CONTENTS

INTRODUCTION

GeneQuant, the RNA/DNA calculator, is a spectrophotometer designed specifically for molecular biologists.

The instrument measures RNA and DNA samples in UV cells at 230 nm, 260 nm, 280 nm and 320 nm simultaneously. The wavelengths 260 nm and 280 nm are used for quantification and purity checking calculations, whereas 320 nm can be used for background compensation. 230 nm can be used as a guide for protein determination using the peptide bond absorbance. After each sample reading the information is stored and used in calculations until the next reading is taken.

GeneQuant can be left on continuously without affecting the lifetime of the light source and this is recommended for the convenience of the user to obtain rapid measurements. The normal status of the lamp is standby mode and it is only powered up during actual reference and sample measurement using a unique demand switching technique. (Patent applied for).

You can use GeneQuant to calculate:

- RNA, ssDNA and dsDNA concentrations in units of weight, molar fraction, moles of phosphate and total molecules
- A_{260}/A_{280} ratio
- Total protein concentration
- Recovery of oligonucleotides
- Purity of nucleic acids
- Melting temperature
- Molecular weight of oligonucleotides

SYMBOLS

Symbols found in the manual:

Please Note

Instrument Ready

Display Panel

Warning

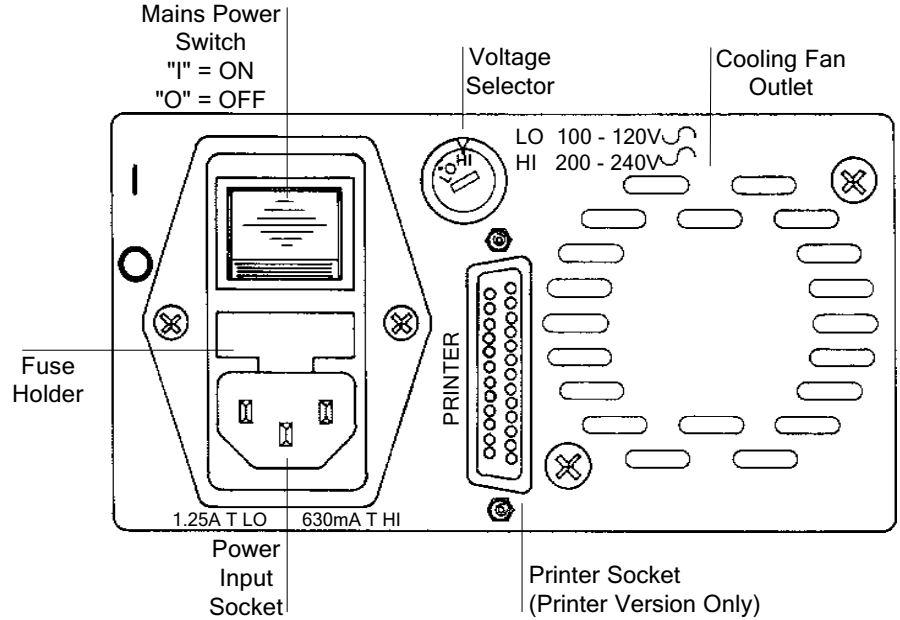
sample

Key Press

Symbols found on the instrument:

Background yellow, symbol black.
Caution - refer to accompanying documents.

REAR PANEL



BEFORE INSTALLATION

Inspect the instrument for any signs of damage caused in transit. If any damage is apparent then inform your supplier immediately and do not proceed with the installation.

Check that:

- the installation site conforms to the environmental conditions for safe operation (see Specifications).
- the cooling fan outlet is not obstructed.

INSTALLATION

This equipment must be connected to the power supply with the power supply cord provided and MUST BE EARTHED.

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not appropriate for safe operation, the protection provided by the equipment may be impaired and instrument warranty withdrawn.

- Select the correct voltage for your local supply, using the voltage selector on the rear panel.
- Select the appropriate fuses for your local supply. Two identical fuses need to be loaded. For LO 100-120V operation use 2 x 1.25A T fuses and for HI 200-240V operation use 2 x 630mA T fuses. (See Maintenance for fitting fuses).
- Connect the power supply cord to the input socket on the rear panel and to the power supply.
- Switch on the instrument.
- For optimum location of the cell, reposition the spring clip located in the sample compartment by pulling firmly upwards and relocating in appropriate central slots and if you are not using 10mm cells reset the path length in 'set up'.
- ^If you are using a printer, check that it is a parallel version and is switched 'on-line' if necessary. Ensure that the printer options are selected in 'set-up'.

