Libra S32
User Manual

English

Deutsch

Français

Español

Italiano

biochrom

Biochrom Ltd
Certificate No. 890333

Declaration of Conformity

This is to certify that the Libra S32 UV/Visible Spectrophotometer
Part number 80-2115-30
Serial number 81000 onwards

manufactured by Biochrom Ltd. conforms to the requirements of the following Directives-: 73/23/EEC & 89/336/EEC

Standards to which conformity is declared

EN 61 010-1: 2001
Safety requirements for electrical equipment for measurement, control and laboratory use.

EN 61326: 1998
Electrical equipment for measurement, control and laboratory use – EMC requirements

Signed: Dated: 23rd October 2002

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Unpacking, Positioning and Installation

- Inspect the instrument for any signs of damage caused in transit. If any damage is discovered, inform your supplier immediately.
- Ensure your proposed installation site conforms to the environmental conditions for safe operation:
  Indoor use only
  Temperature 10°C to 40°C
  Maximum relative humidity of 80 % up to 31°C decreasing linearly to 50 % at 40°C
- The instrument must be placed on a hard flat surface, for example a laboratory bench or table, which can take its weight (13 kg) such that air is allowed to circulate freely around the instrument.
- Ensure that the cooling fan inlets and outlets are not obstructed; position at least 2 inches from the wall.
- This equipment must be connected to the power supply with the power cord supplied and must be earthed (grounded). It can be used on 90 - 240V supplies.
- Switch on the instrument and check that the display works (see Operation). It can be configured to have the display and print outs in English (0), German (1), French (2), Spanish (3), Italian (4) or Russian (5) by pressing the number in brackets as the instrument powers up (default is English).
- The instrument is delivered with a stored baseline. This is required to correct for the wavelength/energy profile of the light sources. A new baseline should be stored when a lamp is changed or if the instrument is not used for a long time (several weeks); refer to Maintenance for details.
- To enter laboratory name, operator name, instrument asset number, current date/time and to configure for a particular type of printer, refer to Instrument Utilities.

> This is a “press to read” instrument, whereas other deuterium / tungsten lamp instruments measure continuously. The lamps will switch off from stand by mode automatically if the instrument is not used for 15 minutes; the message “Turning lamp on…” will appear for a few seconds when the instrument is re-used.

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.

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 is yellow, symbol and outline are black.

WARNING

UV RADIATION
HOT

WARNING

UV RADIATION IS HARMFUL TO YOUR EYES
If power is restored with this cover removed, eye protection must be worn

Accessories

- Care should be taken when handling all heated accessories.
- Ensure that the cell compartment lid is closed when operating cell changers and the sipper.
- It is essential that the baseplate plug supplied with single cell accessories is fitted to optimise air flow and to prevent light ingress.

OPERATION

Introduction

Your UV/Visible spectrophotometer is a stand alone, simple-to-use instrument with a high-resolution liquid crystal display (LCD), and a comprehensive range of spectrophotometry measurements can be undertaken. It fulfils the requirements of the Pharmacopoeia (Appendix).

It works on the basis of light from the deuterium and tungsten lamps being directed by a fixed mirror through the monochromator inlet slit. This passes through one of several (dependent on wavelength selected) filters mounted on a filter quadrant; the filtered light is then directed onto the holographic grating that produces light of the selected wavelength. The light then leaves the monochromator via the exit slit, and mirrors focus and direct the light into the sample compartment. This passes through your cell, containing the sample of interest, and then a defocusing lens to a solid state detector unit. The resulting signal is then filtered and displayed.

Your spectrophotometer:

- Measures standard absorbance, concentration and % transmission.
- Has Application Modes for
  - Wavescan (Wavelength Scanning)
  - Enzyme Kinetics
  - Multiple Wavelength (Equation Entry)
  - Standard Curve
  - Substrate Concentration
- Can store up to 50 user-defined methods
- Can download results directly via the serial interface for manipulation in Excel and for data storage and archiving
- Has GLP self diagnostics

A range of accessories further enhances the capability of the instrument.

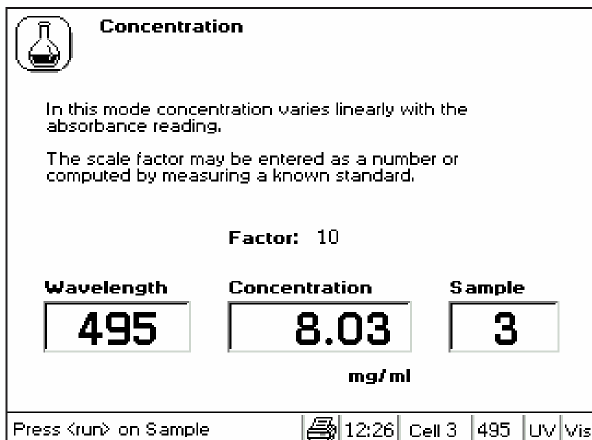
Keypad and display

Navigation around the displays, presented in an index card format, and the options therein is by using the ◀ ▶ ▲ ▼ keys; press **enter** or ▼ to select an option. Number, letter and base entry will become active when appropriate.

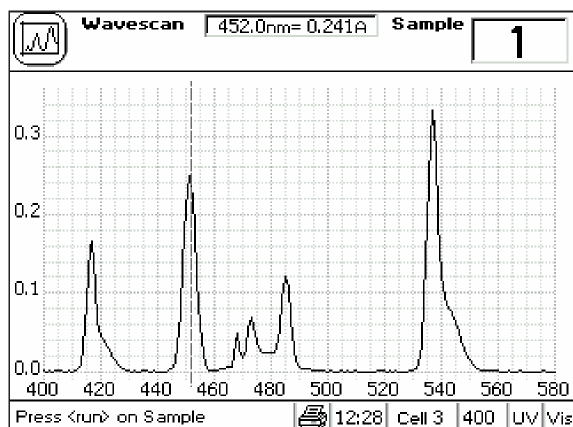
- press **mode** to select measurement mode or to recall set-up pages, enabling selection of post run routines, after obtaining results
- press **function** to access instrument utilities
- press **set ref** to set reference at all wavelengths in the mode selected; this is subtracted from all subsequent samples in the experiment
- press **print** to output graphics and results to a parallel printer or PC; this is automatic if Auto-print is selected (see Instrument Utilities)
- press **enter** to select an option on the display
- press **C** to clear a numeric entry. If in Multi-Wavelength mode, **C** enables set reference in order to restart another experiment
- press **run** to start measurements when operating from within the measurement modes; this may set reference automatically in "non-Basic" modes . Sample number (and cell position) is automatically incremented.
- press **stop** to end current activity; use as an "escape mechanism" to stop making measurements or to return to main menu

