Lightwave 3 & Lightwave 3+

Spectrophotometers

Users Manual

WPA

biochrom

a division of
Harvard Bioscience, Inc.

5061-084-REV-1

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Warranty Policy

IN NO EVENT SHALL BIOCHROM BE LIABLE FOR INCIDENTAL OR CONSQUENTIAL DAMAGES[1]

THERE ARE NO IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR USE, OR OF ANY OTHER NATURE.[1] Biochrom shall not be liable for any claims of any kind whatsoever, as to the equipment. Warranty is void if the instrument is modified, disassembled, repair carried out using unauthorized parts or by a service engineer not previously approved by Biochrom.

Returns

If any defect arises within or outside the warranty period, please contact:

US Office Technical Support

Email: support@hbiosci.com

Telephone (Toll Free, US only): 800-272-2775

Telephone (Outside the US): 508-893-8999

Address:

84 October Hill Road

Holliston, Massachusetts 01746, USA

UK Office Technical Support

Email: support@hbiosci.com

Telephone: +44 (0) 1223 423723

Address:

1020 Cambourne Business Park

Cambourne, Cambridge, UK, CB23 6DW

Goods will not be accepted for return unless the RMA (Return Materials Authorization) number has been issued. The unit must be returned with the completed RMA forms and the Decontamination checklist. The customer is responsible for shipping charges unless the failure is within 30 days of receiving the goods. Please allow a reasonable period of time for completion of repairs or replacement.

Caution Notice

The Biochrom Lightwave systems are intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) and safety intended for laboratory equipment applications. This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen, or nitrous oxide.

1 Where the territory does not allow this exclusion or limitation, this term will not apply.

Hazards and Warnings

This section describes potential hazards which may exist in the operation of these units. A number of warning labels and symbols are affixed to your instrument. These symbols are used to inform you of potential dangers which may exist or where caution is required. Before installing your new unit, please take time to familiarise yourself with these warnings and symbols.

This instrument is subject to the following identified hazards:

This unit uses a Xenon lamp. The lamp energy is mainly confined within the unit but traverses the cell holder when a measurement is being taken. Although the energy present is low and intermittent you are advised not to stare into the beam or attempt to deflect the beam as prolonged exposure could result in permanent eye damage.

High voltages exist within the power supply unit and the Xenon lamp housing. Repair and maintenance should only be carried out by individuals trained to work on these instruments.

There are no biohazardous materials within the unit, however, this unit may be exposed to biohazardous samples during normal laboratory use. To protect users against these hazards we recommend the following decontamination procedures:

- Wipe the exterior casework with disinfectant cleaning wipes.
- Remove cuvettes and cuvette holders.
    - Wash with disinfectant appropriate for the biohazard in question.
    - Rinse with distilled water.
    - Allow to dry thoroughly before reuse.

To further reduce the possibility of biohazards:

- Include an appropriate decontamination certificate for equipment returned for repair.
- Ensure that the operator of the equipment is provided with a safe working environment.
- Use, store and dispose of any chemicals in accordance with manufacturer's guidelines and local safety regulations.
- Provide suitable ventilation when working with volatile solvents or toxic substances.
- Dispose of solvents and chemicals that may be classed as hazardous waste in accordance with local regulatory practice.
- Determine if personal protective equipment (PPE) is required for handling laboratory samples.

All models can be connected to and operated from a PC. To preserve the integrity of the measuring equipment it is essential that the attached PC itself conforms to basic safety and EMC standards and is set up in accordance with the manufacturers' instructions. If in doubt, consult the information that came with your PC.

The following safety precautions should be observed when operating a PC:

- To reduce the chance of eye strain, set up the PC display with the correct viewing position, free from glare and with appropriate brightness and contrast settings.
- To reduce the chance of cross contamination from biological samples, use appropriate personnel protection measures and disinfectant wipes on keyboard and mouse.

In the event of contamination, malfunction or hazard occurring, the operator should disconnect the unit, by removing the power cord, and isolate for decontamination and/or repair.

INTRODUCTION

Biochrom WPA Spectrophotometers

Spectrophotometers are ubiquitous among modern laboratories. Ultraviolet (UV) and Visible (VIS) spectrophotometry has become the method of choice in most laboratories concerned with the identification and quantification of organic and inorganic compounds across a wide range of products and processes. Applied across research, quality, and manufacturing, with continuing focus on life science and pharmaceutical environments, they are equally as relevant in agriculture, animal husbandry and fishery, geological exploration, food safety, environmental monitoring, and many manufacturing industries to name a few.

The WPA Lightwave spectrophotometers are quick, accurate, and reliable. They require only small demands on the time and skills of the operator. This operating manual details the processes for taking basic measurements using the Lightwave 3 and Lightwave 3+ spectrophotometers.

The Lightwave 3 instrument is UV-VIS split-beam spectrophotometers with a 5 nm spectral bandwidth and a 40 mm pathlength cell holder. The hardware of the Lightwave 3+ model variants differ only by having a narrower 3 nm spectral bandwidth.

INSTALLATION

Unpacking

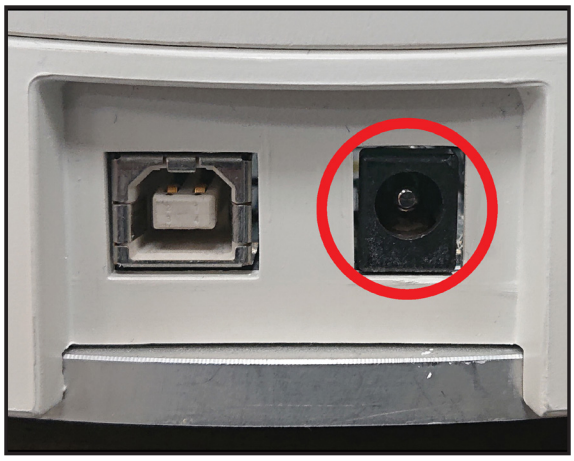
- The unit weighs less than 4 kg. No special handling is required.
- Please keep the original packaging for transport for service or repair as it has been specifically designed to protect the unit from damage during transit.
- Inspect the instrument and its power supply for any signs of damage caused during transit. If any damage is discovered, do not use the instrument and report the problem to your supplier.

Positioning

- Ensure your proposed installation site conforms to the environmental conditions for safe operation:
- Indoor use
- 5°C to 40°C
- Maximum relative humidity 90% up to 31°C decreasing linearly to 50% at 40°C.
- Extremes of temperature may require recalibration of the unit for optimal performance.
- The instrument must be placed on a stable, level bench or table capable of supporting its weight allowing sufficient space around the instrument for air to circulate freely.
- The instrument should be positioned so that the power supply cable may be readily removed in the event of a hazard or malfunction.
- Locate the instrument in an atmosphere free from dust and corrosive fumes. Use the dust cover to further protect the instrument when not in use.

Installing

- If the instrument has been stored in a cold environment, it should be allowed to come to room temperature before turning it on to avoid compromising the internal calibration procedure.
- The equipment is operated using an 18 VDC power supply adapter unit. Always use the power supply adapter and mains cords supplied with the instrument.
- Mains power requirements are as follows:
- 100 to 240 VAC~
- 50 or 60 Hz
- The UK style mains cord plug has a user replaceable 3A fuse. Replace only with the same rating and type 3A BS1362.
- The unit maximum power rating is 40 VA.
- Connect the instrument to the mains power using the main power cord and the 18 VDC power supply adapter unit.
- Switch on the instrument after it has been plugged in. The instrument will perform a series of self-diagnostic checks.



18 VDC power supply socket

INSTRUMENT OVERVIEW

Scope

This user manual covers the following range of WPA UV/Visible spectrophotometers:

Part Number	Description
80-3007-42	WPA Lightwave 3
80-3007-43	WPA Lightwave 3 with Printer
80-3007-44	WPA Lightwave 3 with Bluetooth
80-3007-45	WPA Lightwave 3 with Printer and Bluetooth
80-3007-47	WPA Lightwave 3+
80-3007-48	WPA Lightwave 3+ with Printer
80-3007-49	WPA Lightwave 3+ with Bluetooth
80-3007-50	WPA Lightwave 3+ with Printer and Bluetooth

Spectrophotometer Principle and Intended Use

UV/Visible spectrophotometers measure the transmission of light through a sample. Samples absorb light based on their unique molecular composition. The amount of absorbance is directly proportional to the sample concentration and the pathlength, which is the distance that the light travels through the sample.

The WPA Lightwave spectrophotometers are intended for laboratory use to measure the concentration of nucleic acids and proteins in experimental samples.

Hardware

Your spectrophotometer is a simple-to-use UV/Visible instrument with a CMOS array detector (1024 pixels). It has no moving parts, which is the basis of the rapid scanning operating system. The look and operation of the Lightwave 3 and 3+ are identical; the only difference between them is the bandwidth. Throughout the rest of this manual, the term Lightwave 3 will be used to cover both instruments.

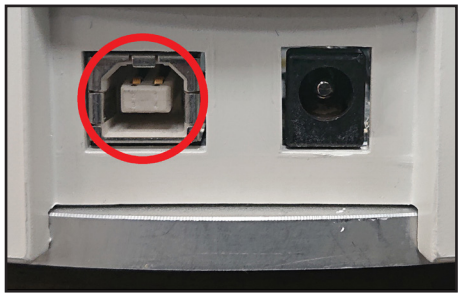
Technical Specifications

Wavelength Range	190 to 1100 nm
Monochromator	Flat grating
Wavelength Calibration	Automatic upon switch on
Beam Height	15 mm
Spectral Bandwidth	5 nm or 3 nm for '+' models
Wavelength Accuracy	±2 nm
Wavelength Reproducibility	±1 nm
Light Sources	Xenon flash lamp
Detector	Twin CMOS array
Photometric Range	-0.300 to 2.500 A, 0.3 to 199 %T
Photometric Linearity	±1.3 % or ±0.008 A whichever is greater at 546 nm
Photometric Reproducibility	±0.002 A to 0.5 A at 546 nm
Stray Light	<0.5 %T 340 nm
Stability	±0.01 A/h at 340 nm
Noise	±0.005 peak to peak ± 0.002 RMS
Digital Output	USB Flash Drive, PC via PVC software, Optional Bluetooth
Data Export	USB Flash Drive: .tsv, native PVC format PC via PVC: .csv, .emf, .xlsx, .xls, .rtf, .tsv, native PVC format
Method Storage	90 with PIN number protection
Sample ID	Yes
Languages	English, German, French, Spanish, Italian, Japanese, Chinese
Dimensions	260 × 390 × 100 mm
Weight	3.00 kg
Power Input	18 VDC at max 40 VA from a supplied 100 to 240 V~, 50/60 Hz Mains Power Adapter

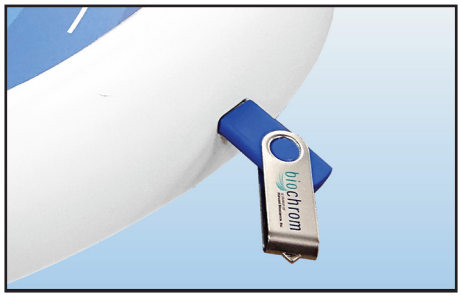
Display and Keypad

The instrument has a ¼ VGA (320 × 240 pixel) resolution backlit LCD display. The instrument's built-in firmware is navigated using the hard-wearing, spill-proof membrane keypad.

