows XP™ to enable a Biowave DNA to transfer data into a PC environment via the USB interface. 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 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

ACCESSORIES

USB cable	source locally
Built-in printer accessory	80-3003-84
PVC Software and USB cable	80-3004-73

MAINTENANCE

After Sales Support

Support agreements that help you to fulfil the demands of regulatory guidelines concerning GLP/GMP are available.

- Calibration, certification using filters traceable to international standards
- Certificated engineers and calibrated test equipment
- Approved to ISO 9001 standard

Choice of agreement apart from break down coverage can include

- Preventative maintenance
- Certification

When using calibration standard filters, insert such that the flat surface is facing away from the spring end of the cell holder.

Observe all necessary precautions if dealing with hazardous samples or solvents.

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

<i>Wavelength range</i>	190 - 1100 nm
<i>Monochromator</i>	Flat grating
<i>Wavelength calibration</i>	Automatic upon switch on
<i>Spectral bandwidth</i>	5 nm
<i>Wavelength accuracy</i>	±2 nm
<i>Wavelength reproducibility</i>	±1 nm
<i>Light sources</i>	Pulsed xenon lamp
<i>Detector</i>	1024 element CCD array
<i>Photometric range</i>	- 0.300 to 2.500A, 0 to 199%T
<i>Photometric linearity</i>	±0.005 Abs or 1% of the reading, whichever is the greater @ 546 nm
<i>Photometric reproducibility</i>	±0.003 Abs (0 to 0.5 Abs), ±0.007 Abs (0.5-1.0 Abs)
<i>Stray light</i>	<1% at 220 nm and 340 nm using NaNO ₂
<i>Zero stability</i>	±0.01 Abs/hour after 20 min warm up @ 340 nm
<i>Noise</i>	0.005 pk to pk 0.002 rms
<i>Digital output</i>	USB port standard, Bluetooth option
<i>Dimensions</i>	260 x 390 x 100 mm
<i>Weight</i>	<4.5 kg
<i>Power input</i>	90-250 V, 50/60 Hz, Max 30 VA

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

- Your supplier 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. The supplier can accept no liability for loss or damage, however caused, arising from the faulty or incorrect use of this product.
- This product has been designed and manufactured by Biochrom Ltd, 22 Cambridge Science Park, Milton Road, Cambridge CB4 0FJ, UK. However, please contact your original supplier in the first instance if you experience technical or sample handling difficulties.