



GeneQuant pro User Manual

English

Deutsch

Français

Español

Italiano

Declaration of Conformity

This is to certify that the GeneQuant *pro* UV/Vis Spectrophotometer
Part number 80-2114-98 / 99 and 80-2115-04 / 05
Serial number 79000 onwards

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

Standards to which conformity is declared

EN 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: 23nd 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 bench or table that can take its weight (4 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 5 cm 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 100 - 240V supplies.
- Switch on the instrument at the rear panel; the instrument initialises for a few seconds and then goes through a calibration procedure for a minute or so, until the display shows “Instrument Ready” together with information relating to serial number, software version, date and sample number.
- 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not appropriate for safe operation, the protection provided by the equipment may be impaired and instrument warranty withdrawn.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

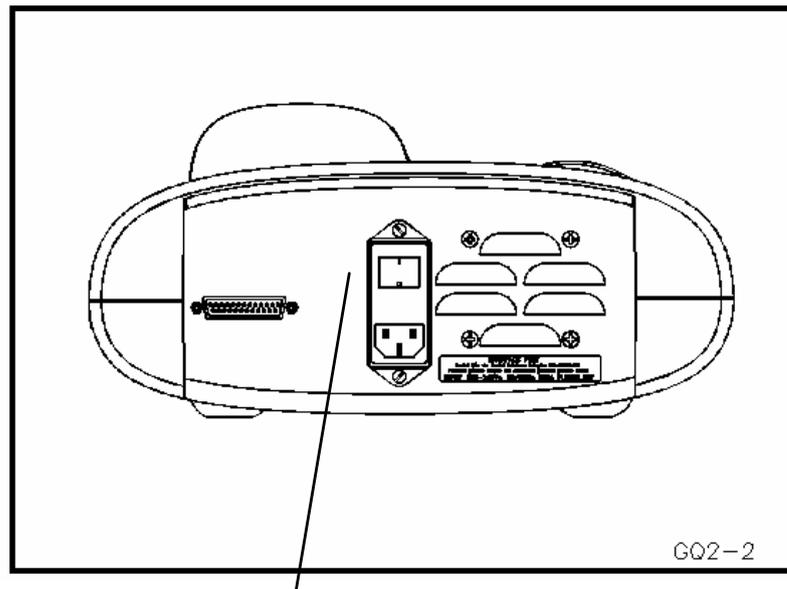
There are a number of warning labels and symbols on your instrument. These are there to inform you where potential danger exists or particular caution is required. Before commencing installation, please take time to familiarise yourself with these symbols and their meaning.



Caution (refer to accompanying documents).

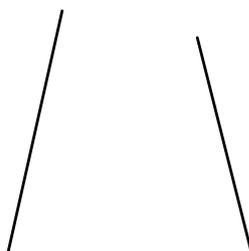
Background colour is yellow, symbol and outline are black.

Rear panel



1 2 3

1. Multi purpose interface socket
2. Power supply switch (1 = on, 0 = off)
3. Power supply socket



OPERATION

Introduction

Your GeneQuant *pro* RNA/DNA Calculator is a unique, yet simple to use spectrophotometer designed specifically for molecular biologists working in the life sciences, including biotechnology and drug discovery research. It has a pulsed deuterium borosilicate glass lamp for long life. It measures absorbance at 230, 260, 280, 320, 546, 562, 595 and 600 nm, and various relevant parameters are calculated automatically, and displayed at the touch of a button. The instrument is ideal for many routine molecular biologist activities:

- it can determine the concentration (in $\mu\text{g/ml}$, $\text{ng}/\mu\text{l}$, $\mu\text{g}/\mu\text{l}$, $\text{pmol}/\mu\text{l}$ or pmol phosphate) and purity of **nucleic acids** (DNA, RNA and oligonucleotides) after PCR amplification, for hybridisation studies and for quantitating minipreps. The wavelength at 260 nm is used to calculate quantification, and, together with those at 230 and 280 nm, for purity checking calculations (via 260/230 and 260/280 ratios). The wavelength at 320 nm is used for background compensation. A variety of cells, including UV transparent disposable cells, is now available for convenient sampling, depending on sample concentration, dilution factor and available sample volume. A nucleic acid survey scan facility is available so that the spectrum of samples can be viewed.
- it can calculate annealing temperatures for **primers** prior to PCR upon entry of the oligonucleotide sequence and concentration together with the total buffer molarity; this is done using the nearest neighbour thermodynamic data for each base in the nucleotide chain in relation to its neighbour (Breslauer et al, Proc. Natl, Acad. Sci. USA, 83, 3746, 1986).
- it can be used for **protein determination** using the Bradford, Biuret and BCA protein assays at 595, 546 and 562 nm, respectively; samples are measured against a stored standard curve and the linear regression results of the straight line are printed out.
- it can measure OD 600 for **bacterial cell culture** solutions in order to optimise induction and harvest times.

To facilitate **electronic project archiving** of experimental results, all data can be downloaded directly to Excel using the appropriate interface adapter lead. Results can also be printed to a standard Centronics parallel printer.

A **calibration check filter set** is available for periodic testing of the instrument for GLP purposes (wavelength accuracy, absorbance accuracy and stray light); results of tests and status of instrument can only be viewed by downloading to PC or printing as an aid to record keeping.