Instrument Connections



USB connector for PC connection



USB connector for USB memory stick

PVC PC Software

The instrument is supplied with the PVC software program (supplied with its own devoted operating manual) on the accompanying USB flash drive. The instrument can be connected to a PC onto which the PVC software has been installed, via a USB A to USB B cable or the factory fit Bluetooth accessory. This enables the operator to “print through” the PC directly to the printer that is connected to it. The data may also be stored as a comma-separated value (.csv), enhanced meta file (.emf), Excel spreadsheet (.xlsx, .xls), rich text format (.rtf), tab-separated value (.tsv) or in a native PVC format file.

Biochrom Resolution PC Software

When connected to a PC the spectrophotometers can be controlled using the Biochrom Resolution PC software packages. Operation using Biochrom Resolution PC software is described in the Resolution user manual or Resolution help file.

Instrument Data Output

A printer accessory is available for the instrument. This may either be supplied preinstalled or as an optional accessory for end-user installation.

Measurement data can also be exported to a USB flash drive via the USB A socket on the side of the instrument, as either a tab-separated value (.tsv) or native PVC format file.

Performing a Measurement

The optical height (z value) of the instrument is 15 mm. The light path is directed from RIGHT to LEFT through the cell chamber.

The cell holder supplied with the instrument accepts standard quartz, glass or plastic cuvettes with pathlengths up to 40 mm. When using a cuvette with a pathlength less than 40 mm ensure the cell is inserted to the far right of the cell holder. Cuvettes with a pathlength of less than 10 mm should be secured using an appropriate packing piece.

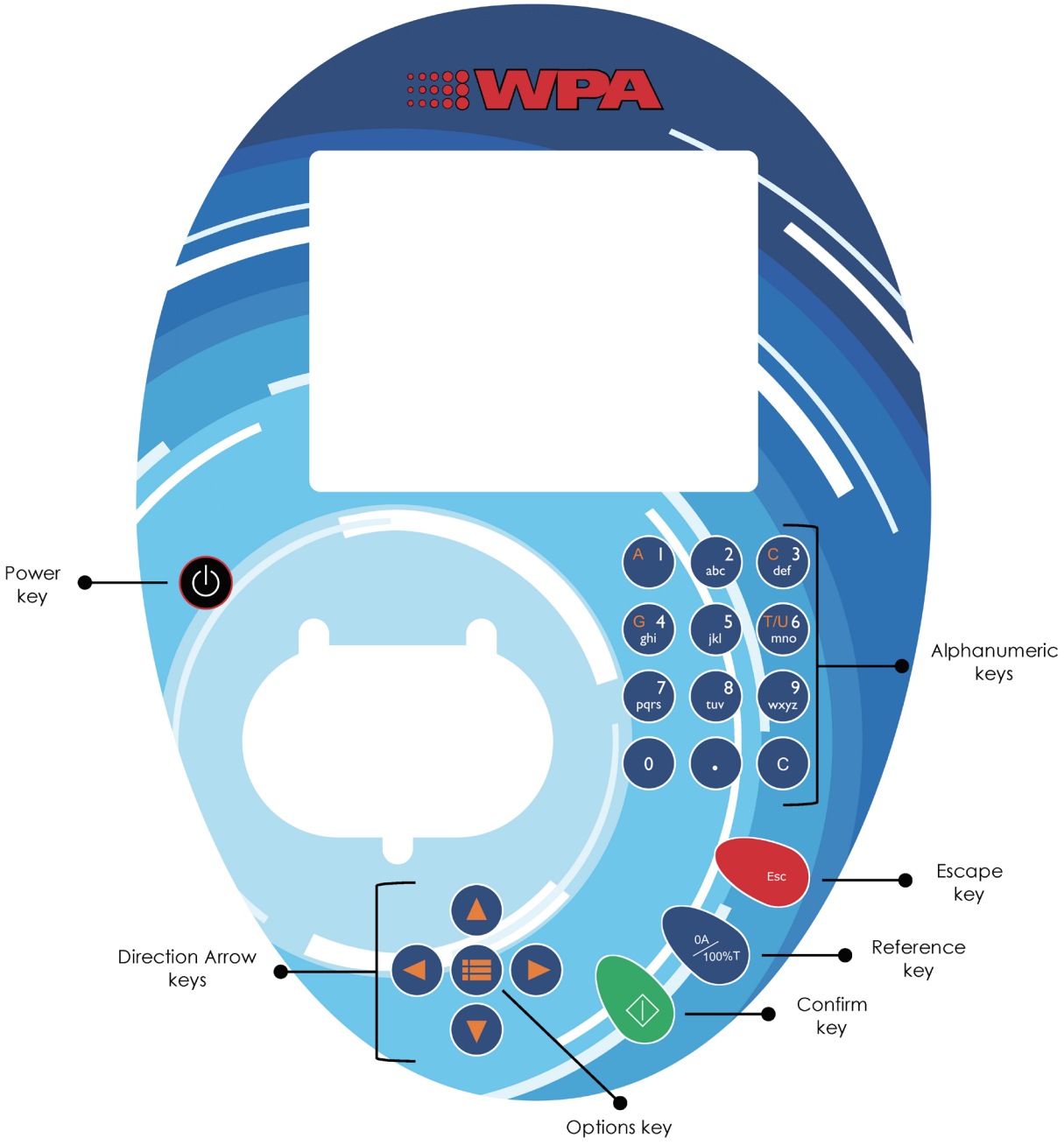
12 mm test tubes may be used (for cell cultures for example), however they are not recommended as higher quality data is produced by using disposable cuvettes. If used, align the indicator line on 12 mm test tubes in the same direction to ensure reproducible positioning of the tube.

Please consult the “User Interface” section of this user manual for more detail on taking a measurement using the spectrophotometer. In summary, how to perform a measurement is outline below.

1. Open the desired application on the spectrophotometer.
2. Insert a cuvette containing the reference sample into the cuvette holder.
3. Take a reference measurement using the reference key; the acquired reference baseline is applied to any subsequent sample measurements until a new reference baseline is taken, or the application is closed.
4. When the reference is complete, remove the reference sample containing cuvette from the cuvette holder, and replace it with a test sample containing cuvette then take a sample measurement using the confirm key.
5. Repeat step 4 until all the sample data has been collected (see the “Saving and Printing” section for post measurement options).

Keypad

The instrument is controlled using the snap-dome switch-keys of the membrane keypad, offering tactile feedback during operation.

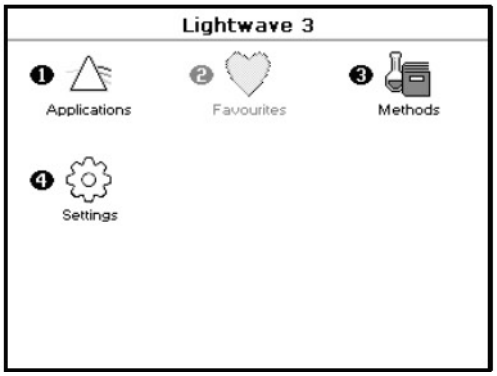


Instrument Firmware

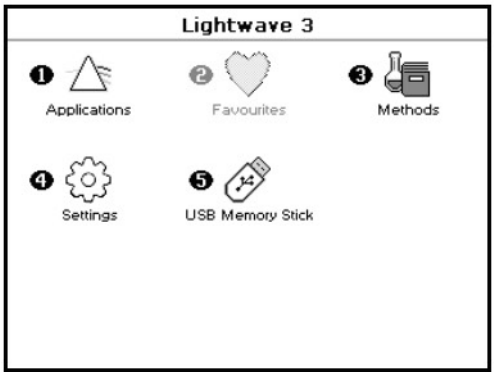
The instrument firmware uses an intuitive menu arrangement that is navigated using the membrane keypad. Upon powering on the instrument, and after completion of the internal calibration, the home screen is displayed.

Home Screen

The instrument home screen is the first screen displayed after initialisation of the instrument, and all applications and settings can be accessed from here.



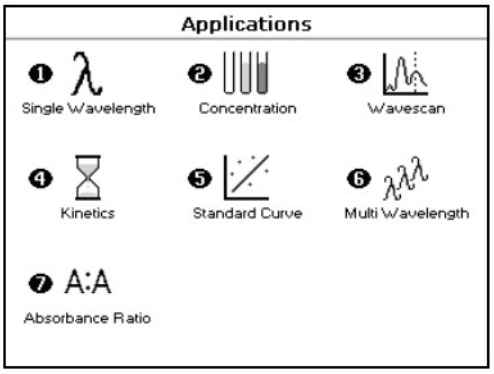
Home screen for the Lightwave 3 spectrophotometer



Home screen for the Lightwave 3 spectrophotometer displaying the USB memory Stick application, made available when a USB Flash drive is inserted

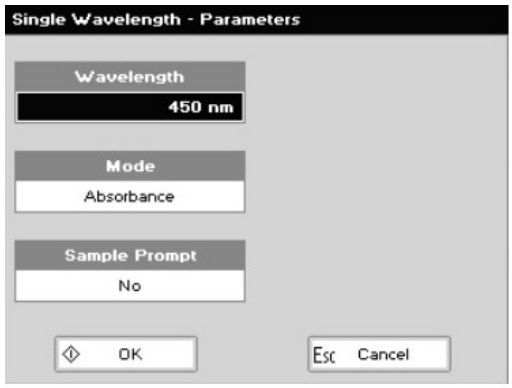
Applications

The Applications screen is accessed from the home screen using the '1' key. It contains basic applications with definable parameters to meet the needs of typical laboratory protocols.



Single Wavelength

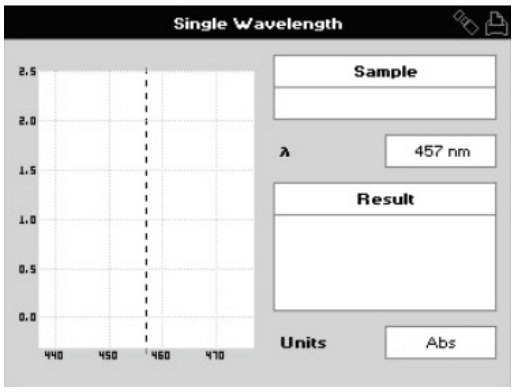
The Single Wavelength application is accessed from the Applications screen using the '1' key. It can be used to performs simple absorbance (A) or % transmission (%T) measurements.



Step 1
Set the wavelength, 190 to 1100 nm, using the left and right arrow or alphanumeric keys.

Step 2
Press the down arrow, select the mode, "Absorbance" or "%Transmission", using the left and right arrow keys.

Step 3
Press the down arrow, set the sample prompt, "Yes" or "No", to bring up the sample number option before each measurement, using the left and right arrow keys.

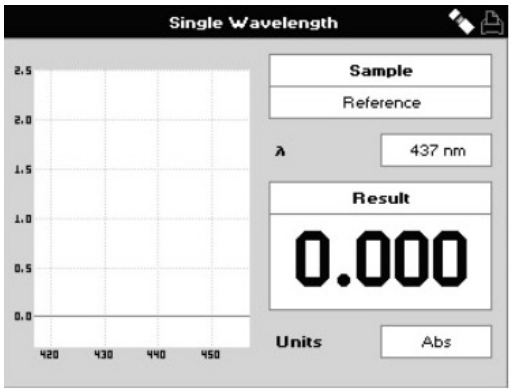


Step 4

Proceed to the measurement screen by selecting "OK" using the confirm key.