There are two standard formats of display, depending on the measurement mode:

a) Basic (Absorbance, %Transmission, Concentration) has a simple layout, with information presented in a box on the LCD.



b) Other measurement modes have a graphical layout with information about the instrument also being provided along a status bar which shows messages, whether a printer is connected or not, time, accessory fitted (cell number for multi-cell holder) and wavelength. There are also messages that tell the user what needs to be done to perform a routine, and the status of the instrument during this routine; for example set-ref, setting-ref, load sample, running sample.



Basic Modes

In the basic modes it is possible to change cell position by pressing the required number on the keypad.

Absorbance

Absorbance mode measures the amount of light that has passed through a sample relative to a blank (this can be air). The procedure is as follows:

- Enter appropriate wavelength and press **enter**
- Enter appropriate sample number and press **enter**
- Insert reference and press **set ref**. The cell changer, if fitted, automatically moves to position 2 and displays the result for the reference measurement (0.000)
  - This is a “press to read” instrument, whereas other deuterium / tungsten lamp instruments measure continuously. Thus to monitor sample stabilisation, the simple kinetics mode must be used
  - This reference value is used for subsequent samples until changed
- Insert samples as required and press **run** (repeat as necessary)
- To go back and change the wavelength press **mode**

% Transmission

Transmission mode measures the amount of light that has passed through a sample relative to a blank (this can be air), but displays the result as a percentage. The relationship between the concentration of the sample and its transmittance at any given wavelength is not linear, and hence transmission mode is rarely used experimentally except for samples having very high absorbances (low transmittances). The procedure is as follows:

- Enter appropriate wavelength and press **enter**
- Enter appropriate sample number and press **enter**
- Insert reference and press **set ref**. The cell changer, if fitted, automatically moves to position 2 and displays the result for the reference measurement (100%)
  - This reference value is used for subsequent samples until changed
- Insert samples as required and press **run** (repeat as necessary)
- To go back and change the wavelength press **mode**

Concentration

There are two concentration modes, Factor and Standard.

Factor Concentration mode is used when a conversion factor is known; this is required to convert the absorbance measurement for a sample at a specific wavelength to a concentration, by a simple multiplication of absorbance * factor.

Standard Concentration mode is used when a sample of known concentration is available; by measuring the absorbance of this at a specific wavelength, the conversion factor is calculated (see above), and this can be applied to other samples of unknown concentration. This is equivalent to a one point calibration, and assumes that sample of zero concentration has zero absorbance.

The procedure is as follows:

- Enter appropriate wavelength and press **enter**
- Enter appropriate sample number and press **enter**
- Select mode, Factor or Standard using ▶
- If Factor
  - Concentration of unknown = Absorbance * factor
  - Enter factor, range 0.01 – 99999
  - Select units using ▶
  - Select units using ▶
  - Insert reference and press **set ref**.
  - Insert samples as required and press **run** (repeat as necessary)
- If Standard
  - $\text{Concentration of unknown} = \text{Absorbance of unknown} * \frac{\text{Concentration of standard}}{\text{Absorbance of standard}}$
  - Enter concentration of standard
  - Select units using ▶
  - Select units using ▶
  - Insert reference and press **set ref**.
  - Insert standard and press **run** (calculates factor)
  - Insert samples as required and press **run** (repeat as necessary)
    - Concentration of sample relative to standard is displayed

Methods

Recall
To recall a stored method from memory
1. Select whether it is between 1-10, 11-20, etc by using ▸
2. Select the method number

The appropriate mode is obtained; load reference and samples, and press **run**

Clear
To clear a stored method from memory
1. Press **C**
2. Select whether it is between 1-10, 11-20, etc by using ▸
3. Select the method number
4. Confirm yes (**enter**) or no (▸ **enter** ▸)

Save
Stored methods, up to a maximum of 50, are saved directly from within an application by selecting the save option. To save a method in memory
1. Select whether is to be between 1-10, 11-20, etc
2. Enter the method number
3. Enter the title (refer to Instrument Utilities); press ▸ to get the an alphanumeric keypad or enter directly from the keypad

Print
Press **print** to obtain a list of all method names and numbers.

Wavescan

A graph of change of absorbance against wavelength is known as an absorption spectrum and is one of the most useful physical characteristics of a compound, both as means of identification (qualitative analysis) and of estimation (quantitative analysis). It arises because of the various electronic transitions that are possible within a molecule, and the peaks are broad (in solution). A derivative of a spectrum can provide additional information; the 1st order derivative enables identification of multiple peaks that are close together, 2nd order enables identification of peak shoulders (inflections) and 4th order identifies both multiple peaks and inflections at the same time. The procedure is as follows:

Set up

- Select Absorbance or Transmission mode
- Enter start wavelength (range 190-1090nm)
- Enter end wavelength (range 200-1100nm)
- Select scan speed as appropriate; slow , medium or fast, using ▸
- Select data interval, 2.0, 1.0, 0.5, 0.2 or 0.1 nm, using ▸
  - Nominal scan speeds are shown in the table below
- Select if a reference scan is required using ▸ ; if yes, the reference scan will act as a temporary baseline
- Save method if required using ▸
- Insert reference and samples, and press **run**

Data interval, nm	2.0	1.0	0.5	0.2	0.1
Fast, nm/min	7300	4600	2600	1100	600
Medium, nm/min	4600	2600	1400	600	300
Slow, nm/min	3300	1800	1000	400	200

- A smaller data interval gives improved peak resolution
- The minimum and maximum wavelength scanning ranges for 0.2 and 0.1 nm data intervals are 10 – 500 nm and 10 – 250 nm, respectively; full scanning range at these data intervals requires the use of Acquire software.