The user friendly measurement system offers ease of use and ensures a long lamp lifetime, minimising the cost of ownership of the product.

Instrument Description

The display is a 128 x 64 pixel back lit graphics liquid crystal and the keypad has 28 keys organised into 3 areas; numeric keypad, **utility** keys and **reading** keys, including **set up** (see inner front cover for diagram). After switch on, initialisation and the calibration process, the instrument is ready for use (“Instrument Ready”). Note the following points -

- it may be necessary to **set up** the instrument for your measurement, particularly for nucleic acid quantification, where low volume and reduced aperture cells are used, and for doing a Bradford protein determination
- set reference is always required at the start of each measurement type; insert reference and sample cells so that the light path direction is in the back to front axis of the instrument
- press the appropriate reading or enter key after the sample has been inserted (length of measurement time is dependent on the number of wavelengths being read, all wavelengths are read in absorbance mode over a period of approx. 12 seconds)

Utility keys

set up	Enables configuration for the various instrument functions (see Set up). Pressing set up after the appropriate reading key will activate the set up process for that mode, with the first option being highlighted.
cal check	Enables instrument to be tested for absorbance accuracy, wavelength accuracy and stray light with special filters (see Performance Verification Check)
select	Enables review of options in the set up parameters and viewing results for base sequence and nucleic acid modes (if scan option is on)
print	Outputs data to printer or PC via multi purpose output port
base seq.	Enables entry of a primer or oligonucleotide base sequence for calculation of Theoretical Absorbance, Molecular Weight and conversion factor (see Base Sequence Entry)
Tm	Enables determination of Tm (from Abs260) and display of calculated Tm for an entered base sequence (see Tm)
stop	Behaves as an “escape” key, moving the user back to “Instrument Ready”
set ref	Reads reference values at appropriate wavelengths
enter	Enter / accept option in set up, and acts as reading key

Reading keys

abs	measures absorbances at calculates measure on	230, 260, 280, 320, 595, 600 nm no results are calculated individual samples
DNA	measures absorbances at calculates measure on	230, 260, 280, 320 nm DNA concentration 260/280 and 260/230 ratio individual samples
RNA	measures absorbances at calculates measure on	230, 260, 280, 320 nm RNA concentration 260/280 and 260/230 ratio individual samples
oligo	measures absorbances at calculates measure on	230, 260, 280, 320 nm oligo / primer concentration 260/280 ratio and 260/230 ratio individual samples
protein 595 assay	measures absorbances at calculates measure on	595, 546 or 562 nm protein concentration individual samples relative to a calibration curve of known standards
protein 280 meas.	measures absorbances at calculates measure on	260, 280, 320 nm protein concentration individual samples
cell culture	measures optical density at calculates measure on	600 nm corrected OD individual samples

TECHNIQUES

DNA, RNA and oligonucleotide characterisation

Nucleic Acid Quantification (NAQ)

- Nucleic acids can be quantified at 260 nm because it is well established that a solution of DNA or RNA with an optical density of 1.0 has a concentration of 50 or 40 µg/ml, respectively, in a 10 mm pathlength cell. Oligonucleotides, as a rule of thumb, have a corresponding factor of 33 µg/ml, although this does vary with base composition; this can be calculated if the base sequence is known (see Base Sequence in Set up and below).

$$\text{Concentration} = \text{Abs}_{260} * \text{Factor}$$

- The instrument uses factors 50, 40 and 33 as defaults for DNA, RNA and oligonucleotides, respectively, and compensates for dilution and use of cells which do not have 10 mm pathlength; dilution factor and cell pathlength are entered via **set up**.
- If using the ultramicrovolume or capillary reduced aperture cells, ensure they are correctly filled by carefully following the instructions supplied with the cell (see Cells) and that the correct pathlength has been entered.

- Default units are µg/ml, but units of ng/µl, µg/µl, pmol/µl and pmol phosphate can be selected via **set up** (the pmol/µl unit is useful for sequencing and PCR calculations involving primers). The following conversions are used:

$$1 \mu\text{g/ml} = 1 \text{ ng}/\mu\text{l} = 0.001 \mu\text{g}/\mu\text{l}$$

$$\text{pmol}/\mu\text{l} = \frac{\mu\text{g/ml} \times 1000}{\text{MW of oligo}}$$

$$\text{MW of oligo}$$

$$\text{pmol phosphate} = \frac{\text{nucleotide concentration, } \mu\text{g/ml}}{315}$$

$$315$$

- The molecular weight (MW) of a DNA oligonucleotide is calculated from
$$\text{MW (g/mole)} = [(dA \times 312.2) + (dC \times 288.2) + (dG \times 328.2) + (dT \times 303.2)]$$

$$+ [(MW \text{ counter-ion}) \times (\text{length of oligo in bases})]$$

(for RNA oligonucleotide, (dT x 303.2) is replaced by (dU x 298.2))
The MW calculated using this equation must be adjusted for the contribution of the atoms at the 5' and 3' ends of the oligo.
Phosphorylated oligos, add: [17 + (2 x MW counter-ion)]
Non-phosphorylated oligos, subtract: [61 + (MW counter-ion)]
The MW (g/mole) of the most common oligo counter-ions are Na (sodium, 23.0), K (potassium, 39.1) and TEA (Triethylammonium, 102.2).