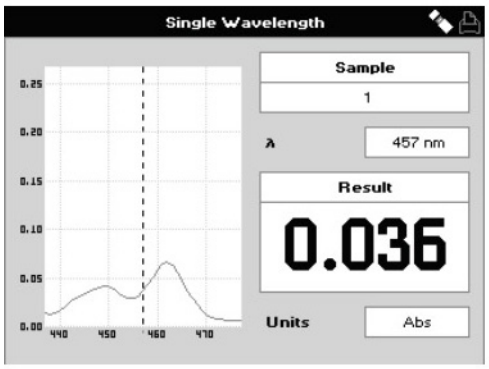
OR

Return to the Applications screen by selecting "Cancel" using the escape key.



Step 5
Insert the reference sample then take a reference measurement using the reference key.

The acquired reference sample baseline will be applied to all subsequent sample measurements.



Step 6
Replace the previous sample with a test sample then take a sample measurement using the confirm key.

Repeat for all samples.

WARNING!

Previous measurements cannot be recalled so should be printed or output to a USB flash drive before any subsequent samples are measured.

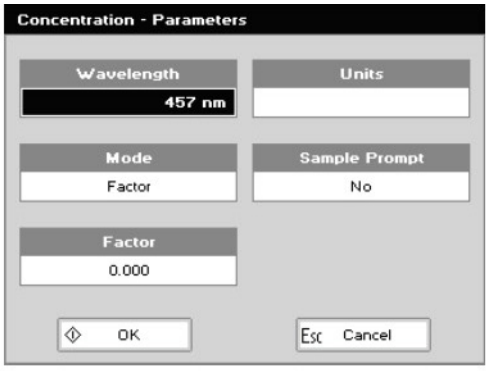
Results

The results are displayed on the screen. Use the left and right arrow keys to move the cursor and display the absorbance at ±15 nm either side of the set wavelength.

Return to the Applications screen using the escape key OR display the options menu using the options key (see the "Additional Options" section).

Concentration

The Concentration application is accessed from the Applications screen using the '2' key. It can be used to apply a known factor, or one determined using a standard of known concentration to a single wavelength absorbance (A) measurement.



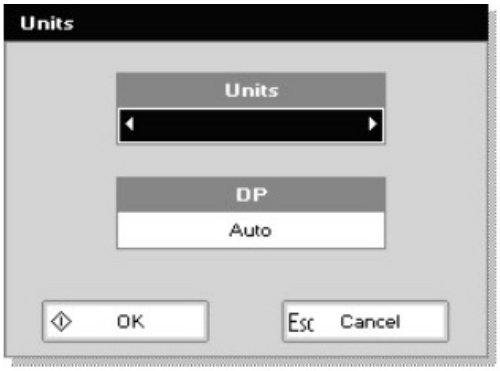
Step 1
Set the wavelength, 190 to 1100 nm, using the left and right arrow or alphanumeric keys.

Step 2
Press the down arrow, select the mode, "Factor", "Standard" or "Negative Factor", using the left and right arrow keys.

For "Factor" or "Negative Factor" mode, press the down arrow and enter a value of up to four significant figures, using the alphanumeric keys.

OR

For "Standard" mode, press the down arrow and enter a value of up to four significant figures, for the known concentration of the standard to be used using the alphanumeric keys.



Step 3

Press the down arrow, enter up to 8-digits to define the units that the measurements will be reported in.

OR

Open the Units option using the options key:

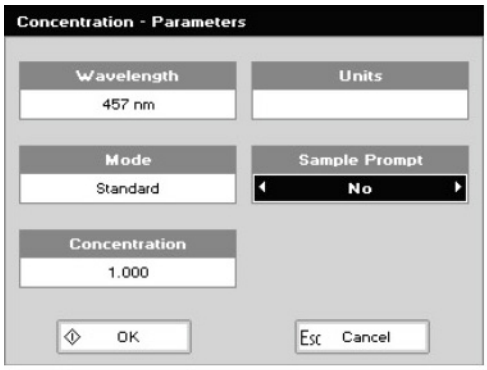
Select one of the predefined units; “µg/ml”, “µg/µl”, “pmol/µl”, “mg/dl”, “mmol/l”, “µmol/l”, “g/l”, “mg/l”, “µg/l”, “U/l”, “%”, “ppm”, “ppb”, or “conc”, using the left and right arrow keys.

Press the down arrow, select the number of decimal places to display the concentration in, “Auto”, “0”, “1”, or “2”, using the left and right arrow keys.

Implement the changes and return to the parameters screen by selecting “OK” using the confirm key.

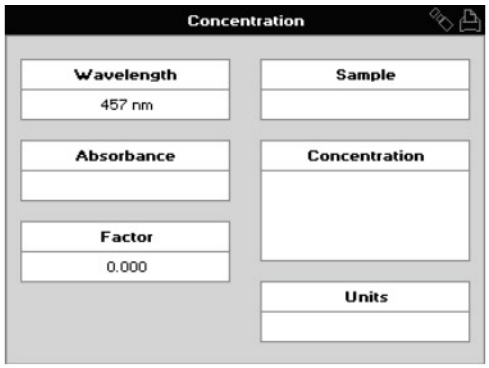
OR

Reject the changes and return to the parameters screen by selecting “Cancel” using the escape key.



Step 4

Press the down arrow, set the sample prompt, “Yes” or “No”, to bring up the sample number option before each measurement, using the left and right arrow keys.

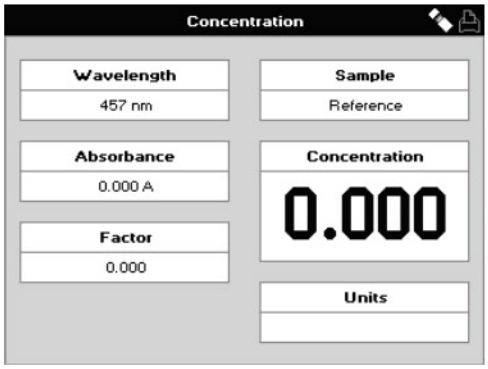


Step 5

Proceed to the measurement screen by selecting “OK” using the confirm key.

OR

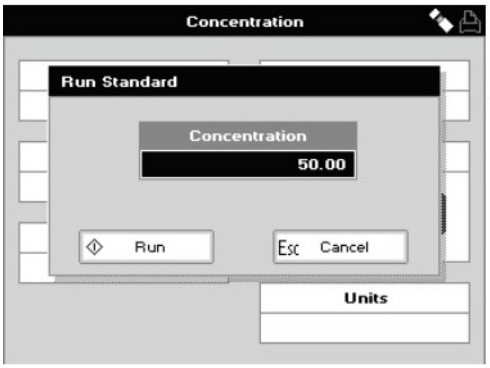
“Return to the Applications screen by selecting “Cancel” using the escape key.

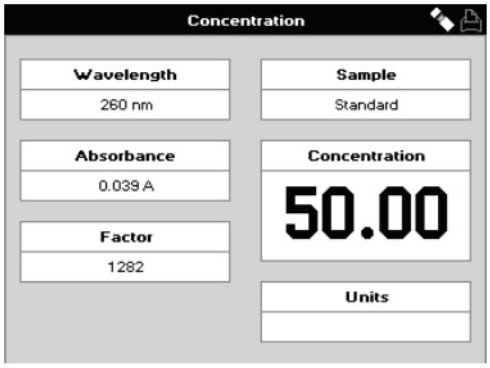


Step 6

Insert the reference sample then take a reference measurement using the reference key.

The acquired reference sample baseline will be applied to all subsequent sample measurements.





Step 7

For “Standard” mode, replace the previous sample with a standard sample then take a standard measurement using the confirm key.

Confirm the concentration of the standard sample by selecting “OK” using the confirm key.

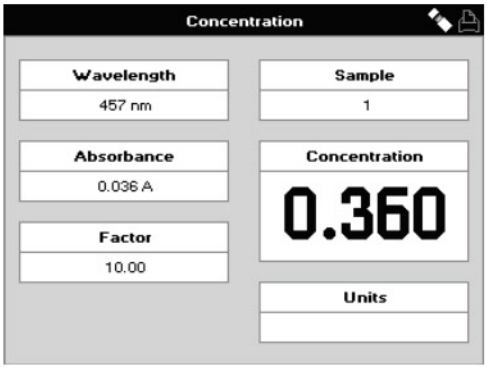
OR

Change the concentration of the standard sample by entering a value of up to four significant figures, using the alphanumeric keys.

Implement the change and return to the measurement screen to run the standard measurement by selecting “OK” using the confirm key.

OR

Reject the changes, return to the measurement and cancel the standard measurement run by selecting “Cancel” using the escape key.



Step 8
Replace the previous sample with a test sample then take a sample measurement using the confirm key.

Repeat for all samples.

WARNING!

Previous measurements cannot be recalled so should be printed or output to a USB flash drive before any subsequent samples are measured.

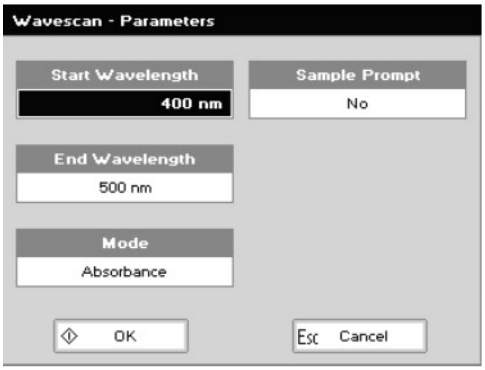
Results

The results are displayed on the screen.

Return to the Applications screen using the escape key OR display the options menu using the options key (see the “Additional Options” section).

Wavescan

The Wavescan application is accessed from the Applications screen using the ‘3’ key. It can be used to perform absorbance (A) or % transmission (%T) measurements across a range of wavelengths creating an absorbance, or transmission, spectrum.

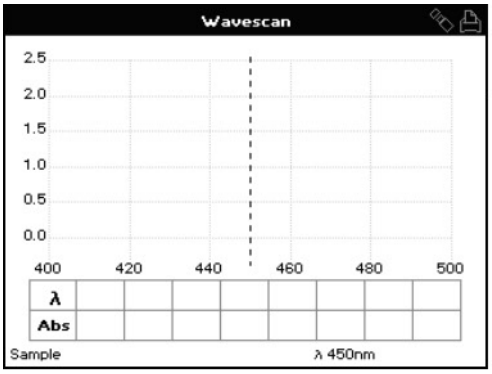


Step 1
Set the start wavelength, 200 to 940 nm, using the left and right arrow or alphanumeric keys.

Step 2
Press the down arrow, set the end wavelength, 210 to 950 nm, using the left and right arrow or alphanumeric keys.

Step 3
Press the down arrow, select the mode, “Absorbance” or “%Transmission”, using the left and right arrow keys.

Step 4
Press the down arrow, set the sample prompt, “Yes” or “No”, to bring up the sample number option before each measurement, using the left and right arrow keys.

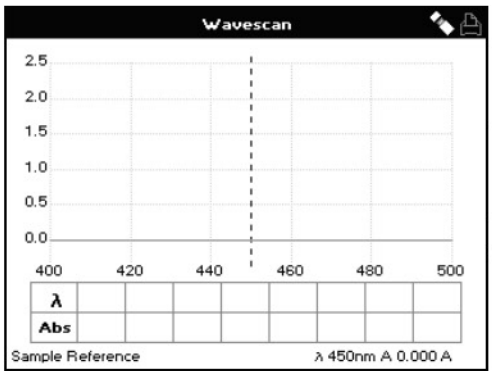


Step 5

Proceed to the measurement screen by selecting “OK” using the confirm key.