Graph

Use this facility to scale data post run for presentation on the display and print out.

- Enter the start wavelength
- Enter the finish wavelength
- Enter if auto-scaling of the absorbance axis is required using ▸
- Enter absorbance maximum
- Enter absorbance minimum

To **zoom**, move the cursor to the peak of interest using ◂ ▸ and press ▴ repeatedly. To return, press ▾.

Overlay

Use this facility to select derivatives and to overlay them with the spectrum, smooth or enhance spectral data and to convert Abs to % T. The overlaid data can be optimised on the LCD for examination and printing purposes by using scale and offset to affect the selected data type. Note that different individual scans cannot be overlaid.

- Select data type as appropriate; off, 1st or 2nd or 4th derivative, smooth, enhance % transmission , using ▸ (not 2 nm data interval)
- Enter a factor to multiply the selected data type by, for clarity
- Enter an offset to move the selected data type from the graph abscissa if required

Peak Table

Use this facility to list the peak absorbance and wavelength values for the whole spectrum. The algorithm is designed to identify narrow peaks; the absorbance maxima of broad peaks having a natural bandwidths of 15 nm or greater are subject to some uncertainty and are indicated by brackets. In this case, use ◂ ▸ to move the cursor for visual identification. Results cannot be edited.

- Select if Peak Table is required using ▸ (not 2 nm data interval)

Multiwave

The measurement of Absorbance values at specific wavelengths and combining these with appropriate factors is a means of overcoming interference effects in several applications.

Abs Ratio

This facility enables the determination of Abs λ1 / Abs λ2 and Abs λ1*factor

- Enter the first wavelength
- Enter the second wavelength
- Select if background correction (for both wavelengths) is required using ▸
    - If yes, enter the wavelength
- Enter the factor to be applied to the first wavelength
- Select units using ▸
- Select units using ▸
- Save method if required using ▸
- Insert reference <u>and</u> samples, and press **run**

Abs Diff

This facility enables the determination of the following equations:

1. [(Abs λ1 – Abs λ2) * Factor 1], for bichromatic measurements, and
2. [(Abs λ1 – Abs λB) * Factor 1] – [(Abs λ2 – Abs λB) * Factor 2], compensating for background absorbance. The use of factors here enables two component mixtures to be studied.

If factors are not required, use 1.0.

- Enter the first wavelength
- Enter the second wavelength
- Select if background correction (for both wavelengths) is required using ▸
    - If yes, enter the wavelength
- Enter the factor to be applied to the first wavelength
- Enter the factor to be applied to the second wavelength
- Select units using ▸
- Select units using ▸
- Save method if required using ▸
- Insert reference <u>and</u> samples, and press **run**

3 point net

This facility enables the determination of true peak height for turbid samples that have a sloping baseline, for example the determination of bilirubin in amniotic fluid.

- Enter the first wavelength; this is wavelength on the “UV” side of the peak
- Enter the second wavelength; this is the wavelength of the peak, and will have a value greater than the first wavelength
- Enter the third wavelength; this is the wavelength on the “visible” side of the peak
- Enter the factor to be applied to the peak height after accounting for the sloping baseline, if required
- Select units using ▸
- Select units using ▸
- Save method if required using ▸
- Insert reference and samples, and press **run**

MultiWave

This facility enables the entry of equations so that post measurement calculations can be done automatically and the end result displayed. This is a very useful for the busy industrial, QC or environmental testing laboratory as the method can be saved. Up to 9 absorbances / transmittances at different wavelengths can be measured and 9 factors applied to them; an overall dilution factor can be applied to the completed equation. Before entry, write the equation out in front of you; a worked example is shown in the Appendix.

Set up

- Select Absorbance or Transmission mode
- Enter the wavelength
- Enter the first wavelength
- Enter subsequent wavelengths
- Enter the integration time using ▸
    - Default is 0.1 second, other options are 1, 2 and 5 seconds. Use long integration times for very low and very high absorbance readings.

Factors

- Enter the overall dilution factor, C
- Enter the first factor
    - Maximum of 2 decimal places
    - Press C on the keypad to enter a negative factor
- Enter subsequent factors

Equation 1, 2 and 3

Enter the equation(s) to be applied to the sample measurement. If only one equation is required, enter it and then go to equation 3 in order to save it as a method.

- Enter the equation name / description using the alphanumeric keypad
- Enter the equation itself using ◂ ▸
    - Define the written down equation one parameter at a time, using ▸ to select open and close bracket, Abs λ, λ value of factor; mathematical operators (+, -, *, /) are available after the first entry. Note that A1 represents Abs λ1.
    - The equation is displayed as it is entered; use C to remove an incorrect parameter entry.
- Select units using ▸
- Select units using ▸
- Enable equation if required using ▸
- Save method if required using ▸ (this facility is in Equation 3 only)

- Insert reference <u>and</u> samples, and press **run**

Kinetics

A graph of change of absorbance with time is known as a kinetics assay, and gives information about the rate at which a reaction proceeds; further information is given in the Appendix. Note that this mode should be used to check sample stabilisation since this instrument does not have a continuous output source.

Set up

- Enter the wavelength
- Enter the factor required to convert the slope to a meaningful unit
- Select units using ▸
- Select if Automatic set reference at time zero is required using ▸

Timing

- Select if serial mode or parallel mode is required using ▸
  - Use parallel mode to measure several assays at the same time. If parallel:
    - Enter the number of samples, maximum is 8 (or 7 if active reference is selected)
    - Select if active reference is required using ▸
      - Use if reference changes with time and needs to be subtracted
- Select time units, seconds or minutes, using ▸
- Enter the delay time over which no measurement should be taken (range 0-1000)
- Enter the length of reaction
  - Note that if using clinical test kits, set the reaction time equal to the time interval below
- Enter the time interval between measurements. The maximum number of data points is 600. A minimum time interval of 8 seconds is required in parallel mode.
- Save method if required using ▸
- If Serial mode:
  - Insert reference if required and press **run**, then insert sample and press **run**
- If parallel mode:
  - Insert active reference and samples as appropriate and press **run**
    - Time and absorbance are updated at every time interval, and the slope (ΔA/minute) is shown in real time. At the end of the assay, the result (slope * factor) is displayed on the basis of the last 5 data points. The slope is calculated over the steepest part of the curve, and can be edited post run.

