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biochrom

Biochrom Ltd
Certificate No. 890333

Declaration of Conformity

This is to certify that the Ultrospec 3100 *pro* UV/Visible Spectrophotometer
Part number 80-2112-31 / 32 / 37 / 38
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: 1993 Safety requirements for electrical equipment for EN 61 010-1: 1993 Safety requirements for electrical equipment for measurement, control and laboratory use.
- EN 61326-2.3: 1998 Electromagnetic compatibility - Generic emission standard part 1. Electrical equipment for measurement, control and laboratory use.
- EN 61000-4-6: 1992 Electromagnetic compatibility - Generic immunity standard part 1. Residential, commercial and light industry.

Signed: Dated: 2nd October 2000

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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).
- 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.

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.

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Essential Safety Notes

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



Caution (refer to accompanying documents).
Background colour 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.

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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 works on the basis of light from the xenon lamp 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.

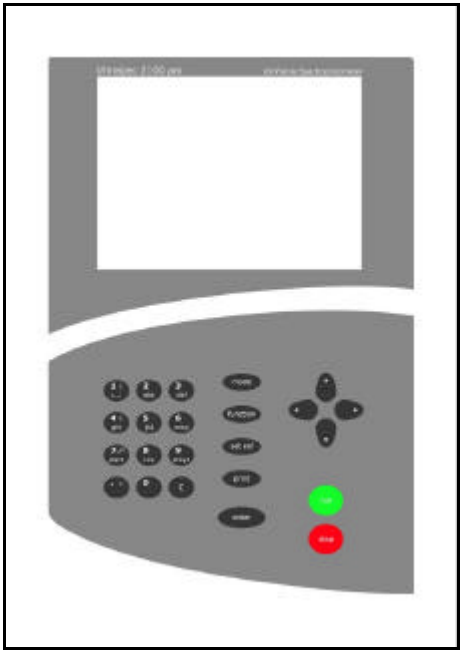
Your spectrophotometer:

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

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Keypad and display



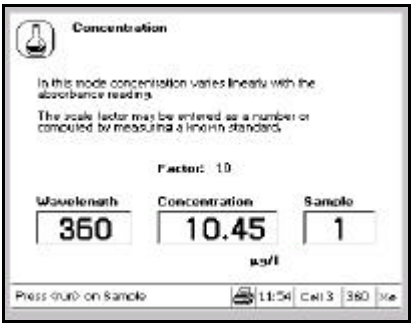
Navigation around the displays, presented in an index card format, and the options therein is by using the ◄ ► ▲ ▼ keys; press **enter** or ▼ to select an option. Number, letter and base entry will become active when appropriate.

- 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 Nucleic Acid or Multi-Wavelength modes, **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

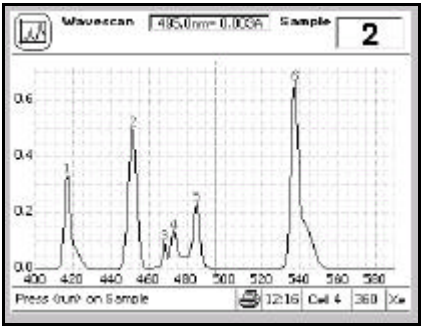
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There are two standard formats of display, depending on the measurement mode:

a) Basic (Absorbance, %Transmission, Concentration), Nucleic (DNA, RNA, Oligo) and Protein (UV methods) have 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.



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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)
    - Xenon lamp based instruments are "press to read", whereas 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**

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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
    - To enter a negative factor, press C first; the reference should have a higher absorbance than the samples
  - Select units using ▸
  - Select units using ▸
  - Insert reference and press **set ref**.
  - Insert samples as required and press **run** (repeat as necessary)
- If Standard
  - Concentration of unknown = 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

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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.

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Nucleic

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

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

Concentration = Abs260 * Factor

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

Absorbance ratio = Abs260 / Abs280

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

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

Concentration = (Abs260 – Abs320) * Factor
Absorbance ratio = (Abs260 – Abs 320) / (Abs280 – Abs 320)

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

Use of 7 µl volume ultramicrovolume cell

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

DNA, RNA, Oligo

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

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cDNA

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

Set up

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

Scan

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

Other dye

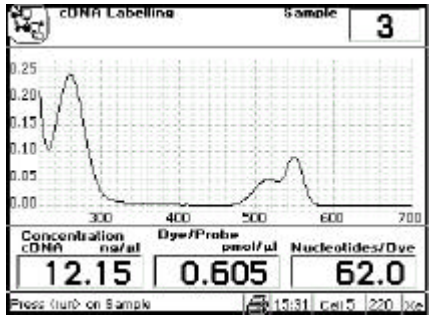
- Enter the name of the dye using the alphanumeric keypad
- Enter the absorption maximum (nm) of the dye
- Enter the Extinction Coefficient of the dye (l μmol^{-1} cm^{-1})