OR

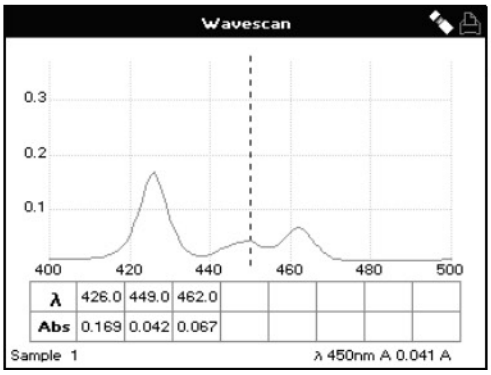
Return to the Applications screen by selecting “Cancel” using the escape key.



Step 6

Insert the reference sample then take a reference measurement using the reference key.

The acquired reference sample baseline will be applied to all subsequent sample measurements.



Step 7

Replace the previous sample with a test sample then take a sample measurement using the confirm key.

Repeat for all samples.

WARNING!

Previous measurements cannot be recalled so should be printed or output to a USB flash drive before any subsequent samples are measured.

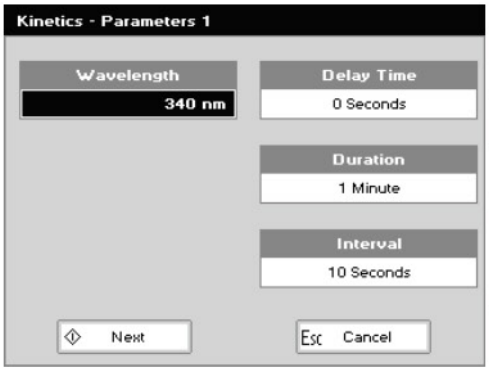
Results

The results are displayed on the screen. Use the left and right arrow keys to move the cursor and display the absorbance at each wavelength position.

Return to the Applications screen using the escape key OR display the options menu using the options key (see the “Additional Options” section).

Kinetics

The Kinetics application is accessed from the Applications screen using the '4' key. It can be used to perform a series of absorbance (A) measurements over a defined timeframe creating a time-course trace.

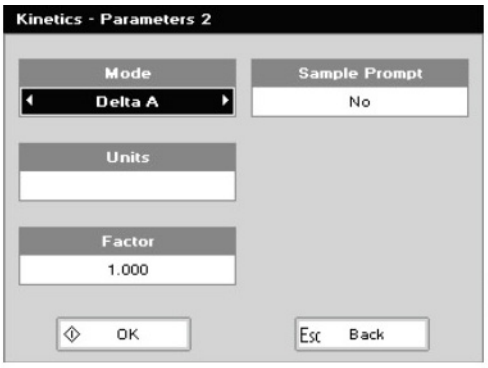


Step 1
Set the wavelength, 190 to 1100 nm, using the left and right arrow or alphanumeric keys.

Step 2
Press the down arrow, set the delay time before the first measurement, 0 to 600 seconds, using the left and right arrow or alphanumeric keys.

Step 3
Press the down arrow, set the duration of the observation, 1 to 60 minutes, using the left and right arrow or alphanumeric keys.

Step 4
Press the down arrow, set the interval between individual measurements, 5 to 60 seconds, using the left and right arrow or alphanumeric keys.



Step 5

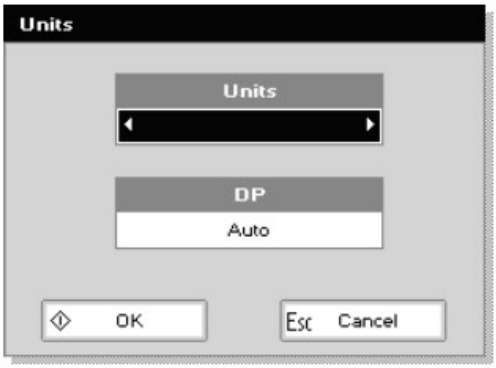
Proceed to the second parameters screen by selecting "Next" using the confirm key.

OR

Return to the Applications screen by selecting "Cancel" using the escape key.

Step 6

Select the mode to define the desired final result, "Delta A", "Final A", or "Slope", using the left and right arrow keys.



Step 7
Press the down arrow, enter up to 8-digits to define the units that the final result will be reported in.

OR

Open the units options using the options key:

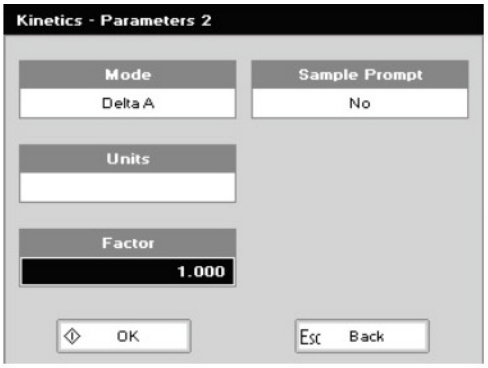
Select one of the predefined units; “µg/ml”, “µg/µl”, “pmol/µl”, “mg/dl”, “mmol/l”, “µmol/l”, “g/l”, “mg/l”, “µg/l”, “U/l”, “%”, “ppm”, “ppb”, or “conc”, using the left and right arrow keys.

Press the down arrow, select the number of decimal places to display the concentration in, “Auto”, “0”, “1”, or “2”, using the left and right arrow keys.

Implement the changes and return to the parameters screen by selecting “OK” using the confirm key.

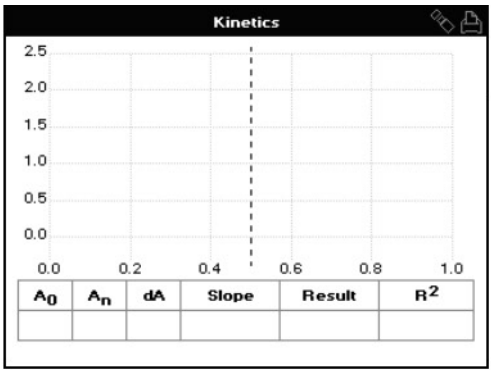
OR

Reject the changes and return to the parameters screen by selecting “Cancel” using the escape key.



Step 8
Press the down arrow, enter a factor to be used to generate the final result of a value of up to four significant figures, using the alphanumeric keys.

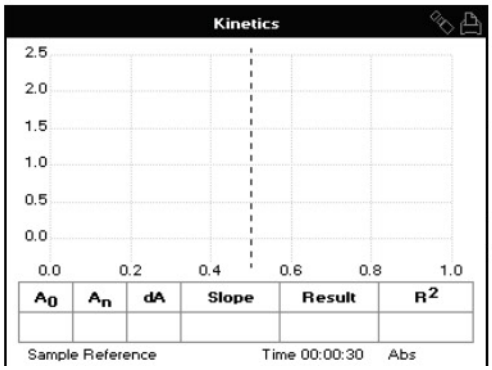
Step 9
Press the down arrow, set the sample prompt, “Yes” or “No”, to bring up the sample number option before each measurement, using the left and right arrow keys.



Step 10
Proceed to the measurement screen by selecting “OK” using the confirm key.

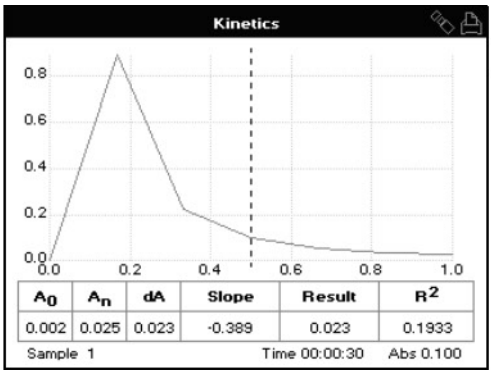
OR

Return to the previous parameters screen by selecting “Back” using the escape key.



Step 11
Insert the reference sample then take a reference measurement using the reference key.

The acquired reference sample baseline will be applied to all subsequent sample measurements.



Step 12
Replace the previous sample with a test sample then take a sample measurement using the confirm key.

Repeat for all samples.

WARNING!

Previous measurements cannot be recalled so should be printed or output to a USB flash drive before any subsequent samples are measured.

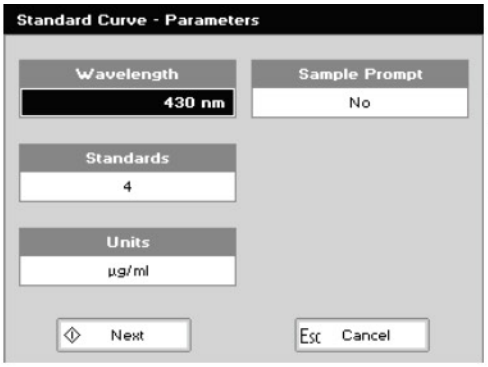
Results

The results are displayed on the screen. Use the left and right arrow keys to move the cursor and display the absorbance at each interval position.

Return to the Applications screen using the escape key OR display the options menu using the options key (see the “Additional Options” section).

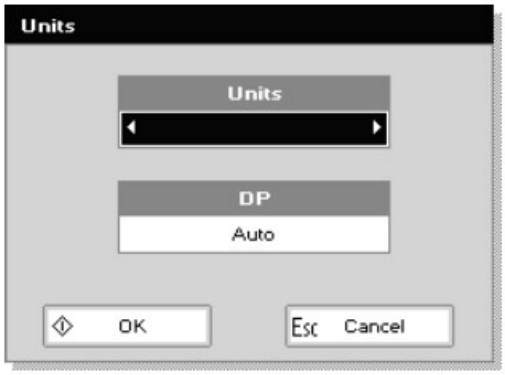
Standard Curve

The Standard Curve application is accessed from the Applications screen using the ‘5’ key. It can be used to create a calibration curve from standard samples of known concentration. The curve fit equation is then applied to the absorbance (A) measurements of any subsequent test samples to determine their concentration.



Step 1
Set the wavelength, 190 to 1100 nm, using the left and right arrow or alphanumeric keys.

Step 2
Press the down arrow, set the number of standard samples of known concentration, 1 to 9, using the left and right arrow or alphanumeric keys.



Step 3
Press the down arrow, enter up to 8-digits to define the units that the primary result will be reported in.

OR

Open the units options using the options key:

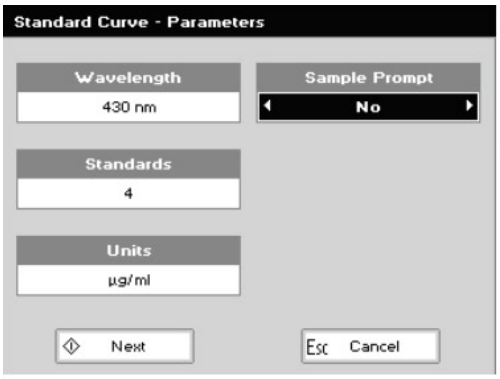
Select one of the predefined units; “µg/ml”, “µg/µl”, “pmol/µl”, “mg/dl”, “mmol/l”, “µmol/l”, “g/l”, “mg/l”, “µg/l”, “U/l”, “%”, “ppm”, “ppb”, or “conc”, using the left and right arrow keys.

Press the down arrow, select the number of decimal places to display the concentration in, “Auto”, “0”, “1”, or “2”, using the left and right arrow keys.