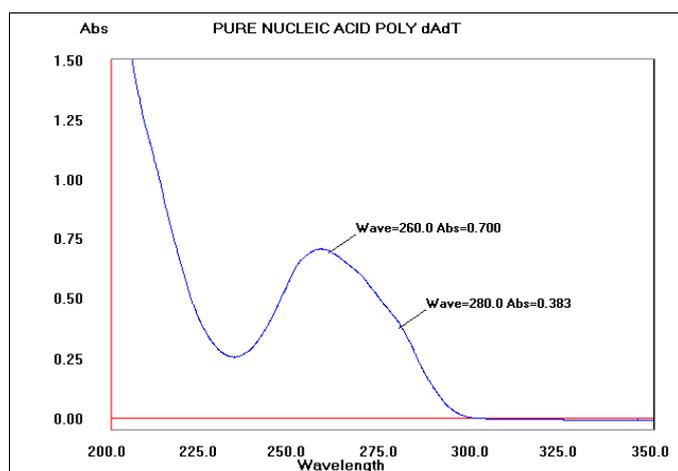
Nucleic Acid Purity Checks

- The extraction of 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 impurity in the sample, but care must be taken in interpretation of results.
- The 260 nm reading is taken near the top of a broad peak in the absorbance spectrum for nucleic acids, whereas the 280 nm reading is taken on a steep slope (i.e. small changes in wavelength cause large changes in absorbance). Consequently, small variations in wavelength at 280 nm will have a greater effect on the 260/280 ratio than will variations at 260 nm. Thus different instruments of the same and different types may give slightly different ratios due to variations in wavelength accuracy, but each instrument will give consistent results within itself.
- Concentration will also affect 260/280 readings. If a solution is too dilute, the readings will be at the instrument's detection limit, and results may vary because there is less distinction of the 260 peak and 280 slope from the background absorbance. This is one reason why absorbances should be > 0.05 for accurate measurements.
- An elevated absorbance at 230 nm can indicate the presence of impurities as well; 230 nm is near the absorbance maximum of peptide bonds and also indicates buffer contamination since 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. The nucleic acid survey scan facility is particularly useful for RNA samples.
- The instrument can display 260/280 and 260/230 ratios, and compensates for dilution and use of cells that do not have 10 mm pathlength; dilution factor and cell pathlength are selected via **set up**.

Use of Background Correction

- Background correction at a wavelength totally separate from the nucleic acid and protein peaks at 260 and 280 nm, respectively, is sometimes used to compensate for the effects of background absorbance. The wavelength used is 320 nm and it can allow for the effects of turbidity, high absorbance buffer solution and the use of reduced aperture cells. The instrument can use background correction; its use is selected via **set up**.
- If it is used, different results will be obtained to when it is not used because Abs320 is subtracted from Abs260 and Abs280 prior to use in equations:
Concentration = (Abs 260 - Abs 320) * Factor
Abs ratio = (Abs 260 - Abs 320) / (Abs 280 - Abs 320)
Abs ratio = (Abs 260 - Abs 320) / (Abs 230 - Abs 320)
- If your laboratory has not used background correction before, set this option to NO.
- The use of background correction can remove variability due to handling effects if the capillary cell or ultramicrovolume cell is being used (see Cells).

Spectral scan of nucleic acid (obtained using SWIFT software)



Note:

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

Protein Determination

Protein Determination at 595, 546 and 562 nm

- The Bradford method depends on quantitating the binding of a dye, Coomassie Brilliant Blue, to an unknown protein and comparing this binding to that of different, known concentrations of a standard protein at 595 nm; this is usually BSA, bovine serum albumin. The Biuret method depends on reaction between Cupric ions and peptide bonds in an alkali solution, resulting in the formation of a complex absorbing at 546 nm. The BCA method also depends on reaction between Cupric ions and peptide bonds, but in addition combines this reaction with the detection of Cuprous ions using bicinchoninic acid (BCA), giving an absorbance maximum at 562 nm. The BCA process is less sensitive to the presence of detergents used to break down cell walls.
- Detailed protocols are supplied with these assay kits, and must be closely followed to ensure accurate results are obtained. Due to the low lamp energy at 546 - 595 nm, absorbances greater than 2.000 cannot be measured.
- The use of plastic disposable cells is recommended. To include the zero concentration standard include it in the number of standards to be entered and enter 0.00 for concentration; use this when required to enter standard 1. If using duplicates, enter the same concentration twice; 2 duplicates of 3 different concentration equals 6 standards. Set reference on water.
- A linear regression analysis of the calibration standard data points is calculated; the result, together with the correlation coefficient, is printed out. A correlation coefficient of between 0.95 and 1.00 indicates a good straight line.

Protein Determination at 280 nm

- Protein can be determined in the near UV at 280 nm due to absorption by tyrosine, tryptophan and phenylalanine amino acids; Abs 280 varies greatly for different proteins due to their amino acid content, and consequently the specific absorption value for a particular protein must be determined.
- The presence of nucleic acid in the protein solution can have a significant effect due to strong nucleotide absorbance at 280 nm. 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:
$$\text{Protein (mg/ml)} = 1.55 * \text{Abs 280} - 0.76 * \text{Abs 260}$$

or, Protein conc. = (Factor 1 * Abs 280) - (Factor 2 * Abs 260)
- This equation can be applied to other proteins if the corresponding factors are known. The instrument can determine protein concentration at 280 nm and

uses the above equation as default; the factors can be changed, and the use of background correction at 320 nm is optional (via **set up**).