Graph

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

- Enter the maximum absorbance to be shown on the display during the assay
- Enter the minimum absorbance to be shown on the display during the assay
- Select if automatic scaling of the results to fit the display is required post run
- Select if the actual absorbance time data should be printed with the results
- Select if data points should be indicated on each assay
- Select if the graph should be printed with the results
- Select if the graphs should be printed overlaid (parallel mode only)

Post Run

This facility enables an assay to be selected (in serial or parallel mode) and the slope to be optimised by redefining the start and end points for the slope.

- Enter the sample number for optimisation
- Enter the start point for the slope
- Enter the end point for the slope
- Select if these points should be defined automatically
    - If no, the slope is calculated on the basis of the start / end points entered above
    - Results shown are Initial Abs, Final Abs, Slope, Slope * Factor (result), Linearity

Standard Curve

The construction of a multi point calibration curve from standards of known concentration in order to quantify unknown samples is a fundamental use of a spectrophotometer. Examples include protein determination using the methods determined previously and the analysis of waste water for metal complexes, salts and disinfectants. Alternatively, a known factor can be applied to measured sample absorbance values.

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

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

Set up

- Enter the wavelength
- Select the curve type from linear regression, linear interpolation, spline or factor using ▸
  - If factor is selected, enter the factor
- Enter the number of standards, maximum is 9
- Enter the number of replicates for each standard, maximum is 3
- Enter the number of replicates for the samples, maximum is 3
- Select units using ▸

Concs

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

Running Standards

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

Running Samples

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

Graph

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

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

Standards

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

Substrate

Reagent test kits are routinely used for the enzymatic determination of compounds in food, beverage and clinical laboratories; an example is measurement of NAD / NADH conversion at 340 nm. The change in absorbance over a specified time period can be used to provide useful information when an appropriate factor is applied; the start and end times as well as the factor are defined in the reagent kit protocol. The curve fit usually used is linear regression.
Note that reaction rate and enzyme activity can be calculated if the factor used takes account of the absorbance difference per unit time, as opposed to the absorbance difference *per se*.
If a factor needs to be applied to a change in absorbance with time, use the kinetics application.

Set up

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

Timing

- Select time units, seconds or minutes, using ▸
- Enter the delay time over which no measurement should be taken (range 0-1000)
- Enter the length of reaction

Concs

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

Running Standards

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

Running Samples

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

Graph

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

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

Standards

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

Instrument Utilities

Accessory

- This identifies the type of cell holder / cell changer that has been fitted
  - A multi-position cell changer can be made to act as a single cell holder if required
- Refer to Accessories section for further information

Printer

- Select the generic printer driver type required; choice is Seiko DPU-414, Epson P40, Epson FX-80+ / Epson 9 pin, Epson 24 pin (ESC P), Epson InkJet (ESC P2 raster), PCL HP DeskJet / HP LaserJet, PCL HP DeskJet / HP LaserJet (A4), Text printer (no graphics)
  - Press **print** in this mode to set to top of page
- Refer to "Output to Printer" for further information

Display

- Select the contrast required using ▸
  - Use the high contrast option to make the background white

GLP

This facility is only available if the GLP option in **Set up** has been enabled; for further information on GLP, refer to the Appendix.

- The GLP Calibration Results are displayed for reference

Entry of alphanumeric characters for print outs and method names

- Remove default characters (highlighted), if necessary, using ◂
- Press appropriate key on keypad to cycle through options of lower case letter, numbers and upper case letters (for example pressing key 2 cycles through abc2ABC). Note that a space is entered using key 1, which cycles between 1_1_)
- Press another key to move to next letter. To enter a doubled letter (eg AA) or number (eg 00), press **print** and then the appropriate key again.
- Delete incorrect characters using **C**
- Complete entry by pressing **stop**

Alternatively, pressing ▸ gives an alphanumeric display that can be navigated using ◂ ▸ ▴ ▾; select letters using **enter** and complete entry by pressing **stop**.

Set up

User

This facility enables user parameters for the instrument to be configured.

- Enter operator name as described previously
- Enter laboratory name as described previously
- Enter instrument asset number or preferred description as described previously
- Select if sound is required for keypad presses, sipper use and time intervals during kinetics reactions
- Select if the GLP enabled option is required. If selected:
  - Pressing enter after calibration confirms that the instrument status is acceptable
  - A header with information to conform with GLP if printed out (note that if GLP is off, instrument calibration routines are not affected)
  - Underlying readings and results are printed out every time **run** is pressed
- Select between output to printer only, output to computer only, output to printer and computer using ▸.
- Select if automatic print out of parameters and graph (if in a graphics mode) is required after the **run** process is completed
  - Ensure Auto Print is on for output to computer, otherwise press the print key. Refer to the "Automatic Output to Computer" section.

Baseline

This facility enables the instrument baseline to be set up

- Select View, New, Save or Restore using ▸
  - View is the default mode required to see current information
  - Use New to create a temporary baseline
  - Use Save to make a temporary baseline permanent
  - User Restore to revert to the saved baseline if a temporary baseline is created

Clock

This facility enables the real time clock and date to be set

- Enter the facility by pressing ▾, and put in appropriate values as required

Service

This facility is password protected and is available for service engineers use only.

Output to Printer

The graphics capability of the instrument means that the following requirements for printer compatibility should be fulfilled:

- The printer must not be USB only style; parallel Centronics is required
- The printer must not be designed to work with MS Windows only (GDI type); these are less expensive printers and can only function when connected to a PC with the appropriate driver installed

If in doubt, check with the printer manufacturer.
Note that printer output is always in black and white even on colour printers.

Seiko DPU-414

If obtained in your country, it should already be configured properly.
If not, set software DIP SW2 to American character set.

Epson FX-80+ / Epson 9 pin

Includes Epson FX 850 and similar.

Epson 24 pin (ESC P)

For use with Epson 24 pin dot matrix printers and older inkjet printers such as the Stylus 400.

Epson InkJet (ESC P2 raster)

For use with more recent Epson Inkjet printers such as the Epson Stylus Color 680.