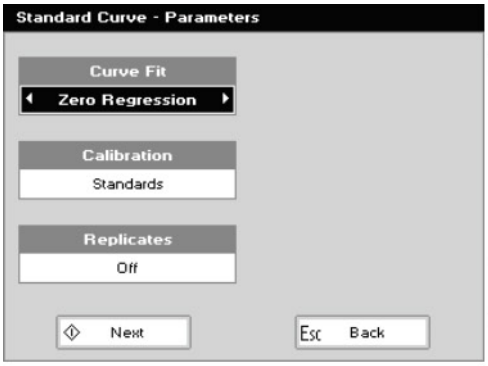
Implement the changes and return to the parameters screen by selecting “OK” using the confirm key.

OR

Reject the changes and return to the parameters screen by selecting “Cancel” using the escape key.



Step 4
Press the down arrow, set the sample prompt, “Yes” or “No”, to bring up the sample number option before each measurement, using the left and right arrow keys.



Step 5
Proceed to the second parameters screen by selecting “Next” using the confirm key.

OR

Return to the Applications screen by selecting “Cancel” using the escape key.

Step 6
Select the curve fit, “Zero Regression”, “2nd Order Polynomial”, “Regression”, “Interpolation”, or “Cubic Spline”, using the left and right arrow keys.

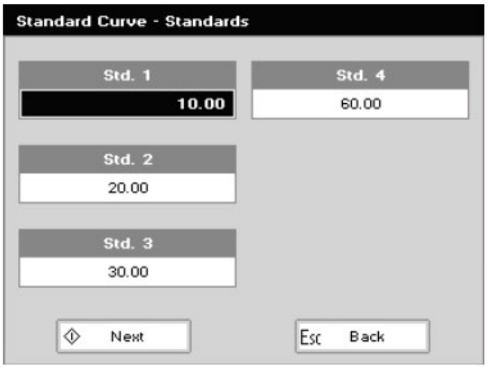
PLEASE NOTE

If only 1 standard sample of known concentration is used to create the standard curve, only a “Zero Regression” fit can be selected. If 2 standard samples are being used to create the standard curve, only “Zero Regression”, “Regression”, and “Interpolation” fits can be selected.

Step 7

Press the down arrow, select the source of the calibration, “Standards”, “Manual”, or “New Standards”, using the left and right arrow keys.

For “Standards” and “New Standards” calibration, press the down arrow and select the number of standard sample replicates, “Off”, “2”, or “3”, using the left and right arrow keys.



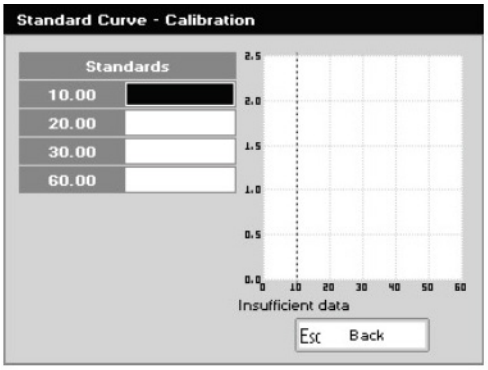
Step 8
Proceed to the Standards screen by selecting “Next” using the confirm key.

OR

Return to the previous parameters screen by selecting “Back” using the escape key.

Step 9

Enter a value of up to four significant figures, for the known concentration of each standard to be used using the alphanumeric keys.



Step 10
Proceed to the Calibration screen by selecting "Next" using the confirm key.

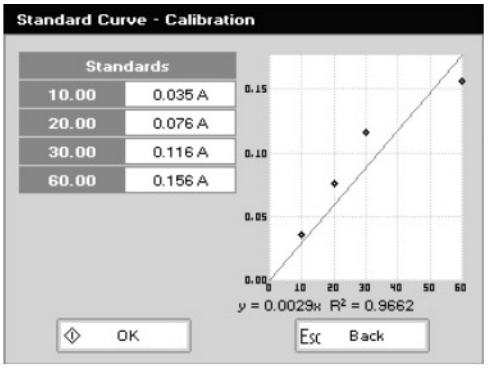
OR

Return to the previous parameters screen by selecting "Back" using the escape key.

Step 11

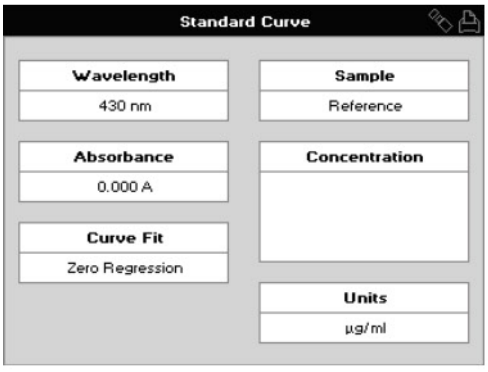
Insert the reference sample then take a reference measurement using the reference key.

The acquired reference sample baseline will be applied to all subsequent standard and test sample measurements.



Step 12
Replace the previous sample with the first standard sample then take a sample measurement using the confirm key.

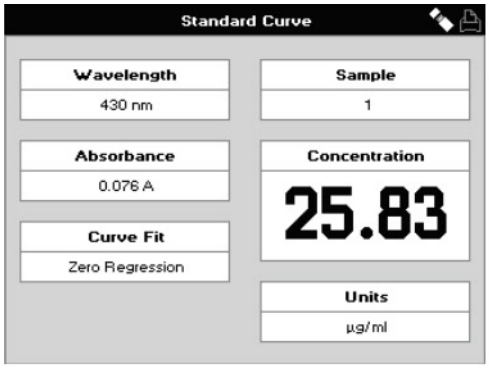
Repeat for all standards samples.



Step 13
Proceed to the measurement screen by selecting "OK" using the confirm key.

OR

Return to the standards screen by selecting "Back" using the escape key.



Step 14
Insert a test sample then take a sample measurement using the confirm key.

Repeat for all samples.

WARNING!

Previous measurements cannot be recalled so should be printed or output to a USB flash drive before any subsequent samples are measured.

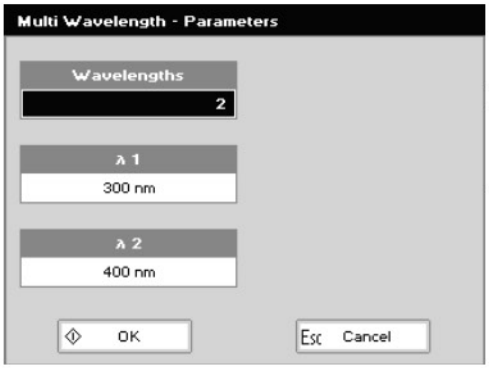
Results

The results are displayed on the screen.

Return to the Applications screen using the escape key OR display the options menu using the options key (see the “Additional Options” section).

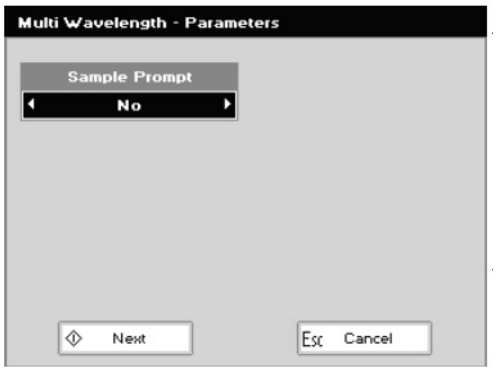
Multi Wavelength

The Multi Wavelength application is accessed from the Applications screen using the ‘6’ key. It can be used to perform simple absorbance (A) measurements at up to five specific wavelengths.



Step 1
Set the number of wavelengths, 2 to 5, using the left and right arrow or alphanumeric keys.

Step 2
Press the down arrow, set the first wavelength, 190 to 1100 nm, using the left and right arrow or alpha-numeric keys.



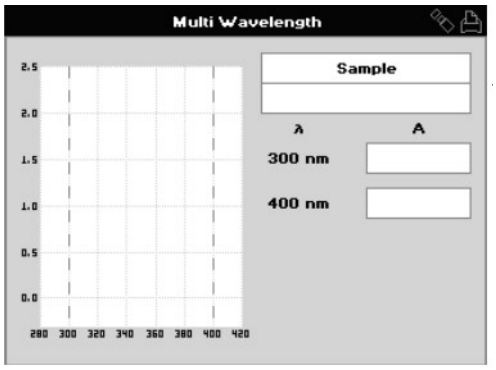
Step 3
Proceed to the second parameters screen by selecting "OK" using the confirm key.

OR

Return to the Applications screen by selecting "Cancel" using the escape key.

Step 4
Press the down arrow, set the sample prompt, "Yes" or "No", to bring up the sample number option before each measurement, using the left and right arrow keys.

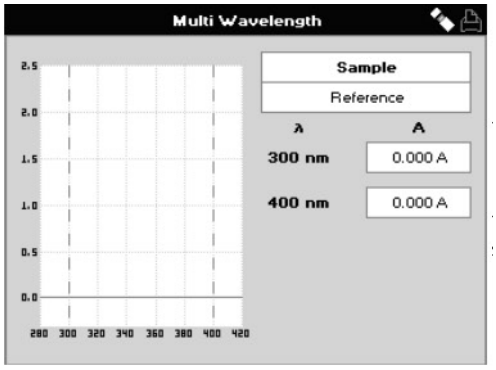
Repeat for all wavelengths.



Step 5
Proceed to the measurement screen by selecting "OK" using the confirm key.

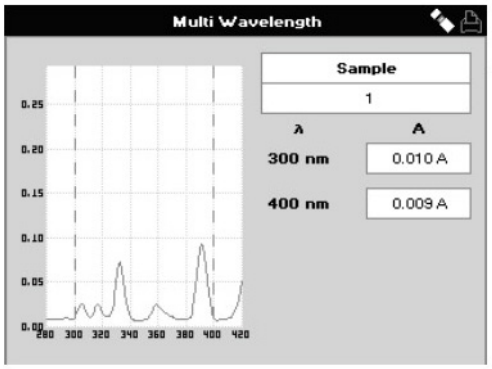
OR

Return to the previous parameters screen by selecting "Cancel" using the escape key.



Step 6
Insert the reference sample then take a reference measurement using the reference key.

The acquired reference sample baseline will be applied to all subsequent sample measurements.



Step 7
Replace the previous sample with a test sample then take a sample measurement using the confirm key.

Repeat for all samples.

WARNING!

Previous measurements cannot be recalled so should be printed or output to a USB flash drive before any subsequent samples are measured.

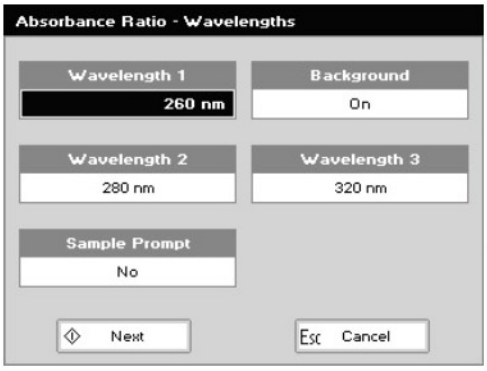
Results

The results are displayed on the screen.

Return to the Applications screen using the escape key OR display the options menu using the options key (see the “Additional Options” section).

Absorbance Ratio

The Absorbance Ratio application is accessed from the Applications screen using the ‘7’ key. It can be used to perform two absorbance (A) measurements and calculate the absorbance ratio between them.