- 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.
- Set Factor 2 = 0.00 for direct λ 280 UV protein measurement; Factor 1 is based on the extinction coefficient of the protein. If BSA (bovine serum albumin) is an acceptable standard, setting Factor 1 = 1.115 will give linear results from 0 to 0.8 mg/ml protein.
$$\text{Protein (mg/ml)} = 1.115 * \text{Abs 280}$$
- Rapid measurements such as this at Abs 280 are particularly useful after isolation of proteins and peptides from mixtures using spin and HiTrap columns by centrifuge and gravity, respectively.

Bacterial Cell Culture Measurement

- Bacterial cell cultures are routinely grown to an OD 600 of approx. 0.4 prior to induction or harvesting. A linear relationship exists between cell number (density) and OD 600 up to approx. 0.600 OD.
- It is important to note that for turbid samples such as cell cultures, the light intensity measured is the proportion of the **scattered** light reaching the detector, and **not** the result of molecular absorption. The amount of scatter is affected by the optics of the system (distance between the cell holder and instrument exit slit, geometry of this slit and the monochromator optics). Different spectrophotometer types therefore give different responses for the same turbid sample; to compare results, they must be normalised using calibration curves.
- A calibration curve can be determined by comparing measured OD to expected OD. Expected OD is determined by counting cell number using an alternative technique (for example microscope slide method) and converting to OD using the rule of thumb that 1 OD 600 = 8×10^8 cells/ml for E. Coli.
- GeneQuant pro has much smaller optics than most conventional spectrophotometers, and more scattered light is transmitted through to the detector resulting in lower than expected OD 600 values. Results obtained by comparing measured OD 600 with expected OD 600 (see above) indicate that a correction factor of 2.0 is required to make the data comparable to larger instruments; this factor is included as a default value in set up.
- The use of 10mm pathlength disposable cells is recommended for optical density measurements of cell culture solutions; to prevent the suspension settling too quickly and giving an OD that changes with time, glycerol should be added to the sample.

SET UP AND SAMPLE MEASUREMENT

Set up

- For instrument set up, press “**set up**”. Press the appropriate “**reading key**” followed by the “**set up**” key to access relevant options (there is no set up for absorbance).
- Press “**select**” and “**enter**” to work through the various options.
- Press “**stop**” at any time to return to Instrument Ready status or to go back a level.

Day	Key in date (adjust daily)
Month	Select appropriate month
Year	Key in year.
Sample No.	Key in required number (increments automatically, default is 1)

Output Mode	Select output to Printer (standard Centronics parallel lead required), Serial (a special serial lead is required for connection to PC) or None
AutoPrint	Automatic output of reading key results to Printer or PC; print key is disabled. Select On or Off
Survey Scan	Select On, Off or Print; relevant to Nucleic Acid modes only
Language	Select English, German, French, Spanish or Italian

Use Date	Select On or Off; off means that date will not be printed
Key Click	Select On or Off

Sample measurement

- Press “**reading**” key to access the mode, then “**set ref**” key to set reference
 - Measurements are taken at all relevant wavelengths, and displayed as 0.000 values. A “Starting Lamp” message is displayed prior to measurement.
- Press “**reading**” key again, or “**enter**”, in order to make readings.
 - Measurements are taken at all relevant wavelengths, and actual absorbance values are displayed together with the results of calculations. A “Starting Lamp” message is displayed prior to measurement.
- Sample number is always displayed; reset using set up.
- After measurement, press “**print**” to obtain a print out comprising header, selected set up options, absorbance readings (as appropriate) and the various calculated results (as appropriate).

- If **Autoprint** is on, results for reading keys only will be output automatically; use of the print key is disabled.

DNA, RNA and oligo

Refer to Techniques for further information.

Pathlength	Select 10, 5, 2, 1 or 0.5mm
Units	Select required concentration units from $\mu\text{g/ml}$, $\text{ng}/\mu\text{l}$, $\mu\text{g}/\mu$, $\text{pmol}/\mu\text{l}$, pmol phosphate . $\text{pmol}/\mu\text{l}$ is only valid for oligonucleotides, and requires base sequence entry in order to be calculated
Use 320nm	Select yes or no
Dilution Factor	Key in the dilution factor for the concentration calculation, range 1 - 1000

Insert reference and press “set ref”

Insert sample	<p>Insert sample and press enter or “DNA”/“RNA” as appropriate. <i>The absorbances are measured and displayed together with the 260/230 and 260/280 ratios and the concentration</i> <i>If the sample is too dilute to give reproducible results, the concentration is displayed as “invalid”</i> <i>Press “stop” to exit</i></p>
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oligo only

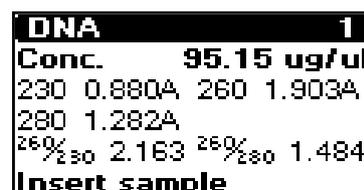
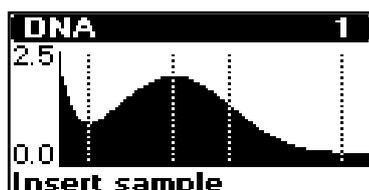
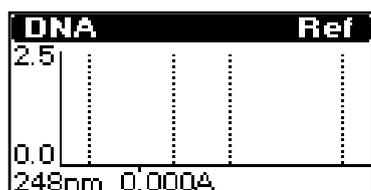
Default Factor	Default factor of 33 $\mu\text{g/ml}$. This can be changed, for example to 37 for ssDNA, if required
Calc Factor	Display shows the conversion factor ($\mu\text{g/ml}$) if base sequence has been entered
Use Default	Select no if calculated factor is to be used; if yes, default factor is used

Insert reference and press “set ref”

Insert sample	<p>Insert sample and press enter or “oligo” <i>The absorbances are measured and displayed together with the 260/230 and 260/280 ratios and the concentration</i> <i>Press “stop” to exit</i></p>
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Survey Scan On

- Survey scan is over the range 220 – 330 nm, and takes about 10 seconds.