PCL HP DeskJet / HP LaserJet

Intended for printers such as HP LaserJet II/III/4, HP DeskJet 500, HP DeskJet 690C.
The printer must be HP PCL level 3 or greater; HP DeskJet 700, 820 and 1000 series printers do not fulfil this requirement and cannot be used.
Use for letter sized paper.

PCL HP DeskJet / HP LaserJet - (A4)

Intended for printers such as HP LaserJet II/III/4, HP DeskJet 500, HP DeskJet 690C.
The printer must be HP PCL level 3 or greater; HP DeskJet 700, 820 and 1000 series printers do not fulfil this requirement and cannot be used
Use for A4 sized paper (European)

Text printer (no graphics)

Use for any class of parallel printer; no graphics or accents on text are printed.

Parameters and graphics are printed automatically if Auto Print is selected (see User). Pressing **print** from within a mode set up page will print the parameters for that mode, whereas pressing **print** after experimental results are displayed will print both parameters and graphics.

Download to Spreadsheet

Results can be downloaded directly to Excel when the PC has the Spreadsheet Interface Software installed (80-2110-73) and the two are linked with the serial cable (80-2105-97); detailed instructions are supplied with the software. Thus absorbance / wavelength data comprising a scan, for example, can be picked up as columns of numbers and converted to a more conventional graph using the spreadsheet; results can then be formatted or manipulated as appropriate prior to inclusion in reports or archiving / saving to hard disk.
Results from all modes of use on the instrument can be output in this way. Output is automatic when **print** key is pressed.

Messages

Most messages are self-explanatory and relate to use of the instrument.
Others relate to the calibration of the instrument on switch on:

Message summary	**Possible causes / remedy**
Calibration status: Fail	One or more of the parameters tested for during GLP calibration is out of specification (see Appendix). You can accept this status and either continue to use the instrument as normal or try again later, but you may wish to contact your local service engineer.
Calibration status: visible only	Visible region only is calibrated; check lamps and replace if necessary
Calibration status: UV only	UV region only is calibrated; check lamps and replace if necessary
Failed to align visible lamp	Detector did not see enough energy during calibration; replace visible lamp
Abs Non-Linear	Instrument cold, dirty filters or misalignment of filter quadrant. Recalibrate later on. Contact local service engineer.
Too much light	Close lid properly and ensure baseplate plug is in place
Beam blocked	Not enough light getting to detector; check light beam is not blocked by a cell
> 3.0	Sample too concentrated or something blocking light path
! C	Accessory not initialised properly

ACCESSORIES

If an accessory is changed, press Function > Accessory to initialise the instrument in order that the appropriate accessory can be identified. Depending on the accessory type, a list of options is presented.

Multiple Cell Holder Accessories

- Install by removing accessory in place, replacing with the new one, turning the central mounting screw until it is finger tight and initialising as above.
- All multiple cell holders have the option of being used as a single cell holder. This means that there will be no rotation after pressing run.

Description	Part number	Comments
4 position cell changer	80-2106-01	Accommodates cells 10-50mmm in pathlength
8 position water heated cell changer	80-2109-70	Requires a water-circulating bath. Locate round extension of tube restrainer into top of cell changer thumb screw. Attach the tube guide to the instrument base using the screws provided. Replace the front blanking plug on the cell compartment lid with the new one that is provided.
6 position Peltier heated cell changer	80-2106-04	Requires Temperature Control Unit (80-2112-49). Insert into socket 1.
8 position cell changer	80-2108-01	Spare, if required

Single Cell Holder Accessories

- Install by removing accessory in place, replacing, if necessary, the baseplate plug supplied and positioning the single cell holder so that the arrow is on the front face and it locates in place. Then push the finger locks backwards so that they lock into position. Initialise as described previously

Description	**Part number**	***Comments***
Cell holder, 10mm pathlength	80-2106-05	
Cell holder, for sample stirring	80-2108-10	Requires magnetic flea and controller
Cell holder, 50mm pathlength	80-2106-07	
Cell holder, 100 mm pathlength	80-2107-14	
Cylindrical cell holder	80-2106-10	Up to 100 mm pathlength cylindrical cells
Water heated cell holder	80-2106-08	10-40 mm pathlength. Requires a water-circulating bath. Replace the front blanking plug on the cell compartment lid with the new one that is provided..
HPLC cell holder	80-2106-11	Flowcell volume is 8 µl, pathlength is 2.5mm. Thread wires through one hole of the tube guide and attach this to the instrument base using the screws provided. Replace the front blanking plug on the cell compartment lid with the new one that is provided.
Peltier cell holder	80-2106-13	Set required temp in range 20-49 °C. Insert into socket 2.
Electrical cell holder	80-2106-12	Set required temperature: off, 25, 30, 37 °C. Insert into socket 2.

Other Accessories, consumables etc

Description	Part number	Comments
Sipper	80-2112-25	Use if a large number of samples for single readings are required. Requires single cell holder (80-2106-05 or 80-2106-13). 10mm flowcell and tubing supplied, together with separate user instructions.
Temperature Control Unit	80-2112-49	Required to supply the extra power required by the 6 position Peltier heated cell changer (80-2106-04).
Printer stand	80-2112-18	For thermal printer
Dust cover	80-2106-19	Spare

Consumables and other items

Pump head tubes (6) for Sipper	80-2080-74
PTFE flowcell tubing with connectors	80-2055-13
Replacement flowcell (including tubing)	80-2080-60
Autosampler Interface kit	80-2104-96
Serial interface cable for connection to PC (D9 male instrument to D9 PC)	80-2105-97
Spreadsheet Interface Software	80-2112-23
Centronics parallel printer interface cable	80-2071-87

Separate information giving details on serial and parallel interface connections, if required, is available from a Service Engineer with your local supplier, whom you should contact for further details.

Acquire Applications Software

Acquire comprises application modules for wavelength scanning, reaction kinetics, quantification, multi wavelength, time drive, and can be used to enhance the software already included on the spectrophotometer.