^applies to printer output version only

CALCULATION KEYS

Ensure all parameters in 'set up' are appropriate for your sample.

abs

to display absorbance reading.
Press 'select' to cycle through the 4 wavelength values 230nm, 260nm, 280nm and 320nm.
** indicates that a cell path length of 10mm has not been selected.
To convert to corresponding OD values for a 10mm cell, multiply by 2, 10 or 20 for 5, 1 and 0.5mm path length cells, respectively.

ratio

to display A_{260}/A_{280} absorbance ratio with or without background correction at 320nm. See 'set up'.

RNA DNA

to display concentrations of RNA, dsDNA or ssDNA.
Press 'select' to cycle through choice of units:
Conc 1: µg/ml - range 1 - 4000µg/ml
Conc 2: µg/µl - range 0.001 - 0.2µg/µl
Conc 3: pmol/µl - range 0.001 - 200pmol/µl
(Note: ensure the [+] oligonucleotide length and factor value are correct. See 'set up')
Conc 4: Phosphate concentration pmol - range 0.001 - 0.200pmol

protein

to display protein concentration in mg/ml - range 0 - 600mg/ml.
(Note: ensure the coefficients are correct. See 'set up').

[x]

to display molecules/ml.
(Note: ensure the correct molecular weight is entered. See 'set up').

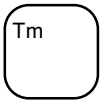
purity

to display percentage purity by comparing actual ratio to expected ratio. (Note: ensure the expected ratio is entered. See 'set up').

recovery

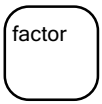
to calculate percentage recovery by comparing actual to expected concentration (pmol/µl).
(Note: ensure the expected concentration and the oligonucleotide length are entered. See 'set up').

[+] does not apply to printer output version

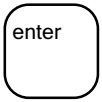


to calculate melting temperature. ^Press 'Select' to cycle between short oligonucleotide and primer calculation.
(Note: ensure that the number of bases and ^molarity are correct. See 'set up').
'!!' indicates that the equation is not strictly applicable for the base numbers used. (See Factors and Formulae).

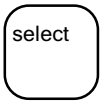
Other Keys



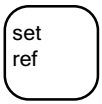
to change factor values for RNA, ssDNA and dsDNA and select the current mode for calculations.



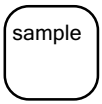
to enter a value or option.



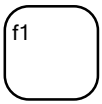
to choose a value or option within a function.



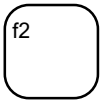
to measure reference.



to measure sample.



^to print out the list of 'set-up' options.



undefined - available for future development.

^applies to printer output version only

OPERATION

To get started:

- switch on the instrument at the rear panel

Instrument Initialising

Note: Instrument initialises for a few seconds.

Instrument Ready

- select 'set up' values. This step can be omitted for quick absorbance readings
- set reference
- insert the cell so that the light path direction is in the front-to-rear axis of the instrument as indicated by the arrow
- measure your sample

The instrument can be used directly as a calculator for Tm and molecular weights without measurement of a sample.

A security facility which locks the instrument keypad is available. When 'Instrument Ready' or a calculation result is displayed, key in 4080 to switch facility on. The instrument will then display 'Instrument Locked'; key in 4080 again to unlock. The instrument remains locked through power off/on.

Calculation keys, except Tm, will only function after a sample measurement has been taken.

Instrument Set Up

If you key in a number incorrectly, press 'C' and start again.

set-up and enter to step through 'set up' options and enter the parameters which relate to your sample. To exit 'set up' press any calculation key. To return to the beginning, press 'set up'.