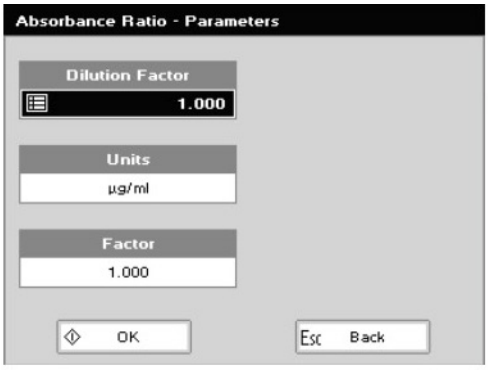
Step 1
Set wavelength 1, 190 to 1100 nm, using the left and right arrow or alphanumeric keys.

Press the down arrow, repeat for wavelength 2.

Step 2
Press the down arrow, set the sample prompt, “Yes” or “No”, to bring up the sample number option before each measurement, using the left and right arrow keys.

Step 3
Press the down arrow, set the background, “On” or “Off”, using the left and right arrow keys.

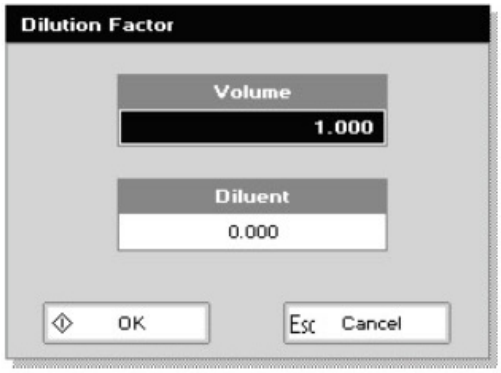
For background set to “On”, press the down arrow and set wavelength 3, 190 to 1100 nm, using the left and right arrow or alphanumeric keys.



Step 4
Proceed to the parameter screen by selecting “OK” using the confirm key.

OR

Return to the Applications screen by selecting “Cancel” using the escape key.



Step 5
Enter any dilution factor to be applied to the absorbance measurement of a value of up to four significant figures, using the alphanumeric keys.

OR

Open the dilution factor options using the options key:

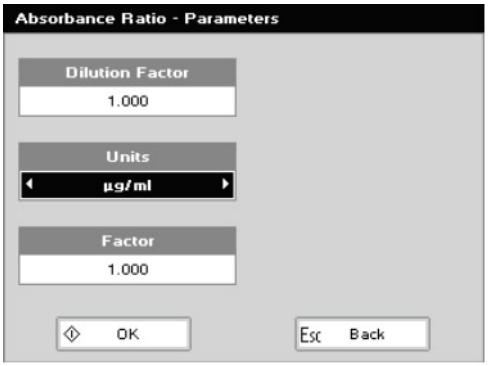
Set the initial sample volume of a value of up to four significant figures, using the alphanumeric keys.

Press the down arrow, set the amount of diluent added to the initial volume of a value of up to four significant figures, using the alphanumeric keys.

Implement the changes and return to the parameters screen by selecting “OK” using the confirm key.

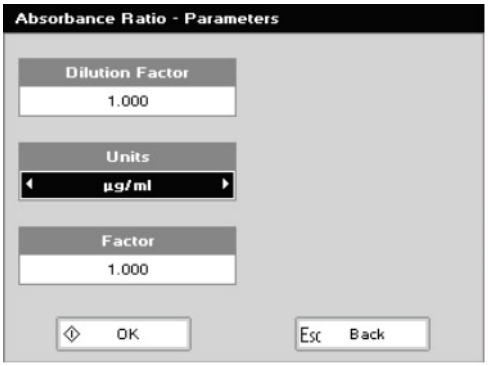
OR

Reject the changes and return to the parameters screen by selecting “Cancel” using the escape key.



Step 6
Press the down arrow, select one of the predefined units, “µg/ml”, “ng/µl”, or “µg/µl”, using the left and right arrow keys.

Step 7
Press the down arrow, enter a concentration factor of up to four significant figures, using the alphanumeric keys.

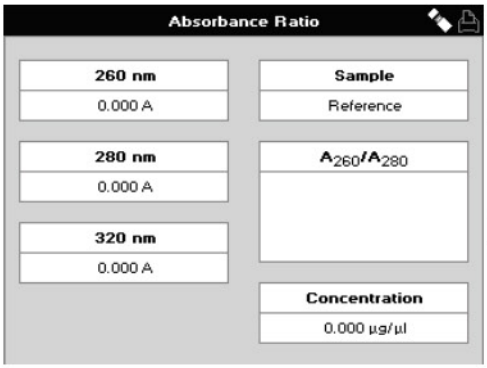


Step 8

Proceed to the measurement screen by selecting "OK" using the confirm key.

OR

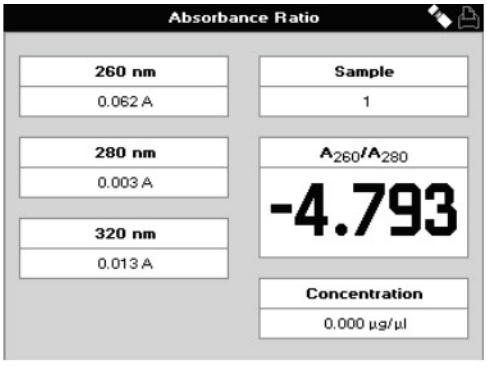
Return to the wavelengths screen by selecting "Back" using the escape key.



Step 9

Insert the reference sample then take a reference measurement using the reference key.

The acquired reference sample baseline will be applied to all subsequent sample measurements.



Step 10

Replace the previous sample with a test sample then take a sample measurement using the confirm key.

Repeat for all samples.

WARNING!

Previous measurements cannot be recalled so should be printed or output to a USB flash drive before any subsequent samples are measured.

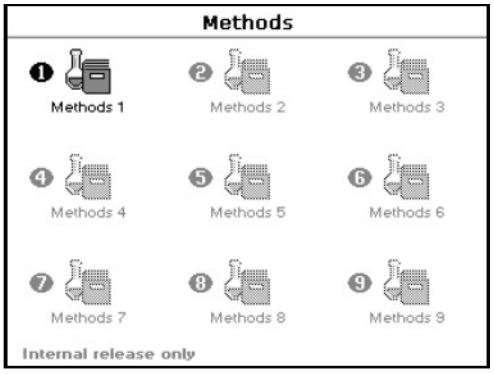
Results

The results are displayed on the screen.

Return to the Applications screen using the escape key OR display the options menu using the options key (see the "Additional Options" section).

Favourites, Methods, and USB Memory Stick

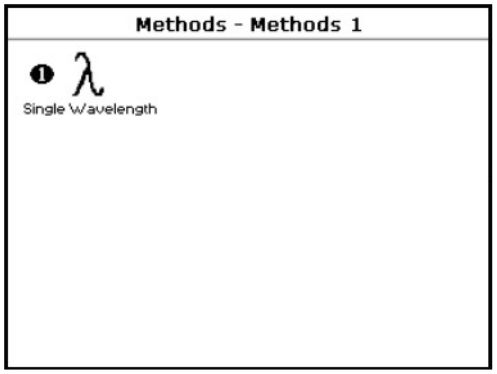
The Favourites, Methods, and USB Memory Stick screens are accessed from the home screen using the ‘5’, ‘6’, or ‘8’ key respectively. They are directories to save custom methods to, using the options menu from the results screen (see the “Additional Options” section).



Select the methods subdirectory, where the custom method is saved, using the appropriate alphanumeric key.

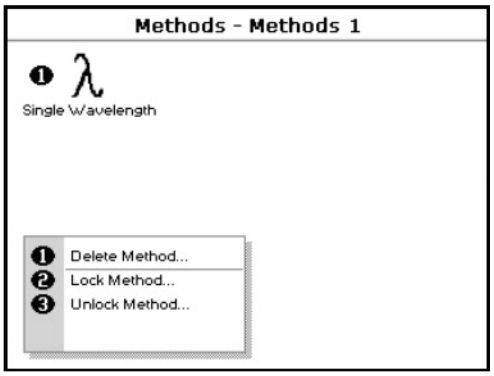
PLEASE NOTE

This only applies to the Methods screen. When selected from the home screen, the Favourites and Memory Stick screens directly display the saved custom method applications

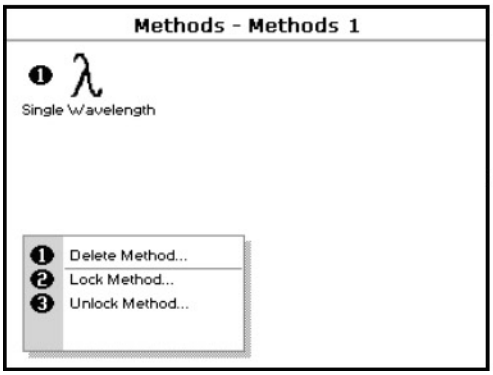


Once the directory containing the saved custom method application is accessed, there are several options available OR return to the home screen using the escape key.

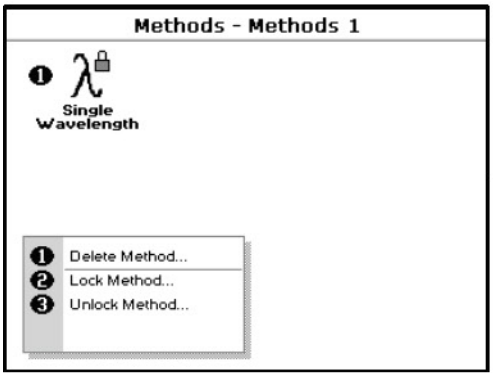
1. To open a saved custom method application, use the appropriate alphanumeric key.



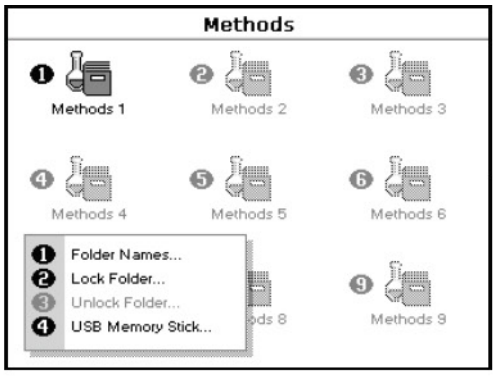
2. To delete a saved custom method application, use the options key then press the ‘1’ key.

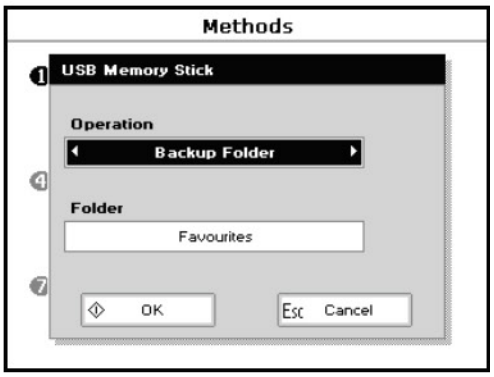


3. To lock protect a saved custom method application, use the options key then press the ‘2’ key.



4. To unlock a protected saved custom method application, use the options key then press the ‘3’ key.





5. To back up or restore custom method applications, insert a USB flash drive then use the options key then press the ‘4’ key.

Select the operation, “Backup Folder”, “Restore Folder”, “Backup All Folders”, or “Restore All Folders”, using the left and right arrow keys.

For “Backup Folder” and “Restore Folder” operations, press the down arrow and select the folder to perform the operation on, “Favourites”, “Methods 1”, “Methods 2”, “Methods 3”, “Methods 4”, “Methods 5”, “Methods 6”, “Methods 7”, “Methods 8”, or “Methods 9”, using the left and right arrow keys.