80-2115-31	**Acquire Applications Software** Wavelength Scanning, Reaction Kinetics, Quantification, Time Drive, Multi Wavelength

Recommended PC for proper operation

For optimum performance, an IBM compatible 486 or greater personal computer running Microsoft Windows 95, 98 or NT is required. The PC should have a minimum of 8MB RAM, 200Mb hard disk, a 1.44 MB 3.5 inch floppy disk drive, CD-ROM, a serial mouse installed, and free COMMS serial port and VGA graphics. Any printer supported by Microsoft Windows 95 can be used. Contact your supplier for further information.

MAINTENANCE

After Sales Support

We supply support agreements that help you to fulfil the demands of regulatory guidelines concerning GLP/GMP.

- 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

User maintenance is restricted to changing the instrument lamps and mains fuse. For any other maintenance operation or rectification contact your local supplier.

Lamp Replacement

Replacement lamps are available from your supplier using the following part numbers:

Deuterium lamp 80-2106-17 (includes tungsten lamp as well)
Tungsten lamp 80-2106-16

The deuterium lamp is supplied fitted into a mounting and pre-adjustment plate; a new tungsten lamp is also included.

NOTE:

- Although deuterium lamps are covered by their own warranty, an engineer's call out fee is not, and users are advised to change their own lamps. Lamp replacement is very easy, and the process has been designed so that the user can do it without the need to call out a local service engineer. No lamp alignment is required as the lamp select mirror is automatically aligned for maximum lamp energy throughput during the instrument calibration procedure.
- The lamps become very hot in use. Ensure they are cool before changing.
- Do not touch the optical surfaces of either lamp with your fingers (use tissue); if touched, the area should be cleaned with iso-propanol.

To change a lamp, proceed as follows:

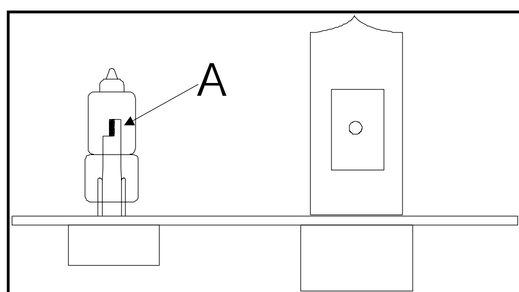
To change a lamp, proceed as follows:

1. Switch off the instrument, remove sample from cell holder and disconnect the power supply cord. Allow time for lamps to cool.
2. Locate the lamp access cover on the left-hand side of the instrument, and push the cover back.
3. Undo the black knurled screw on the lamp cover plate with your fingers and remove the plate.
4. Slide the lamp plate out and unplug the cable connector.

- If the tungsten lamp has failed, the replacement should be inserted onto the plate, pushing it all the way down into its holder *.
- If the deuterium lamp has failed, insert the tungsten lamp onto the plate as above and then replace the whole assembly with the new one.

5. Reconnect the cable connector and slide the lamp plate in until it locates.
6. Replace the lamp cover plate by attach by re-tightening the black knurled screw.
7. Replace the lamp access cover.
8. Reconnect the power supply cord and switch the instrument on.
9. After the lamp has warmed up sufficiently (30 minutes), run a new baseline.

* For reliable tungsten lamp alignment fit the tungsten lamp as shown in the diagram below, with the straight length of wire A closest to the deuterium lamp.



Deuterium Lamp Warranty

Criteria for lamp replacement are that it must:

- be less than 15 months old

Fuse Replacement

1) Switch off the instrument and disconnect the power supply cord. The fuse holder can only be opened if the power supply plug has been removed, and is located in the power input socket on the back panel of the instrument.
2) Slide the fuse holder open by pulling at the notch.
3) Place fuses (1.6A, 5mm x 20mm, FST) into the fuse holder and slide back into position.
4) Reconnect the power supply cord and switch on the instrument.

Fuses are not normally consumed in an instrument's lifetime. If they blow repeatedly contact your supplier

Cleaning and General Care

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.

- **Sample compartment spillages**

- Switch off the instrument and disconnect the power cord.
- The cell holders, baseplate and sample compartment are coated in a chemical resistant finish. Strong concentration of sample, however, may affect the surface, and spillages should be dealt with immediately.
- Observe all necessary precautions if dealing with samples or solvents that are hazardous.
- There is a small drain hole in the sample compartment to allow excess liquid to drain away. Liquids will drain onto the bench or table under the spectrophotometer or if preferred, this drain hole can be connected to waste using suitable tubing.
- Remove the cell holder and clean separately.
- Use a soft dry cloth to mop out the sample compartment. Replace the cell holder.
- Reconnect the power cord and switch on the instrument.

APPENDIX

Pharmacopoeia

In general, there has been an increase in laboratory requirements to conform with Good Laboratory Practice techniques; this is particularly the case in Pharmaceutical companies and in Biotechnology facilities, where the interest in finding solutions to gene therapy opportunities is great. Typically, scientists working in pharmaceutical and bio-pharmaceutical research, be it University or Industry, require a high specification instrument with the ability to develop methods.

The British Pharmacopoeia (A88 Appendix II B) states that for resolution:

- To verify the resolution of the instrument, the spectrum of a 0.02% (v/v) solution of toluene in hexane should b recorded; the ratio of the absorbance at the maximum (269nm) and minimum (266nm) should be at least 1.5. It can be shown that this requires an instrument having 1.8nm bandwidth or less.

The European Pharmacopoeia (1984, v.6.19, 2nd Edition) states that for stray light:

- To verify the stray light of the instrument, the absorbance of a 1.2 % w/v solution of potassium chloride with a pathlength of 1cm should be more that 2.000 when compared with water as reference liquid.

This instrument fulfils the requirements of the Pharmacopoeia, and is delivered with the appropriate factory final test certificate to show this. An “Instrument Qualification and Performance Verification Logbook” is also included; this details the various tests that have to be done to prove Pharmacopoeia compliance and enables the results to be plotted as a function of time.

Good Laboratory Practice

Good laboratory Practice (GLP) concerns being able to trace experimental results to an instrument, an operator and the time the result was obtained so that a laboratory can prove that the instrument was functioning correctly or not. Laboratory, operator and internal instrument reference names can be entered on the spectrophotometer.