The default parameters used in the instrument may be altered as follows:

- **Cell path length (mm).** Press 'select' to choose from: 0.5 1 5 10.
- **^Printer.** Press 'select' to choose from ON/OFF. Press 'enter'.
- **^Sample number.** Key in the required number. Press 'enter'. The sample number increments automatically each time the sample is measured.
- **^Date.** Key in the date. Press 'enter'. (Adjust this daily).
- **^Month.** Select appropriate month. Press 'enter'.
- **^Year.** Key in the year. Press 'enter'.
- **Background compensation 320nm.** Press 'select' to choose from: YES/NO.
- **^Dilute?** Key in the dilution factor for concentration calculation. Range 1.00-99999.9.
- **Factor.** Press 'select' to choose from: RNA dsDNA ssDNA. For synthetic oligonucleotides use ssDNA and key in new factors if defaults are not suitable. Press 'enter'.
- **Bases.** Key in the number of A C G T U to calculate molecular weight from base composition of nucleic acids and to show results as molecules/ml. Press 'enter' to cycle through the bases. Range 0-1000.
- **Oligo length.** ⁺Key in the oligonucleotide length of the sample in base units to show results as pmol/µl. Press 'enter'. Range 1-9999.
- **Molecular weight (MW).** Press 'select' to choose between the calculated value (from A, C, G, T, U numbers) or user-entered value for molecular weight (if known). Press 'enter'.
- **Ratio.** Key in the A_{260}/A_{280} absorbance ratio expected for your sample (if known). Press 'enter'.
- **Concentration.** Key in the concentration expected (in pmoles/µl) for your sample (if known). Press 'enter'.
- **Protein.** Key in coefficients for equation if they are different to defaults. (See Factors and Formulae).
- **^Molarity.** Key in molarity of salts in hybridisation solution.

^ applies to printer output version only
⁺ does not apply to printer output version

Reference Measurement

Take a reference reading. This reading is stored and used as the base reading for all sample measurements until a new reference reading is taken.

If you do not insert and remove the sample cell in time, the display will show:

Remove Cell Press Key Again

1 set ref

Please Wait

2 Wait for the tone and insert the reference cell into the sample compartment:

Insert Reference

3 Wait for the tone and remove the reference:

Remove Reference

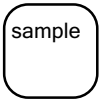
4 After you have removed the reference the display will show:

ABSORBANCE 260 nm 0.000 AU

When using the capillary cell and holder, place the holder in the sample compartment before taking measurements. Then insert and remove the capillary as above.

Sample Measurement

You can now measure a sample. A similar procedure is followed to the one used for measuring a reference. Use the 'sample' key instead of 'set ref'. Ensure correct cell path length and sample type have been selected in 'set up'.

1. 

Please Wait

or

^

Date 23 Aug 1993 Sample No. 9164

2. Wait for the tone and insert the sample cell into the sample compartment:

Insert Sample

3. Wait for the tone and remove the sample cell:

Remove Sample

4. After the first sample, the display will show absorbance at 260nm:

ABSORBANCE	
260 nm	1.000 AU

'Absorbance**' indicates that a 10mm path length cell has not been selected. (See 'set up').

Stored readings can now be manipulated using the calculation keys.

For subsequent samples the display defaults to the previous function used.
This facility provides useful single key operation if you require the same type of measurement on successive samples. Operation of the 'sample' key automatically displays the result after sample measurement.

^ displayed on printer output version only

Printer Output Version

This section should be read in addition to the details given in the previous sections.

Installation

- Connect the printer via its interface cable to the socket on the rear panel of the instrument.
- Switch on the printer and ensure it is on-line.

Operation

The sample number and date are stored permanently until altered. Sample number is incremented automatically each time a sample is measured. The date does not increment automatically.

If you do not wish to alter any further parameters in 'set up', take reference readings and sample measurements as described previously. The displays and printouts appear as shown below (when the printer option in 'set up' is set to 'on', printouts of the display occur automatically).

After you have removed the reference:

ABSORBANCE	
260 nm	0.000 AU

```
Absorbance
260 nm  0.000 AU
```

select

ABSORBANCE	
280 nm	0.000 AU

```
Absorbance
280 nm  0.000 AU
```

The remaining absorbance values can be printed out in the same way.

When a sample is measured:

sample

Date 23 Aug 1993 Sample No. 9164	Operator _____ Date 23 Aug 1993 Sample No. 9164
Insert Sample	
Remove Sample	
ABSORBANCE 260 nm 0.957 AU	Absorbance 260 nm 0.957 AU

RNA
DNA

dsDNA CONC 1 47.9 µg/ml	dsDNA CONC 1 47.9 ug/ml

If required press 'select' to cycle through and print out the other choices of units. Other calculations are printed out automatically when the appropriate key is pressed.