- Lines on the graph indicate 230, 260, 280 and 320 nm.
- After the scan is complete, results are displayed; use “select” to view the graph.
- Press “set up” to change absorbance scaling of graph (use “select” and “enter” to choose between 0.1, 0.2, 0.5, 1.0, 2.0, 2.5 and Autoscale).

Examples of output:

DNA determination

Date: 22 May 2000

Name:

Factor : 50.0
Units : µg/ml
Pathlength : 10mm
Dilution Factor : 1
Use 320 nm : No

No.	230nm	260nm	280nm	320nm	260/280	260/230	Conc.
1	0.230	0.471	0.255	-----	1.850	2.045	23.5

Oligonucleotide determination

Date: 22 May 2000

Name:

Dflt Factor : 33.0
Units : µg/ml
Pathlength : 10mm
Dilution : 1
Use 320 nm : No

No.	230nm	260nm	280nm	320nm	260/280	260/230	Conc.
1	0.168-	0.325	0.183	-----	1.780	1.934	10.7

Base Sequence

Refer to Techniques for further information.

Base Type	Select DNA or RNA
Phosphorylated?	Select Yes or No
Counter Ion	Select Na (sodium), K (potassium), TEA (triethylammonium) or other counter ion
Other	Enter Molecular Weight of the counter ion being used <i>Press "enter" to initiate base sequence entry facility for oligonucleotide or primer; press A, C, G, T/U keys as appropriate; minimum 10, maximum 64 mer. Press the "delete" to remove the last base entered, and "cancel" to remove a base sequence (press "select" to change from yes or no). Press "select" to display the parameters calculated from the base sequence calculations:</i>
Theor Abs	Display shows theoretical absorbance (in units of AU/ μ mole)
Calc MW	Display shows molecular weight of entered base sequence and counter ions, including corrections for phosphorylation; this value is used in the calculation of concentration in pmol/ μ l and the conversion factor
Calc Factor	Display shows the conversion factor (μ g/ml) of entered base sequence; calculated from MW divided by Theor Abs

Example of output:

base sequence	
Date:	22 May 2000
Name:
AGC,AGC,AGC,AGC,AGC	
Base type	: DNA
Phosphorylated?	: Yes
Counter Ion	: Na
Theor Abs	: 149.1
Calc MW	: 5051
Calc Factor	: 33.9

Tm

Refer to Techniques for further information; a minimum base sequence of 10 mer needs to have been entered.

Primer Conc.	Key in concentration of primer, range 1.0 – 100.0 pmol/μl
Buffer Molarity	Key in molarity of salts in hybridisation solution, range 0.1 - 10.0
Calculated Tm	Display shows Tm calculated using the nearest neighbour method applied to the entered base sequence and the primer concentration and the salts molarity entered above. <i>Insert reference and press “set ref”</i>
Insert sample	Insert sample and press enter or “Tm” <i>The melting temperature based upon the oligonucleotide concentration actually measured in the sample is displayed; this is done by applying the nearest neighbour method to the entered base sequence, and uses the salts molarity entered above.</i> <i>Press “stop” to exit</i>

Example of output:

Tm		
Date:	22 May 2000	
Name:	
Base Sequence		
AGC,AGC,AGC,AGC,AGC		
Phosphorylated	:	Yes
Counter Ion	:	Na
Buffer Molarity	:	0.100
Primer Conc.	:	1.000
Calculated Tm	:	57.0 degC
No.	260nm	Measured Tm
1	0.123	57.8

Protein 595 Assay

Refer to Techniques for further information.

Method	Select Bradford, Biuret or BCA <i>The selected method becomes the default.</i>
No of standards	Key in the number of standards, range 3 - 27
Pathlength	Select 10, 5, 2, 1 or 0.5 mm
Units	Select µg/ml, mg/ml, µg
New standards	Select Yes or No (appears only if no method has been entered) <i>If No, last standards entered are used.</i> <i>If Yes, insert reference and press “set ref”</i>
Standard 1	Key in the concentration of the first standard, range 0.0 – 1000.0 <i>Insert Standard 1 and press “enter”</i> <i>The absorbance is measured and displayed together with the concentration of the standard. Press “enter”.</i>
Standard 2	Key in the concentration of the next standard, range 0.0 – 1000.0 <i>Insert Standard 2 and press “enter”</i> <i>The absorbance is measured and displayed together with the concentration of the standard. Press “enter”.</i> <i>Repeat procedure until all standards have been measured</i> <i>The standard curve details (slope, correlation coefficient and intercept) are displayed</i> <i>Insert reference and press “set ref”</i>
Insert sample	Insert sample and press “enter”. <i>The absorbance is measured and displayed together with the concentration of the sample relative to the standard curve.</i> <i>Repeat procedure until all samples have been measured</i> <i>Press “stop” to exit</i>

Example of output:

protein 595

Date: 22 May 2000

Name:

Method : Bradford

No. of standards 6

No.	Abs.	Conc.
1	0.693	0.139
2	0.842	0.278
3	0.934	0.417
4	1.026	0.556
5	1.150	0.650
6	1.285	0.834

Slope : 0.746

Corr Coeff : 0.996

Intercept : 0.593

No.	Abs.	Conc.
1	0.995	0.520
2		0.162

Protein 280 Measurement

Refer to Techniques for further information.