If the GLP option is on, during calibration or re-calibration the instrument self-checks its integrity for GLP purposes. The GLP test of this instrument is essentially a “confidence test” that it is performing as it was when manufactured and tested. For absolute measurements, an annual certification service agreement with your supplier is recommended. The integrity of the instrument for GLP purposes is quantified from:

- the calibration status of the instrument
- the age of the lamps
- the wavelength accuracy by comparing to the 656nm deuterium line
- the values of a built in absorbance filter compared to when the instrument was manufactured (or last serviced by an accredited engineer).
- the bandwidth at 656nm
- the instrumental stray light

The expected values are given in parentheses on the GLP print out after calibration; the range of acceptance is defined by the technical specification of the instrument.

In the unlikely event that the instrument fails calibration or goes out of specification, a message will appear on the display. In this event, the following should be checked:-

- is the cell compartment lid closed properly
- is a sample in the light beam - if so, remove it
- is the baseplate plug in place (single cell accessory)
- is the in-fill panel at the front of the cell compartment in place

Pressing **OK** after the message "GLP Calibration Fail" appears confirms that you have accepted the instrument status. If you are working in a regulated environment such as a drug discovery laboratory that generates data for GLP/GMP activities or reports, you should not use the instrument and contact your local service engineer.

GLP print out at instrument calibration (GLP enabled)

Libra S32 UV/Vis Spectrophotometer

Lab name ....

Instrument ....

Serial no : 81012
Software : 6094 V1.0, Slave 6094 V1.0
Last serviced : 14/03/02 13:36

Instrument state at calibration

GLP Calibrated 09/03/02 at 12:56
Calibration Full UV/Visible

Bandwidth (1.3- 1.8nm): 1.7nm	PASS
Wavelength (656.1nm) : 656.0nm	PASS

Absorbance at

220nm	(1.763-1.781A) : 1.772A	PASS
340nm	(1.633-1.665A) : 1.649A	PASS
500nm	(1.477-1.491A) : 1.484A	PASS

Stray light at

220nm (<0.025%T) : 0.013%T	PASS

Current instrument state
Accessory: Eight Position Cell Changer

UV lamp :
installed 14/03/02 13:35, use 5 hours
baseline in use: 14/03/02 13:38
baseline stored: 14/03/02 13:38

Vis lamp :
installed 14/08/00 13:35, use 5 hours
baseline in use: 14/03/02 13:38
baseline stored: 14/03/02 13:38

GLP enabled causes printed results to have calibration status included.
GLP results can also be sent to PC for electronic archiving (Spreadsheet Interface Software required).

Equation Entry using MultiWave

Always write out the equation(s) in front of you before using this mode.
Step by step entry of the following equations is shown in the example below:

Cobalt (g/l) = ((12.26 * A511) - (0.30 * A720)) * 100
Nickel (g/l) = ((-0.40 * A511) - (27.41 * A720)) * 100

Enter numbers using the keypad; press enter or ▼ after each entry.

<u>Setup</u>

No of λ's	2	
λ1	511	
λ2	720	
Integration time	Default	
<u>Factors</u>		
c	100	
K1	12.26	
K2	0.30	
K3	-0.40	press C first to get the negative sign
K4	27.41	press enter repeatedly to get to end of page

<u>Equation 1</u>

Press enter or V after each parameter entry; use C to remove an incorrect parameter entry.

Description	Cobalt	>	to get	alphanumeric keypad; press stop after entry of name
Equation	press	< >	(	
		< >	(	
		< >	K1	already defined as 12.26
		< >	*	
		< >	A1	already defined as absorbance at 511 nm
		< >	)	
		< >	-	
		< >	(	
		< >	K2	already defined as 0.30
		< >	*	
		< >	A2	already defined as absorbance at 720 nm
		< >	)	
		< >	)	
		< >	*	
		< >	C	already defined as 100
Units		< >	g	grams (unit will appear in print out, not in title)
		< >	l	litre (unit will appear in print out, not in title)
Enable equation		>	✓	Check the equation!!

Equation 2

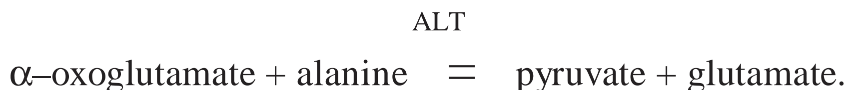
If there is no equation 2, go directly to save method.

Press enter or V after each parameter entry; use C to remove an incorrect parameter entry.

Description	Nickel	>	to get	alphanumeric keypad; press stop after entry of name
Equation	press	< >	(	
		< >	(	
		< >	K3	already defined as -0.40
		< >	*	
		< >	A1	already defined as absorbance at 511 nm
		< >	)	
		< >	-	
		< >	(	
		< >	K4	already defined as 27.41
		< >	*	
		< >	A2	already defined as absorbance at 720 nm
		< >	)	
		< >	)	
		< >	*	
		< >	C	already defined as 100
Units		< >	g	grams (unit will appear in print out, not in title)
		< >	l	litre (unit will appear in print out, not in title)
Enable equation		>	✓	Check the equation!!
Save method		>	✓	Refer to Methods

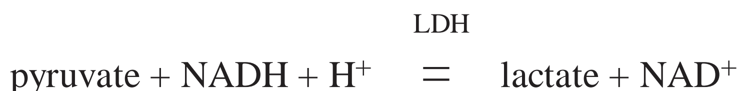
Kinetics

The usual way of measuring the rate of an enzyme reaction is to monitor the change in concentration of one of the substrates involved in, or of one of the products produced by, the reaction. Take for example the alanine transaminase (ALT) enzyme reaction: -



$$\alpha\text{–oxoglutamate} + \text{alanine} \overset{\text{ALT}}{=} \text{pyruvate} + \text{glutamate.}$$

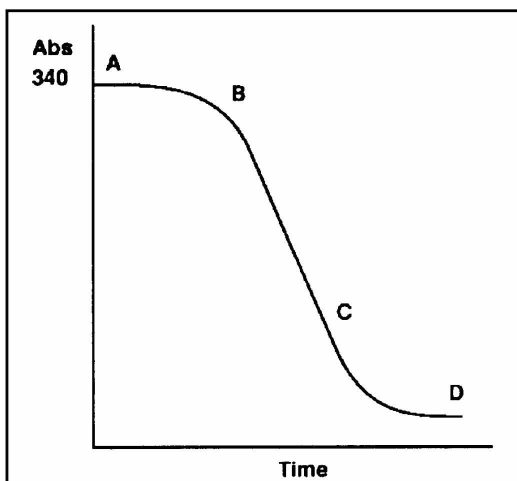
If we want to measure the rate of production of pyruvate, then as this cannot be done directly we can link it to another enzyme reaction involving NADH and the enzyme lactate dehydrogenase (LDH). Thus: -



$$\text{pyruvate} + \text{NADH} + H^{+} \overset{\text{LDH}}{=} \text{lactate} + \text{NAD}^{+}$$

We can follow the rate at which NADH is used up by measuring the absorbance of the reaction mixture at 340 nm. Since LDH is in excess, this rate is directly proportional to the rate of pyruvate produced in the first reaction (about 80% of all enzyme measurements are monitored in this way).