The instrument facilitates routine analysis as the display and print out always show the selected parameters automatically after a measurement, as shown in the examples on the following page.

If the printer is switched off or off-line during use while selected 'on' in 'set up' and a key is pressed, the display shows:

Printer off-line Press any key

Normal functioning resumes after any key is pressed. If the printer is switched on again, normal printing resumes. If the printer remains off, the printer option in 'set up' is automatically set to off.

Examples:

'setup' options

```
GeneQuant        SETUP
Path Length         10
Printer             ON
Sample No.         507
Date                18
Month              Jan
Year              1993
Use 320 nm          NO
Dilute?           1.00

FACTOR
ssDNA?            37.0

BASES
Number A?            5
Number C?            5
Number G?            2
Number T?            1
Number U?            1

OLIGO
Length              13

MOLECULAR WEIGHT
CALC            3961.6

RATIO
Expected?        1.800

CONCENTRATION
Expected?        2.000

PROTEIN
Coeff 1?         1.550
Coeff 2?         0.760

Molarity         0.100
```

Complete list of calculations for a nucleotide

```
Operator________
Date 18 Jan 1993
Sample No.  0505

ssDNA CONC 1
29.3 ug/ml

ssDNA CONC 2
0.029 ug/ul

ssDNA CONC 3
0.197 pmol/ul

Phosphate CONC
0.093 pmol

RATIO
1.997

PURITY
 99%

RECOVERY
 98%

MOLECULES/ML
4.459395 Exp 15

MELTING TEMP
40 C

MELTING TEMP PRI
48.5 C
```

Routine analysis of proteins

```
Operator________
Date 23 Feb 1993
Sample No.  8273

PROTEIN CONC
0.7 mg/ml

Operator________
Date 23 Feb 1993
Sample No.  8274

PROTEIN CONC
0.4 mg/ml

Operator________
Date 23 Feb 1993
Sample No.  8275

PROTEIN CONC
1.5 mg/ml

Operator________
Date 23 Feb 1993
Sample No.  8276

PROTEIN CONC
3.8 mg/ml

Operator________
Date 23 Feb 1993
Sample No.  8277

PROTEIN CONC
32.2 mg/ml

Operator________
Date 23 Feb 1993
Sample No.  8278

PROTEIN CONC
0.2 mg/ml
```

Parallel Printer Interface

Specifications:

Data transmission:	8 bit parallel
Synchronisation:	Timing for attached printer is provided by external strobe signals.
Handshake protocol:	By BUSY and ~STROBE signals.
Signal levels:	The levels of output data and interface control signals are all TTL compatible.

Interface Connector

The printer interface is a standard 25-pin D-shell female connector. The data lines (D0-D7) on the connector are driven by drivers capable of sourcing 15mA and sinking 24mA.

Pin No.	I/O	Signal Name
1	O	~STROBE
2	O	Data Bit 0
3	O	Data Bit 1
4	O	Data Bit 2
5	O	Data Bit 3
6	O	Data Bit 4
7	O	Data Bit 5
8	O	Data Bit 6
9	O	Data Bit 7
10	I	~ACK
11	I	BUSY
12-17	N/A	Unconnected
18-25	N/A	Ground

ACCESSORIES AND CONSUMABLES

Selecting the Appropriate Cell

GeneQuant has a suitable measuring range between 0.1 to 2.5 OD for a 10mm pathlength cell. Choose a suitable cell depending on sample concentration range, dilution factor and available sample volume.

Pathlength, mm	**Suitable Sample OD_{260} range**	**Concentration range (µg/ml) given that 1.0 OD_{260} = 50µg/ml for dsDNA**
10	2.5 - 0.1	125 - 5
5	5 - 0.2	250 - 10
1	25 - 1.0	1,250 - 50
0.5	50 - 2.0	2,500 - 100