Coeff. 1	Key in the factor to be used for 280nm; default is 1.550, range is 0.01 - 10.00
Coeff. 2	Key in the factor to be used for 260nm; default is 0.760, range is 0.00 - 10.00
Use 320nm	Select yes or no <i>Insert reference and press "set ref"</i>
Insert sample	Insert sample and press enter or "protein 280" <i>The absorbances are measured and displayed together with the calculated concentration</i> <i>Press "stop" to exit</i>

Example of output:

Protein 280nm Measurement				
Date:	22 May 2000			
Name:			
Coeff 1	:	1.115		
Coeff 2	:	0.000		
Use 320 nm	:	No		
No.	260nm	280nm	320nm	Conc
1	0.123	0.275	-----	0.307

Bacterial Cell Culture

Refer to Techniques for further information.

Factor	Key in the correction factor you have derived from calibration plot to make results directly comparable with other spectrophotometers used in your lab; default is 2.0, and is typical <i>Insert reference and press "set ref"</i>
Insert sample	Insert sample and press enter or "cell culture". <i>The OD at 600 nm is measured and displayed together with the corrected result</i> <i>Press "stop" to exit</i>

MESSAGES

The following messages may appear during use of the instrument:

<i>> 2.000</i>	Protein 595 and Cell Culture readings only valid up to 2.000 A
<i>Invalid Curve</i>	Standards have not been inserted in corrected order or absorbance values do not increase sequentially
<i>Solution too dilute</i>	For reproducible results, a more concentrated sample or a longer pathlength cell should be used
<i>Too much light</i>	Sample compartment lid not closed properly
<i>Ref 1 error</i>	Check sample compartment area for blockage
<i>Ref 2 error</i>	Check sample compartment area for blockage
<i>Lamp failure</i>	Lamp failed to strike; switch off and try again. Lamp may need replacing if symptom persists

Contact your supplier if the messages below, or any other obvious fault messages appear, and cannot be cleared by re-initialising the instrument:

<i>Lamp too hot</i>	Fan has failed or air grill is blocked
<i>Filter error</i>	Filter wheel has not aligned properly
<i>Grating error</i>	Grating has not aligned properly

OUTPUT OF RESULTS

Use with parallel printer

- We recommend the use of the Seiko DPU-414 thermal printer, however, any Centronics parallel printer can be used together with the appropriate cable. If using a thermal printer, ensure it is set up to print out in condensed printing mode (80 characters) so that there is no wrap round (refer to printer user manual; for the Seiko DPU-414, this is in the DIP SW settings section). Ensure output to printer is on in the Set-up.
- Output is alphanumeric only and automatic when the Print key is pressed, and a printer is connected and switched on. Umlauts and accents are not printed out with letters if the instrument is set up to be in German, French, Italian or Spanish.
- The Survey Scan can be printed using the Seiko DPU-414 printer only; ensure “Survey Scan” in set up is set to Print, as On enables the facility on the instrument display only.

Use with PC

NOTE: A standard serial interface will not work.

1) Download to Spreadsheet

The serial interface adapter lead (80-2109-02) is required; it is also supplied with the Spreadsheet Interface Software for direct download to Excel. This macro is supplied on a floppy disc together with instructions for installation and use.

2) Use with Hyperterminal

The serial interface adapter lead (80-2109-02) is required; ensure output to serial is on in Set-up. The ASCII stream is output at 19,200 Baud via the 25 way D connector on the rear panel, and can be picked up by a PC with Windows 3.1 installed. Use the Hyperterminal emulator in Accessories to pick this up (settings are Handshake None, 19,200 Baud, 1 stop bit, 8 data bits, 0 parity, Comm port depends on which port the lead is connected to). Output is automatic if the interface lead is connected to the instrument.

CELLS

Selecting the appropriate cell

The instrument measures absorbance in the range 0.005 to 3.000 using a 10 mm pathlength cell; in general, spectrophotometers are at their most accurate in the absorbance range 0.100 to 1.000. The cell should be chosen based on sample concentration, dilution factor and available sample volume. Readings < 0.05 approach the detection level of the instrument, which decreases reproducibility; ideally, such levels should be avoided. Use the table below as a guide to selecting the correct cell. GeneQuant *pro* has the standard optical height of 15 mm; cells of 8.5 mm optical height require the use of an adapter.