If a curve of the absorbance of the reaction mixture at 340 nm versus time is plotted, a graph similar to that illustrated will be obtained.



The curve can be split into 3 phases:-

Phase 1, A–B; reactant mixing, thermal equilibrium and attainment of linear phase,

Phase 2, B–C; the linear phase,

Phase 3, C–D; tail off as one of the reactants becomes rate limiting, thus reducing the net reaction rate to zero.

The rate of the reaction is defined by the slope of the linear portion of the plot and therefore, from Beers Law, the change in absorbance per unit time, dA/dt, is given by:

$$dC/dt = (dA/dt) \times (1/EL)$$

where dC/dt = rate of change in concentration (mol/litre), L = cell path length (normally 1 cm) and E = molar absorptivity (molar extinction coefficient) of the compound being measured (for NADH, E = 6300 litres/mol/cm).

The rate of change of concentration can then be used to calculate enzyme activity, which is defined as:

$$\text{Enzyme activity} = (dC/dt) \times (Vt/Vs)$$

where Vt = total volume of the reaction mixture and Vs = volume of sample.

There are two internationally accepted units for enzyme activity:

1) International Unit of Enzyme Activity, U or IU, defined as that amount of enzyme activity which will convert 1 micromole of substrate per minute at $25^{o}C$.

2) Katal, kat, defined as that amount of enzyme which will convert 1 mole of substrate per second:
$1 \text{ IU} = 1.67 \times 10^{-6} \text{ kat}$ $\quad$ $1 \text{ kat} = 6 \times 10^{7} \text{ IU}$
The katal is not often used although it is a recognised SI unit.

Since we have defined our calculations as the rate of change of concentration (mol/litre), the results are therefore as activity per unit volume, IU/litre or kat/litre.

In order to simplify the calculation of enzyme activity, variables such as molar absorptivity and sample volume can be combined to produce a conversion factor, since by consideration of the above equations enzyme activity is proportional to dA/dt. The variables, in the correct units when working in IU/litre, are given in the following equation:

$$\text{Factor} = Vt.10^{6} / E.L.Vs$$

where Vt= total reaction volume (ml), Vs= sample volume (ml), E= molar absorptivity (L/mol/cm), L= path length (normally 1 cm).
The factor has the units of µmol/L

For example, in the alanine transaminase enzyme reaction above, if we have 0.2 ml of test sample in a total reaction volume of 2.20 ml, the conversion factor is calculated as follows:

$$\text{Factor} = 2.20 \times 10^{6} / 6300 \times 1 \times 0.2 = 1746 \ \mu\text{mol/litre}$$

The rate of change of absorbance, dA/dt, is calculated by performing a linear regression analysis on the data points from the linear portion of the Abs vs time plot to give a value for the slope in change in Abs per minute. This is convenient

for working in the IU unit, but to work in microkatals the conversion factor must be divided by 60.

To calculate the enzyme activity, multiply the rate of change in absorbance by the conversion factor:

Enzyme activity (IU/litre) = dA/dt x Factor.

Least squares regression analysis and linearity

The slope (or best straight line) and intercept in a kinetics assay or standard curve determination is calculated from a least squares linear regression of the data. The following equations are used, where n is the number of data points:

$$Slope = \frac{\sum x \sum y - n \sum xy}{\sum x \sum x - n \sum x^2}$$

$$Intercept = \left(\sum y - \sum x * slope\right) / n$$

Linearity is an estimate of the "goodness of fit" of the least squares linear regression analysis, a perfect fit being 1. It is used in both the Kinetics and Standard Curve modes, and is expressed by a correlation coefficient, calculated using the following equation:

$$Quality = 100 * \frac{\sum x \sum y - n \sum xy}{\sqrt{\left(\left(\sum x\right)^2 - n \sum x^2\right)\left(\left(\sum y\right)^2 - n \sum y^2\right)}}$$

SPECIFICATION AND WARRANTY

Wavelength range	190 -1100nm in 0.1nm data intervals
Monochromator	1200 lines/mm Aberration corrected concave grating
Maximum scanning speed	7300 nm/minute at 2 nm intervals
Spectral bandwidth	< 1.8nm
Wavelength accuracy	± 0.7nm
Wavelength reproducibility	± 0.2nm
Light source	Tungsten halogen and deuterium lamps
Detectors	silicon photodiode
Photometric range	- 3.000 to 3.000A, -99999 to 99999 concentration units, 0.1 to 200%T
Photometric accuracy	± 0.5% or ± 0.003A to 3.000A at 546 nm, whichever is the larger
Photometric reproducibility	within 0.5% of absorbance value to 3.000A at 546nm
Stability	± 0.001A per hour at 340nm at 0A after warm up (deuterium lamp)
Stray light	<0.025 %T at 220nm using NaI and <0.025 %T at 340nm using $NaNO_2$
Digital output	9 pin serial and Centronics parallel
Sample compartment size	210 x 140 x 80mm
Dimensions	520 x 370 x 230mm
Weight	13kg
Power requirements	90 - 265V AC, 50/60Hz, 150VA
Safety Standard	EN61010-1
EMC emissions	EN 61326-2.3 Generic emissions
EMC immunity	EN 61000-4-6 Generic immunity part 1
Mains harmonics	EN 61000-3-2
Quality System	Designed and manufactured in accordance with an ISO9001 approved quality system

Specifications are measured 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. They 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.