UV/Visible Cell Order Information

Pathlength and Description	**Minimum Sample Volume**	**Part Number**
10mm, standard cell with lid	2,000µl	80-2002-58
10mm, semi micro cell with lid	500µl	80-2002-77
10mm, micro cell with lid	250µl	80-2002-95
10mm, ultra microvolume cell	70µl	80-2103-69
5mm, standard cell with lid	1,000µl	80-2002-57
5mm, ultra microvolume cell	5µl	80-2103-68
1mm, standard cell with lid	200µl	80-2002-54
0.5mm, quartz capillary cell (includes 100 capillaries)	3µl	80-2104-66
Spare quartz capillaries (100)	3µl	80-2104-67

Using Cells

Ensure that the cell faces are clean before measurement. After use cells should be cleaned with a dilute alkali (e.g. 0.1M NaOH) and a dilute acid (e.g. 0.1M HCl) wash, followed by rinsing several times with distilled water. More rigorous cleaning after difficult samples should be performed with a suitable liquid detergent, following the manufacturer's instruction.

The 0.5mm quartz capillary is filled by dipping into the sample. After use, it can be emptied using a Pasteur pipette bulb attached to narrow bore silicone tubing. The quartz capillary cell can be dismantled for cleaning and removing a broken capillary by unscrewing the two screws on each side using the tool provided.

Instructions are provided with the 0.5mm quartz capillary and 5mm ultra microvolume cells.

Other

GeneQuant	80-2103-98
GeneQuant (printer version)	80-2104-98
GeneQuant II	80-2105-98
Deuterium lamp	80-2104-56
GeneQuant User Manual	80-2105-20
GeneQuant II User Manual	80-2105-58
Dust Cover	80-2105-18
Parallel Interface Cable (Centronics)	80-2071-87
Introduction to Basic UV/Visible Spectrophotometry	80-2005-60

FACTORS AND FORMULAE

- Grams (g) are converted to moles using 309 (the average molecular weight of the ATCG bases)

- Factors for molecular weights (MW) of bases:-

  A=312.2 C=288.2 G=328.2 T=303.2 U=289.2

- For dephosphorylated oligonucleotides subtract 61 from the calculated MW

- For phosphorylated oligonucleotides add 17 to the calculated MW

- A_{260} x factor = µg/ml concentration

  Default factors are: 40 for RNA, 37 for ssDNA, 50 for dsDNA

  For synthetic oligonucleotides use ssDNA mode and change factor

- A_{260}/A_{280} ratios are 1.8 and 2.0 for pure DNA and RNA preparations, respectively.

- $\text{pmol/µl} = \frac{\text{µg/ml x 1000}}{\text{309 x oligo length}}$ ^ $\text{pmol/µl} = \frac{\text{µg/ml x 1000}}{\text{M W}}$

- $\text{pmol phosphate} = \frac{\text{Nucleotide concentration, µg/ml}}{315}$

- $\text{Molecules/ml} = \frac{\text{concentration, µg/ml x } 6.023 \times 10^{23}}{\text{MW x } 10^{6}}$

- Protein (mg/ml) = $1.55 \times (A_{280} - A_{320}) - 0.76 \times (A_{260} - A_{320})$

  Coefficients 1 and 2 (defaults 1.55 and 0.76, respectively) can be changed for different proteins

- Tm 1 (for short oligonucleotides) = 2 (nA + nT) + 4 (nG + nC), n refers to number of individual base units - equation is valid linearly from 10 to 18 mer, but may be used as a guideline for values above 18 mer.

- ^ Tm primers = 81.5 + 16.6 (log molarity) + 0.41 (% guanosine + cytosine) - 500/primer length
  Equation is valid from 14 to 60 mer

^ applies to printer output version only

MAINTENANCE

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

User maintenance is restricted to changing the instrument lamp, changing external fuses and instrument cleaning. For any other maintenance operation contact your local supplier.

Cleaning and General Care

External Cleaning:

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

Sample Compartment Spillages:

- Switch off the instrument and disconnect the power supply cord.
- The sample compartment is coated in a chemical resistant finish. However, strong concentrations of sample may affect the surface and spillages should be dealt with immediately.
- A small drain hole in the sample compartment 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 compartment.
- Reconnect the power supply cord and switch on the instrument.

Fuse Replacement

Select the appropriate fuses for your local supply. Two identical fuses need to be loaded. For LO 100-120V operation use 2 x 1.25A T fuses and for HI 200-240V operation use 2 x 630mA T fuses.