Concentration range after dilution (ng/μl) *	Available sample volume (μl)	Suggested cell type	Pathlength (mm)	Part number
5 - 125	> 2000	standard	10	80-2002-58
	> 500	semi-micro	10	80-2002-77
	> 70 μl	microvolume	10	80-2103-69
10 - 250	> 7 μl	ultra microvolume**	5	80-2103-68
100 - 2500 ***	> 3 μl	capillary spare capillaries (100)	0.5	80-2104-66 80-2104-67

* assuming dsDNA with Abs 260 = 1.0 for 50 μg/ml (= ng/μl) in a 10 mm pathlength cell

** this is supplied with a micro sample viewer accessory

*** note that dilution may not be required for a miniprep / PCR amplification where typical concentrations are between 50 and 200 ng/μl

- The 7 μl ultramicrovolume cell has a minimum detection limit of 2 μg/ml dsDNA assuming an absorbance of 0.020; this equates to 14 ng dsDNA if 7 μl of solution is used. This very low level is not sufficient for reproducible results. Use of background correction is recommended.
- The capillary cell has a minimum detection limit of 20 μg/ml dsDNA assuming an absorbance of 0.020; this equates to 60ng dsDNA if 3 μl of solution is used. This very low level is not sufficient for reproducible results. Use of background correction is recommended.
- The use of standard 1 mm and 5 mm pathlength cells is not recommended due to the requirement of a packing piece (not required for the ultra microvolume and capillary cell described above).

Comparison of instrument with other spectrophotometers

- You must compare like with like, using the same sample with the same cell, and obtain absorbance measurements on both at the same wavelengths.
- GeneQuant *pro* has a bandwidth of 5 nm, and this should be compared with a spectrophotometer having the same bandwidth, since this parameter affects absorbance values.

Filling cells

Ensure cells are always clean and dry before use and between samples

Standard 10 mm pathlength cells

- fill so that the level of the liquid measures at least 20 mm from the cell bottom

Microvolume cell (80-2103-69)

- fill so that the liquid meniscus cannot intercept the light beam - we recommend at least 70 μ l is used

Ultramicrovolume cell (80-2103-68)

- fill so that the liquid meniscus cannot intercept the light beam - we recommend at least 7 -10 μ l is introduced into the pipette fitted with an appropriate tip
- insert tip into one of the conical openings of the cell and ensure the tip is pushed in firmly - deliver the sample slowly into the cell while tilting it with the other conical opening upwards to ensure all air is expelled
- place cell in micro sample viewer and hold up to the light; when viewed through the eye piece, the 3 X magnification lens enables confirmation that there are no bubbles or other impediments in the light path
- ensure the faces of the cell are wiped clean

Capillary cell (80-2104-66) and capillaries (80-2104-67)

- dipping the capillary into the solution will normally introduce enough liquid for measurement
- liquid can be prevented from leaking out using the Cristaseal supplied

Taking measurements

- Ensure cell pathlength is set to 10 mm (or 5mm for the ultramicrovolume cell, 80-2103-68); ensure cell orientation is always the same

For capillary cells:

- Ensure cell pathlength is set to 0.5 mm
- Place capillary cell holder in sample compartment
- Insert capillary

Cleaning cells

- After use, cells should be cleaned with a dilute alkali (e.g. 0.1 M NaOH) and a dilute acid (e.g. 0.1 M HCl) wash, followed by rinsing several times with distilled water. More rigorous cleaning after difficult samples should be performed with a suitable liquid laboratory glassware detergent (e.g. Decon), following the manufacturer's instructions.
- The capillary cell can be dismantled for cleaning and removing a broken capillary by unscrewing the two screws on each side using the tool provided.

Reproducibility using reduced aperture ultramicrovolume and capillary cells

- Check for correct operation range and sample integrity by inspection of all 4 wavelength readings for nucleic acid measurements
- Ensure that set reference is renewed periodically (preferably every 10 consecutive readings)
- Ensure background correction at 320 nm is on
- Ensure sample dilution is such that sample Abs 260 is greater than 0.100
- Ensure cell is scrupulously clean

ACCESSORIES

Deuterium Lamp	80-2109-86
Printer Stand	80-2109-96
Seiko DPU-414 printer	enquire
Parallel interface cable (Centronics)	80-2071-87
Interface adapter for serial connection (includes spreadsheet interface software)	80-2109-02
Spare GeneQuant <i>pro</i> User Manual (multi language)	80-2110-89
GeneQuant calibration check filter set	80-2109-88
Spare dust cover	80-2109-13
Basic UV/Visible Spectrophotometry Booklet	80-2108-63

MAINTENANCE

After Sales Support

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

- 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

Performance Verification Check (Cal Check Utility Key)

- Verification of instrument performance is important for Good Laboratory Practise (GLP) purposes. GLP requires that results obtained in an experiment can be traced back to an instrument, and that the instrument can be proved to be working correctly.
- Using a calibration check filter set, the instrument can be checked for absorbance accuracy at 230, 260, 280, 320, 595 and 600nm, wavelength accuracy at 260 and 280nm and stray light at 260nm.
- An accredited service engineer with your supplier should have a set of filters (secondary standards traceable to NIST), and be able to verify the instrument performance; a certificate for record keeping will be provided. A **calibration check filter set** (part number 80-2109-88) is available for periodic testing of the instrument to ensure that it is working to specification if you prefer to do it in house; instructions for carrying this out are provided with the filters.
- Observe all necessary precautions if dealing with hazardous samples or solvents.