Perform the operation and return to the Methods screen by selecting “OK” using the confirm key.

OR

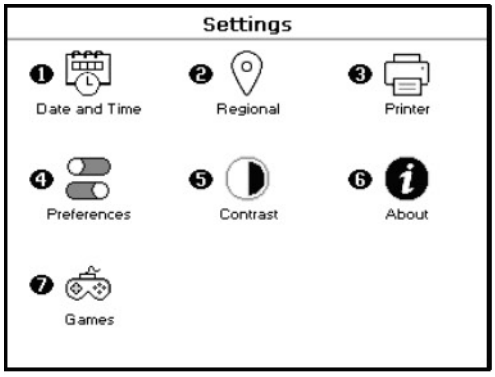
Cancel the operation and return to the Methods screen by selecting “Cancel” using the escape key.

WARNING!

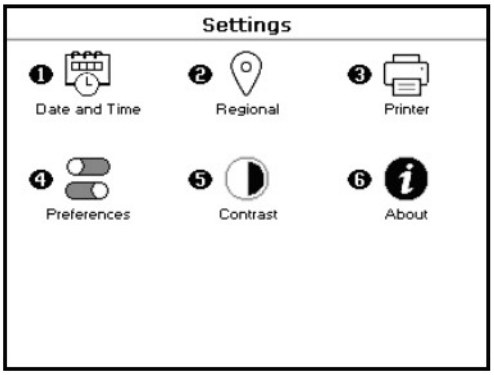
This operation cannot be undone and even locked methods are effected. Therefore, it is recommended to make a separate backup before restoring form a previous one.

Settings

The Settings screen is accessed from the home screen using the '7' key. It can be used to access application to adjust the instrument settings, such as date, time, language, and number format.



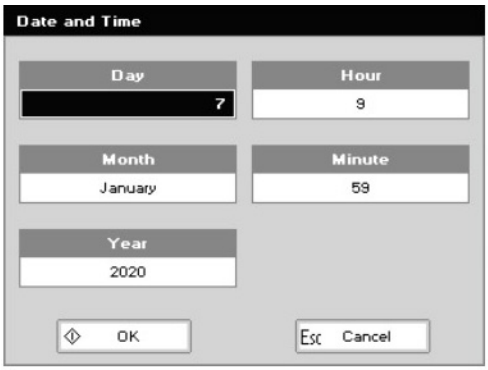
Default Settings screen displaying the Games application



Settings screen with the Games application hidden

Date and Time

The Date and Time application is accessed from the Settings screen using the '1' key. It can be used to adjust the date and time stamp applied to measurement data outputs.



Step 1
Set the day using the left and right arrow or alphanumeric keys.

Step 2
Press the down arrow, set the month using the left and right arrow keys.

Step 3
Press the down arrow, set the year using the left and right arrow or alphanumeric keys.

Step 4
Press the down arrow, set the hour (24-hour format) using the left and right arrow or alphanumeric keys.

Step 5
Press the down arrow, set the minute using the left and right arrow or alphanumeric keys.

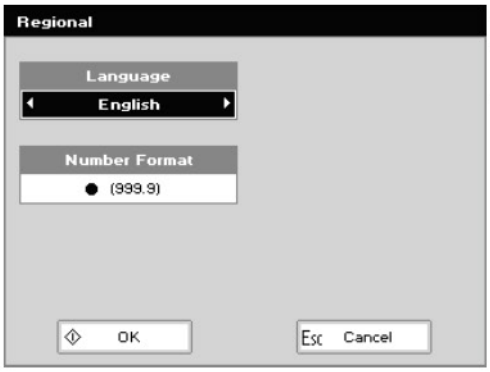
Implement the changes and return to the Settings screen by selecting OK using the confirm key.

OR

Reject the changes and return to the Settings screen by selecting "Cancel" using the escape key.

Regional

The Regional application is accessed from the Settings screen using the '2' key. It can be used to change the language and decimal separator number format.



Step 1
Set the language using the left and right arrow keys.

Step 2
Press the down arrow, set the number format decimal separator using the left and right arrow keys.

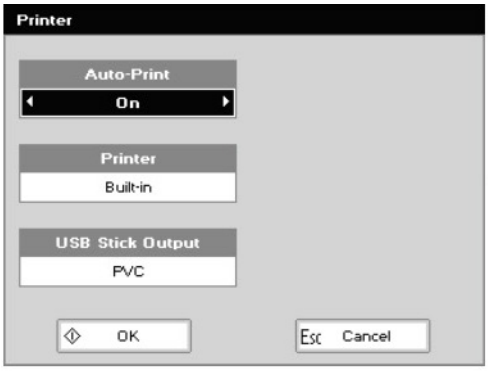
Implement the changes and return to the Settings screen by selecting OK using the confirm key.

OR

Reject the changes and return to the Settings screen by selecting "Cancel" using the escape key.

Printer

The Printer application is accessed from the Settings screen using the '3' key. It can be used to define the default printer and data output settings.



Step 1
Set auto-print "On" of "Off" using the left and right arrow keys.

Step 2
Press the down arrow, set the printer output to a "Built-in" printer, a computer via a USB cable ("Computer (USB)"), or a computer via Bluetooth ("Computer (Bluetooth)") depending on what is connected to the instrument, using the left and right arrow keys.

Step 3
Press the down arrow, set the USB file type for output to a connect USB flash drive ("PVC" or "TSV") using the left and right arrow keys.

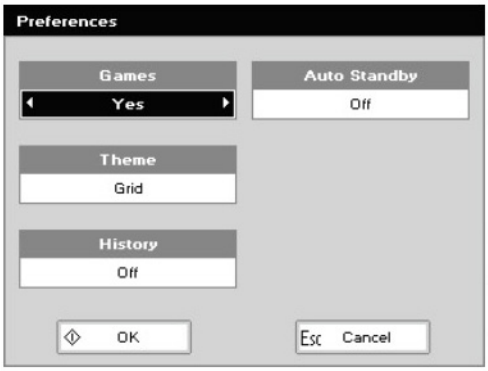
Implement the changes and return to the Settings screen by selecting OK using the confirm key.

OR

Reject the changes and return to the Settings screen by selecting "Cancel" using the escape key.

Preferences

The Preference application is accessed from the Settings screen using the ‘4’ key. It can be used to set the access to the games application, the menu layout theme, parameter history, and standby settings.



Step 1
Set access to the games application or not, using the left and right arrow keys.

Step 2
Press the down arrow, set the application menus layout theme using the left and right arrow keys.

Step 3
Press the down arrow, set the parameter history to store application settings for future use or not, using the left and right arrow keys.

Step 4
Press the down arrow, set the auto standby interval using the left and right arrow keys.

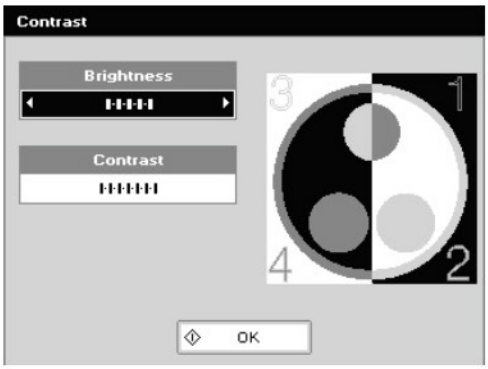
Implement the changes and return to the Settings screen by selecting OK using the confirm key.

OR

Reject the changes and return to the Settings screen by selecting “Cancel” using the escape key.

Contrast

The Contrast application is accessed from the Settings screen using the ‘5’ key. It can be used to set the display brightness and contrast.



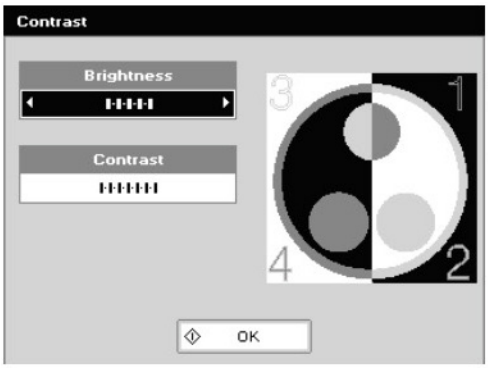
Step 1
Set the brightness using the left and right arrow keys.

Step 2
Press the down arrow, set the contrast using the left and right arrow keys.

Implement the changes and return to the Settings screen by selecting OK using the confirm key.

About

The About pop-up window is opened from the Settings screen using the '6' key. The pop-up window displays information about the instrument, including its serial number, firmware version, and build.



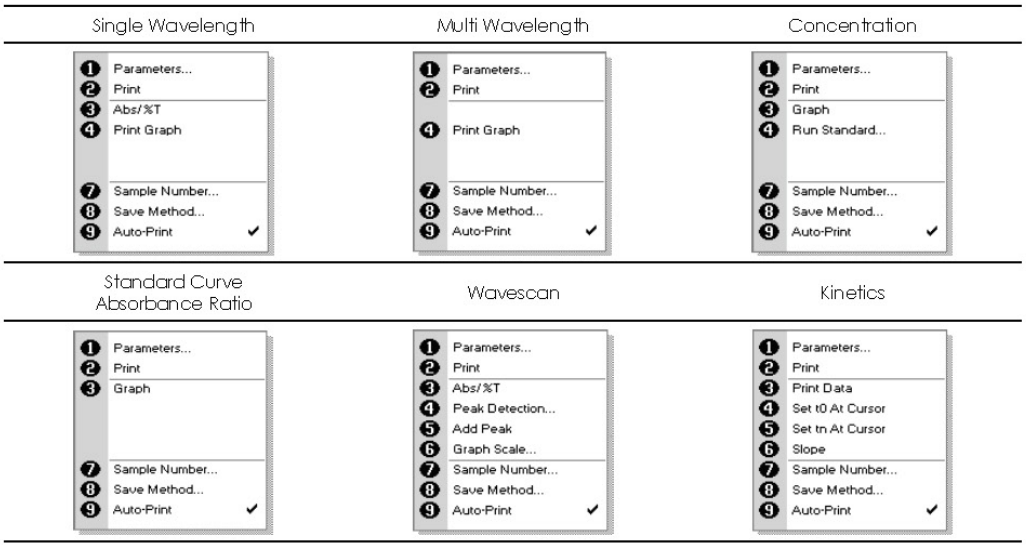
Step 1
Set the brightness using the left and right arrow keys.

Step 2
Press the down arrow, set the contrast using the left and right arrow keys.

Implement the changes and return to the Settings screen by selecting OK using the confirm key.

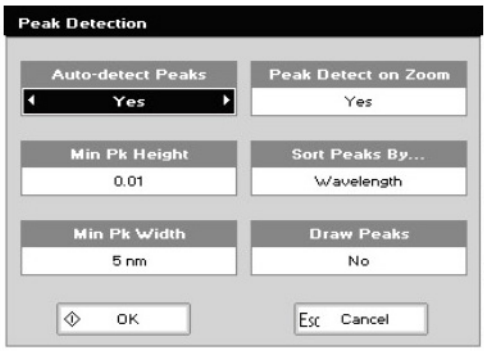
Additional Options

Additional options are available from the measurement screen using the options key. The available options for key press 3, 4, 5, and 6 varies between applications.



Key Press	Description
1	**Parameters** – Return to method parameters screen.
2	**Print** – Print result via selected method.
3	**Abs/%T** – Toggle between Absorbance and %T mode. **Graph** – Displays the graph showing the measurements' local spectrum. **Print Data** – Prints tabulated time-point data.