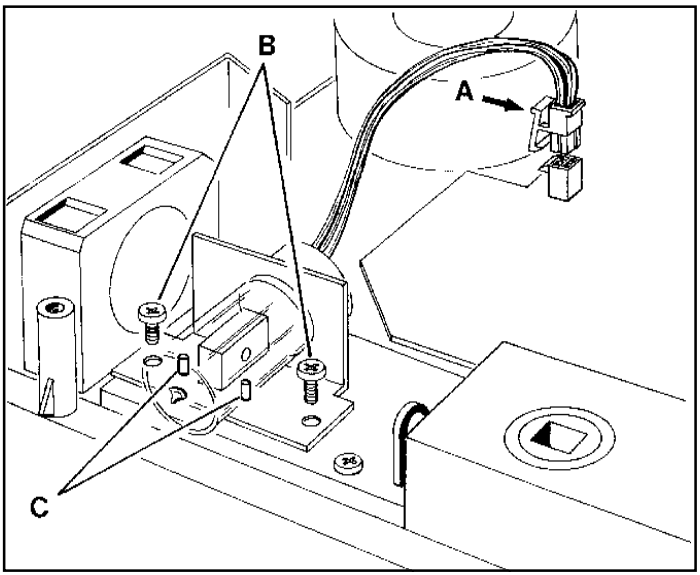
- Switch off the instrument and disconnect the power supply cord. The fuse holder can only be opened if the power supply plug has been removed.
- The fuse holder is located between the power input socket and the on/off switch on the back panel of the instrument.
- Slide open the fuse holder by pulling at the notch.
- Place fuses into the fuse holder and slide back into position.
- Reconnect the power supply cord and switch on the instrument.

Deuterium Lamp Replacement

Replacement lamps are available from your local supplier.

The deuterium lamp becomes very hot in use, so allow at least 10 minutes before changing. Care should be taken not to touch the optical surface of the new lamp with your fingers; if touched, the area should be cleaned with methanol.

- Switch off the instrument and disconnect the power supply cord.
- Release the instrument cover by unscrewing the seven screws in the base.
- Carefully lift top cover upwards, tilt and place on the right side of the instrument taking care not to damage the ribbon cables.
- Depress catch 'A' and lift connector away from circuit board.
  Remove two screws 'B'.
  Lift deuterium lamp and bracket assembly away from mounting plate.
- Place new deuterium lamp and assembly into position, locating pins 'C' into holes and slot in mounting plate.
  Refit two screws 'B' and tighten.
  Refit connector 'A' pushing downwards until the catch snaps shut.
- Refit the instrument top cover, taking care not to trap the ribbon cable.
- Refit the seven screws in the base.
- Reconnect the power supply cord and switch on the instrument.



SPECIFICATIONS

Light source	Deuterium arc lamp
Wavelength range	Fixed at 230, 260, 280 and 320 nm
Wavelength calibration	Factory set
Wavelength accuracy	±2 nm
Wavelength reproducibility	Better than ±0.1nm
Bandwidth	5 nm
Stray light	Less than 0.1%T at 320 nm with $NaNO_2$
Photometric reproducibility	0.5% of Abs reading or 5mA which ever is the greater
Photometric range	0 to 3.000 Abs
Photometric linearity	±1% of reading or ± 0.005 A to 3 A, whichever is the greater
Cell size accommodated	0.5mm pathlength capillary cell 1 mm, 5 mm and 10 mm pathlength standard cell
Dimensions	270 (w) x 320 (d) x 130 (h)
Weight	3.5 kg
Environmental conditions	Indoor use only, away from inflammable materials Temperature 5 °C to 40 °C Maximum relative humidity 80 % up to 31 °C decreasing linearly to 50% at 40 °C Installation Category II
Safety standard	IEC 1010
EMC standard	CISPR 22
Quality system	Designed and manufactured in accordance with an ISO 9001 approved quality system
Digital Output	Centronics parallel output (printer version only)
Electrical specifications	
External fuse ratings	100-120V - 1.25 A T 200-240V - 630 mA T
Internal fuse ratings	F101 - 3.15 A T F102 - 2 A T
VA rating	100 VA
Voltage	100 - 120 V 200 - 240 V } ± 10 %
Frequency	50/60 Hz

Specifications are measured after the instrument has warmed up at 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.