Cleaning and General Care of the Instrument

External cleaning

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

Sample compartment area spillage

- Switch off the instrument and disconnect the power cord.
- The sample area is manufactured from a chemical resistant material. Strong concentrations of sample, however, may affect the surface and spillages should be dealt with immediately.
- A small drain hole in the sample area allows excess liquid to drain away onto the bench or table from under the instrument.
- Use a soft dry cloth to mop out the sample area.
- Reconnect the power supply cord and switch on the instrument.

Fuse Replacement

- 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 between the power input socket and the on/off switch on the back panel of the instrument; do not touch the power supply socket prongs.
- Slide the fuse holder open by pulling at the notch.
- Place fuses (1.6AT, 5mm x 20mm, FST) into the fuse holder and slide back into position.
- 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.

Deuterium Lamp Warranty

The criterion for lamp replacement under warranty is that it must be less than 36 months old.

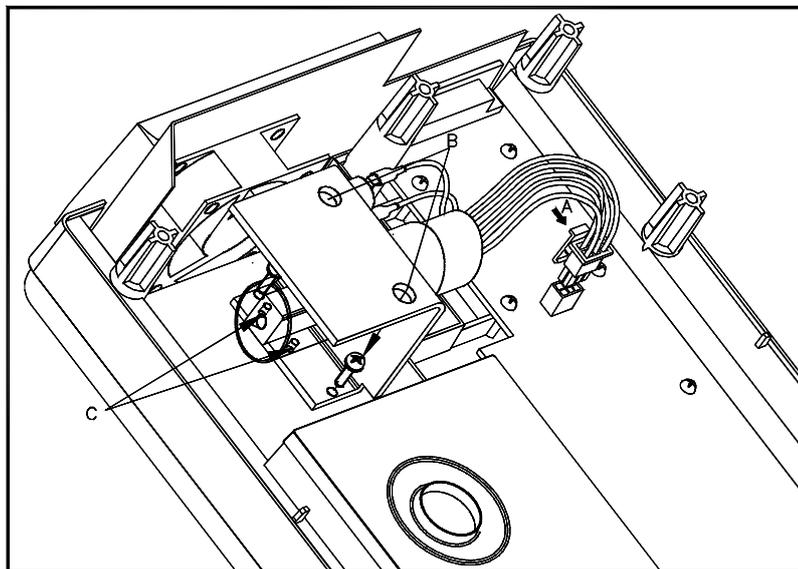
Deuterium Lamp Replacement

Replacement lamps are available from your supplier using the part number **80-2109-86**, deuterium lamp assembly.

The design of the lamp area is such that users are able to change their own lamps. No lamp alignment is required as the lamps are pre-aligned at manufacture. The lamp becomes very hot in use. Ensure it is cool before changing it. Do not touch the optical surface of the lamp with your fingers (use tissue); if touched, the area should be cleaned with iso-propanol.

To replace a lamp proceed as follows:

- 1) Switch off the instrument and disconnect the power supply cord.
- 2) Release the instrument cover by unscrewing the seven screws in the base.
- 3) Carefully lift top cover upwards, tilt and place on the right side of the instrument taking care not to damage the ribbon cables and not to touch any of the circuitry (in particular the heat sink).
- 4) Depress catch "A" and lift connector away from circuit board.
Remove two screws "B".
Lift deuterium lamp and bracket assembly away from mounting plate.
- 5) Place new deuterium lamp assembly into position, locating pins "C" into holes and slot in mounting plate.
Refit two screws "B" and tighten carefully.
Refit connector "A", pushing downwards until the catch snaps shut.
- 6) Refit the instrument top cover, taking care not to trap the ribbon cable.
- 7) Refit the seven screws in the base.
- 8) Reconnect the power supply cord and switch the instrument on.



SPECIFICATION

<i>Wavelength range</i>	Fixed at 230, 260, 280, 320, 546, 562, 595, 600 nm Survey scan from 220 – 330 nm
<i>Monochromator</i>	Czerny-Turner configuration with 1200 lines/mm holographic grating
<i>Wavelength calibration</i>	Automatic upon switch on
<i>Spectral bandwidth</i>	5 nm
<i>Wavelength accuracy</i>	± 1 nm
<i>Wavelength reproducibility</i>	Better than ± 0.5 nm
<i>Light source</i>	Long life borosilicate glass pulsed deuterium lamp
<i>Detector</i>	Silicon photodiode
<i>Photometric range</i>	0 to $\pm 3.000A$ for 230, 260, 280, 320 nm 0 to $\pm 2.000A$ for 546, 562, 595, 600 nm
<i>Photometric linearity</i>	$\pm 1.0\%$ or $\pm 0.005A$ to 3.000A, whichever is the greater
<i>Photometric reproducibility</i>	0.5% of absorbance value
<i>Stray Light</i>	$<0.1\%T$ at 280nm using Acetone
<i>Digital output</i>	Centronics parallel as standard 9 pin serial via interface adapter lead
<i>Dimensions</i>	320 x 400 x 160 mm
<i>Weight</i>	4 kg
<i>Power requirements</i>	100-240V AC $\pm 10\%$, 50/60Hz, 80VA
<i>Safety standard</i>	EN61010-1
<i>EMC emissions</i>	EN 50 081-1 Generic emissions part 1
<i>EMC immunity</i>	EN 50 082-1 Generic immunity part 1
<i>Susceptibility standard</i>	IEC 801
<i>Quality System</i>	Designed and manufactured in accordance with an ISO 9001 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.