- Save method if required using ▸
  - Several dyes can be investigated if each is saved as a separate method

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

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



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

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

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

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

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Scan

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

- Start wavelength is fixed at 200nm. If using a UV transmitting disposable plastic cell such as UViMicro, strange optical effects between 200 and 220 nm may be observed. These can be ignored.
- Enter end wavelength and press **enter** (range is 350 – 600 nm)
- Enter λ1; this is used for quantification and absorbance ratio calculations
- Enter λ2; this is used for absorbance ratio calculations
- Select if background correction is required using ▸
  - If yes, enter λB
- Enter the sample dilution factor
- Enter the factor to be applied to λ1
- Save method if required using ▸
- Insert reference and samples, and press **run**

λ1 can be changed post run using ▸. This is very useful for optimisation; note that concentration and ratio results are updated automatically.

Tm Calculation

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

- Select mode, DNA or RNA, using ▸ (T is replaced by U in RNA)
- Enter the molar concentration of buffer / salts
- Enter the concentration of primer / oligo in µg/ml (or ng/µl)
- Enter the base sequence using the keypad (keys 1, 4, 7, • for C, G, T/U, A, respectively)
  - Note that the length (in mer) and the molecular weight of the oligo are shown as the sequence is entered; once 10 mer is reached the concentration in pmol/µl, conversion factor (µg/ml) and theoretical Tm (°C) are displayed

Info

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

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Protein

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

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

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

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

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

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

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Information
Information relating to proteins and amino acids.

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

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

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

Set up

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

Concs

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

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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.

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Protein Impurity

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

Protein (mg/ml) = 1.55 * Abs 280 – 0.76 * Abs 260

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

Protein (mg/ml). = (Factor 1 * Abs 280) – (Factor 2 * Abs 260)

Set Factor 2 = 0.00 for direct λ280 UV protein measurement; Factor 1 is based on the extinction coefficient of the protein. If BSA (bovine serum albumin) is an acceptable standard, setting Factor 1 = 1.115 will give linear results from 0 to 0.8 mg/ml protein.

Protein (mg/ml) = 1.115 * Abs 280

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

The procedure is as follows:

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

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280, 205 nm method

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

Protein (mg/ml) = 27 + (120 * Abs280 / Abs205)

The procedure is as follows:

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

215, 225 nm method

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

Protein (µg/ml) = 144 * (Abs215 / As 225)

The procedure is as follows:

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

Info

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

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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

- Enter start wavelength (range 190-890nm)
- Enter end wavelength (range 200-900nm)
- Select scan speed as appropriate; slow , medium or fast, using ▸
  - The maximum scan speed can only be attained in the UV section of the wavelength range due to variations in baseline energy, filter changes and dark current measurements. In the table below, the nominal wavelength is the average speed over the complete wavelength range.
- Select data interval, 1.0 or 0.5 nm, using ▸

Data interval	1.0 nm		0.5 nm	
Scan speed, nm/min	Maximum	Nominal	Maximum	Nominal
Fast	3000	1800	1500	900
Medium	1200	750	600	375
Slow	400	250	200	125

- 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**

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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 ▸
- 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 ▸

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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 and 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 λ1 – 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 and samples, and press **run**

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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**

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MultiWave

This facility enables the entry of one or two 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 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

- 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 and Equation 2

Enter the equation(s) to be applied to the sample measurement. If only one equation is required, enter it and then go to equation 2 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 2 only)
- Insert reference and samples, and press **run**

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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 the xenon lamp is not 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 10 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.

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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

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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.

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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.

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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.

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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.

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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**.

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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:
  - On calibration, there is a delay of 10 minutes to enable the instrument to warm up properly; 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.

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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)

Intended 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.

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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); these items are both optional accessories. 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.
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

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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 (80-2105-49). Insert into socket 3.
8 position cell changer	80-2108-01	Spare, if required

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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	
Ultramicrovolume cell holder	80-2106-06	Use with 5 µl cell (80-2103-68) and 70 µl cell (80-2103-69)
Microvolume cell holder	80-2106-09	Use with 50 µl cell (80-2076-38)
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. 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...
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.
Tm Programmable heated cell holder	80-2106-14	Supplied with SWIFT Tm software. Temp range 20-105°C.