4



Print Graph – Print graph via selected setting.

Run Standard – Re-run and standard sample measurement.

Peak Detection – Open the peak detection parameters to define the auto-peak detection settings.

Step 1
Set the method to auto-detect peaks or not using the left and right arrow keys.

For "Yes" to auto-detect peaks, press the down arrow and set the minimum peak height (0.00 to 9999) using the alphanumeric keys. Then press the down arrow and set the minimum peak width (1 to 190 nm) using the alphanumeric keys. Then press the down arrow and set to peak detect on zoom or not using the left and right arrow keys.

Step 2
Press the down arrow, set the sort by peaks by "Wavelength", "Peak Height", or "Peak Width", using the left and right arrow keys.

Step 3
Press the down arrow, set to draw peaks or not using the left and right arrow keys.

Implement the changes and return to the parameters screen by selecting "OK" using the confirm key.

OR

Reject the changes and return to the parameters screen by selecting "Cancel" using the escape key.

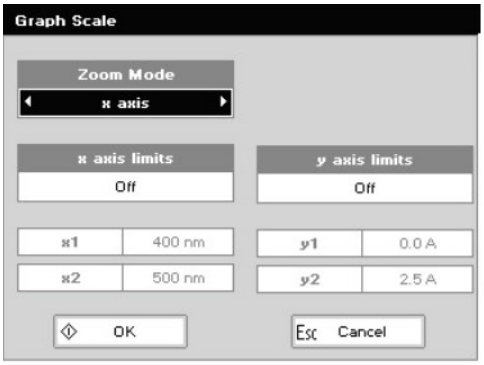
Set t0 At Cursor – Sets the zero time-point of the slope at the current cursor position.

5

Add Peak – Adds the measurement at the current cursor position to the peak table.

Set tn At Cursor – Sets the final time-point of the slope at the current cursor position.

6



Graph Scale – Open the graph scale parameters

Step 1
Set the zoom mode using the left and right arrow keys.

Step 2
Press the down arrow, apply x axis limits or not using the left and right arrow keys.

For “On” to x axis limits, press the down key and set the x-axis start value (“x1”) using the alphanumeric keys, then press the down arrow and set the x-axis end value (“x2”).

Step 3
Press the down arrow, apply y axis limits or not using the left and right arrow keys.

For “On” to y axis limits, press the down key and set the y-axis start value (“y1”) using the alphanumeric keys, then press the down arrow and set the y-axis end value (“y2”).

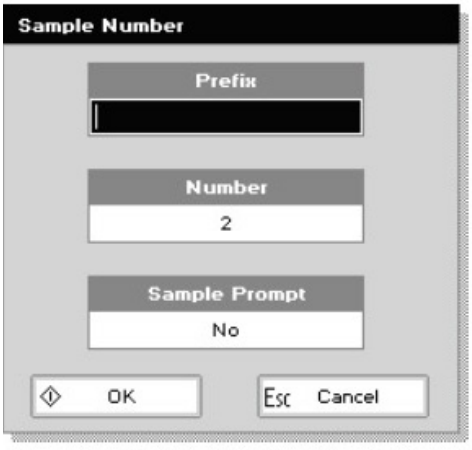
Implement the changes and return to the parameters screen by selecting “OK” using the confirm key.

OR

Reject the changes and return to the parameters screen by selecting “Cancel” using the escape key.

Slope – Fits a trend line across the time-course data or between the set t0 and tn points.

7



Sample Number…

Open sample number options.

Insert up to an 8-digit prefix using the alphanumeric keys.

Press the down arrow, insert a number up to 9999.

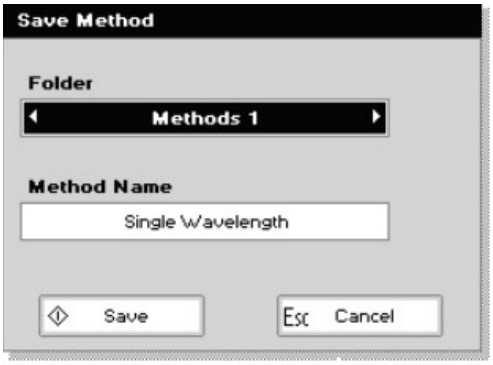
Press the down arrow, change the sample prompt setting using the left and right arrow keys.

Implement the changes and return to the measurement screen by selecting OK using the confirm key.

OR

Reject the changes and return to the measurement screen by selecting “Cancel” using the escape key

8



Sample Number…

Open save method options.

Selection the folder location in which to save the method using the left and right arrow keys.

Press the down arrow, Insert up to a 25-digit method name using the alphanumeric keys.

Save the method and return to the measurement screen by selecting OK using the confirm key.

OR

Return to the measurement screen without saving the method by selecting “Cancel” using the escape key.

9

Auto-Print – Toggle auto-print on or off.

Status Bar Icons

During the measurement process, various status icons are displayed in the top righthand corner status bar. Which icon are displayed depends on the current process being undertaken and the defined settings.


Auto-print to computer via Bluetooth is active.


Auto-print to built-in printer is active.


Auto-print to computer via USB cable is active.


USB flash drive is inserted.


Taking measurement.


Printing to computer via the Bluetooth connection.


Printing to the built-in printer.


Printing to computer via the USB cable connection.


Sending data output to inserted USB flash drive.

USEFUL CALCULATIONS

Beer-Lambert Law

$$A = c\varepsilon l$$

A is the absorbance, which although unit-less is usually described as A or AU (absorbance units).

c is the concentration in molar units (M).

ε is the molar extinction coefficient in per molar unit per cm (M-1cm-1).

l is the pathlength in centimetres (cm).

As the absorbance value is the known quantity, the Beer-Lambert equation can be rearranged to make concentration (c) the product:

$$c = \frac{A}{\varepsilon \times l}$$

Alternative extinction coefficients can be applied to calculate the concentration in alternative units

ε	c
Molar extinction coefficient (M-1cm-1)	Molar, or moles per litre, concentration (M, mol L-1)
Mass extinction coefficient (g-1cm-1)	0.1 % Mass per volume concentration (g L-1)
E1% extinction coefficient (mg-1mL-1cm-1)	1 % Mass per volume concentration (10 g L-1)

Conversions between molar, mass, E1% extinction coefficients:

$$\frac{\text{Molar Extinction Coefficient}}{\text{Molecular Weight (g mol-1)}} = \text{Mass Extinction Coefficient}$$

$$\text{Mass Extinction Coefficient} \times 10 = \text{E1\% Extinction Coefficient}$$

When E1% extinction coefficient are used, the absorbance is multiplied by 10 to present the concentration as a 0.1 % weight per volume (w/v) unit in keeping with convention:

$$c = \frac{A \times 10}{E1\% \times l}$$

TROUBLESHOOTING

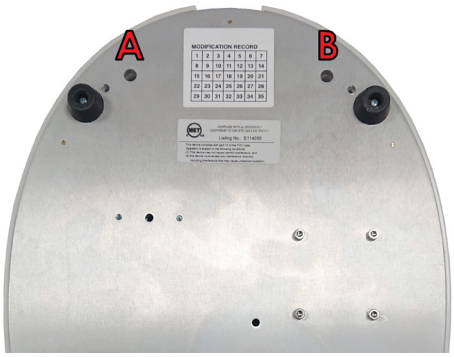
Negative absorbance readings	• Sample measurements will be negative absorbance reading if the absorbance value of the reference is higher than the sample. • Negative readings can also result if reference and sample are interchanged or if the sample is very dilute and close to the absorbance of the reference.
Unexpected results	• Bubbles or contamination in the sample or reference can result in considerable errors. • Incorrect cuvette orientation. Rotate by 90˚ and repeat. • Incorrect cuvette material for UV measurement wavelengths. • Wrong pathlength selected in software. • Contact your supplier for advice on the minimum concentrations that can be measured.
Absorbance higher than expected	• Incorrect sample reference. • Incorrect cuvette orientation. • Incorrect cuvette material for measurement wavelengths. • Wrong pathlength selected in software. • Contamination in sample or on cuvette. • Check baseline, if greater than 0 A toggle background correction or us an appropriate reference sample. • Possible incorrect optical alignment. Contact technical support.
Absorbance lower than expected	• Incorrect sample reference. • Check sample and reference for contamination. • Check sample and reference samples are not the same. • Incorrect cuvette material for measurement wavelengths. • Wrong pathlength selected in software. • Check the beam height and buffer sample volume. • Check baseline, if greater than 0 A toggle background correction or us an appropriate reference sample. • Possible stray light issue. Contact technical support.
Poor reproducibility	• Insufficient sample in cuvette. • Cuvette in wrong orientation. • Cuvette material unsuitable for wavelengths used. • Concentration of sample too low or too high. For best results, the measured sample absorbance using a 10 mm pathlength cuvette should ideally be between 0.1 and 1.0 A. If absorbance is >1 A, measurement is no longer in the most linear range. • Particulates in sample. Absorbance measurements will not be accurate with turbid samples. • Possible noise or measurement stability issue. Contact technical support
Instrument start up reported failure	• Check the cell holder is empty. • Check original 18V dc supply is connected and is fully engaged. • Report persistent failures to technical support.

Built-in Printer Accessory Part Numbers

80-3003-84 – Built-in Printer accessory

80-3004-07 – Spare paper for printer (20 rolls)

Printer Installation Guide



Step 1
Invert the instrument and remove the screws from positions A and B.

Step 2
Return the instrument to its upright position and lift the accessory cover vertically upwards.

Step 3
Remove any tie-wraps from the printer cable and insert its connector to the socket on the underside of the printer.

Step 4
Lower the printer onto the locating bosses and push down firmly.

Step 5
Invert the instrument and replace the accessory cover screws at A and B.

Refilling the Printer Paper

Step 1
Lift off the printer cover using the tabs and discard the spent roll.

Step 2
Open the platen lock, feed in the paper so that the loose end is underneath the roll pointing towards the front of the instrument, then close the platen lock and draw the loose end through using the platen wheel.

Step 3
Replace the printer cover so that the loose end sits above it.

Ordering Information

Part Number	Description
80-3007-42	WPA Lightwave 3
80-3007-43	WPA Lightwave 3 with Printer
80-3007-44	WPA Lightwave 3 with Bluetooth
80-3007-45	WPA Lightwave 3 with Printer and Bluetooth
80-3007-47	WPA Lightwave 3+
80-3007-48	WPA Lightwave 3+ with Printer
80-3007-49	WPA Lightwave 3+ with Bluetooth
80-3007-50	WPA Lightwave 3+ with Printer and Bluetooth

Contact Us

Biochrom Ltd.

Building 1020, Cambourne Business Park
Cambourne, Cambridge, UK, CB23 6DW

phone +44 1223 42 723
fax +44 1223 420 164
e-mail support@hbiosci.com
web www.biochrom.co.uk
www.biochromspectros.com

Biochrom U.S.

84 October Hill Road
Holliston, Massachusetts 01746 USA

phone +1 508-893-8999
toll-free +1 877-246-2476
fax +1 508-429-5732
e-mail support@hbiosci.com
web www.biochrom.co.uk
www.biochromspectros.com

Harvard Bioscience (Shanghai) Co., Ltd.

Room 8C, Zhongxi Tower, 121 Jiangsu Road,
Changning District, Shanghai, China, 200050

phone +86 21 6226 0239
e-mail support@hbiosci.com
web www.biochrom.co.uk
www.biochromspectros.com