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		Used for DNA/RNA denaturing studies. Requires Temperature Control Unit (80-2105-49). Insert into socket 3

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Other Accessories, consumables etc

Description	Part number	Comments
Sipper	80-2112-15	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	80-2105-49	Required to supply the extra power required by the 6 position Peltier heated cell changer (80-2106-04) and Tm Programmable heated cell holder (80-2106-14).
Printer stand	80-2112-13	For Seiko DPU-414 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	80-2110-73
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.

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SWIFT II Applications Software

SWIFT II comprises application modules for wavelength scanning, reaction kinetics, quantification, multi wavelength, time drive and fraction analysis, and can be used to enhance the software already included on the spectrophotometer. There are two application packages available; SWIFT II - LAB and SWIFT II - METHOD.

80-2108-26	**SWIFT II - LAB - for general analytical purposes,** Wavelength Scanning, Reaction Kinetics, Quantification
80-2108-31	**SWIFT II - METHOD - for method development,** Wavelength Scanning, Reaction Kinetics, Quantification, Time Drive, Multi Wavelength, Fraction Analysis

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.

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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

User maintenance is restricted to changing the mains fuse. For any other maintenance operation, including fitting a replacement xenon lamp, contact your local supplier.

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 (1.0A, 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.

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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 all 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.

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APPENDIX

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 % energy of the lamp compared to its value when new
- the bandwidth (this is assessed during calibration by measuring the zero order beam width)
- the wavelength accuracy by comparing to the 881.9 nm xenon emission line
- the values of built in absorbance filters compared to when the instrument was manufactured (or last serviced by an accredited engineer)
- 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.

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GLP print out at instrument calibration (GLP enabled)

Ultrospec 3100*pro* UV/Vis Spectrophotometer

Lab name .....

Instrument .....

Serial no : 81012
Software : 4194 V1.0, Slave 4194 V1.0
Last serviced : 09/10/00 12:13

Instrument state at calibration

GLP Calibrated 09/10/00 at 12:56
Calibration Full UV/Visible

Bandwidth (2.0 - 3.0nm): 2.9nm	PASS
Wavelength (881.9nm) : 881.9nm	PASS

Absorbance at

220nm	(0.996-1.006A) : 1.000A	PASS
340nm	(1.902-1.114A) : 1.102A	PASS
500nm	(1.201-1.123A) : 1.204A	PASS

Stray light at

220nm	(<0.050%T) : 0.008%T	PASS

Xe lamp : 97 % of original energy

Current instrument state
Accessory: Eight Position Cell Changer

Xe lamp :
installed 14/08/00 13:35, use 5 hours
baseline in use: 14/08/00 13:38
baseline stored: 14/08/00 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).

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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.

Setup

No of λ's	2	
λ1	511	
λ2	720	
Integration time	Default	

Factors

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

Equation 1

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

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			title)
	< >	l	litre (unit will appear in print out, not in title)
Enable equation	>	✓	Check the equation!!

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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

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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: -

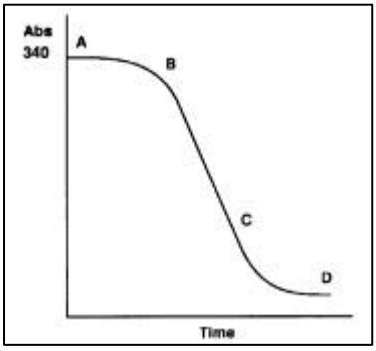
$$\alpha\text{–oxoglutamate} + \text{alanine} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{glutamate.}$$

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: -

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

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:

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

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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).

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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:

$1\ IU = 1.67 \times 10^{-6}$ kat $1\ kat = 6 \times 10^{7}$ 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

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

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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 100%. It is used in both the Kinetics and Standard Curve modes, and is expressed by a coefficient of determination (r^2), calculated using the following equation:

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

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SPECIFICATION AND WARRANTY

Wavelength range	190 - 900nm
Monochromator	1200 lines/mm Aberration corrected concave grating
Maximum scanning speed	3000 nm/minute
Spectral bandwidth	< 3nm
Wavelength accuracy	± 1nm
Wavelength reproducibility	± 0.5nm
Light source	xenon lamp
Detectors	two silicon photodiodes
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
Stray light	<0.05 %T at 220nm using NaI and <0.05 %T at 340nm using NaNO2
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 ± 10%, 50/60Hz, 80VA
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
British Design Registration No.	2097049

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

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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.

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