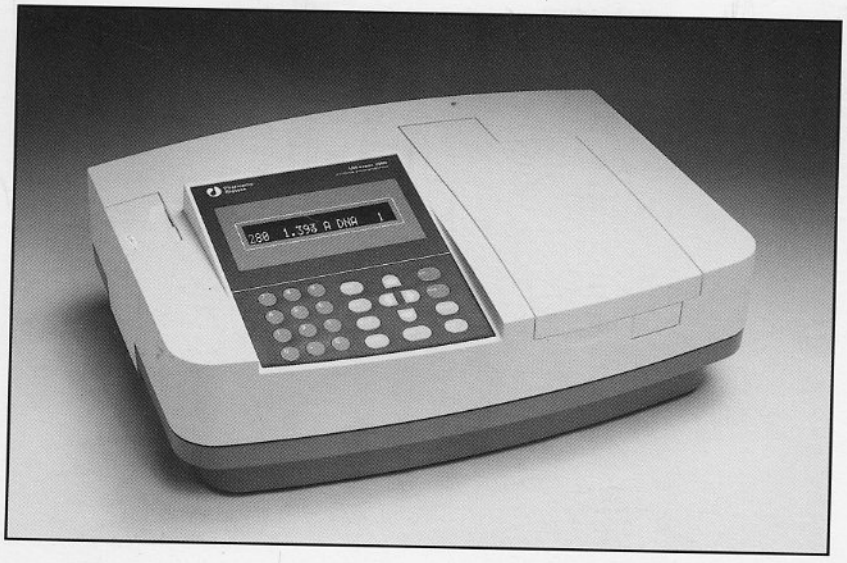


Ultrospec 2000
UV/Visible Spectrophotometer
User Manual

English

Deutsch

Français

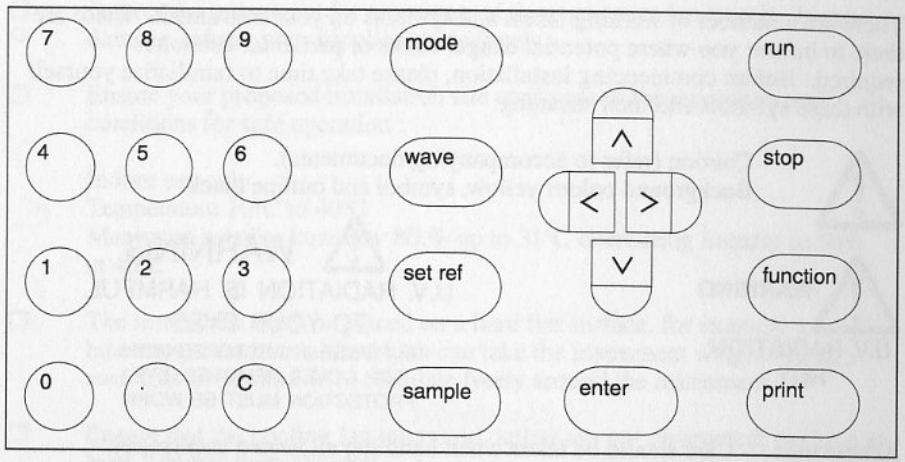
Español

Italiano

80-2106-24

Pharmacia Biotech

Keyboard



Ignoring the numeric keypad, the keyboard is designed to be operated from top to bottom and from left to right. Basic absorbance measurements are very easy to make. In addition, a range of instrument utilities and instrument modes is accessible when the **function** or **mode** key is pressed; these options are described later.

(mode) Select measurement mode required using [<] [>] [∧] [∨] keys. Press (enter)

(wave) Select wavelength either from the numeric keypad or by using [<] [>] keys. Press (enter)

(set ref) Set absorbance and transmittance readouts to 0.000 absorbance units and 100 % T, respectively, on a reference solution.

(sample) Select sample position in cell changer either from the numeric keypad or by using [<] [>] keys. Press (enter)

(enter) Select options on the display. (C) Clears a numeric entry.

(run) Start measurements when operating from within the measurement modes.

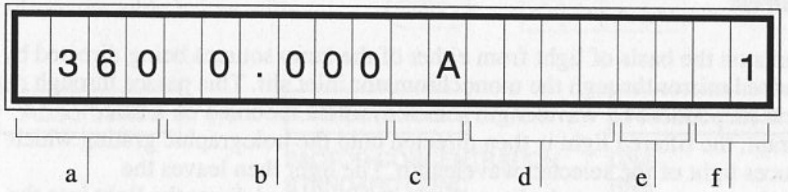
(stop) Ends current programme.

(function) Access to Set-up and Parameters.

(print) Output information on the display panel to a parallel printer.

Display Panel

The display panel has 20 characters divided up as indicated. Default start-up values are shown, and represent a wavelength of 360nm in absorbance mode, with cell position number 1 in the light path.



a) **Wavelength**
Display of current wavelength in nm.

b) **Numeric Result**
Display of absorbance, % T, concentration, absorbance ratio result.

c) **Unit of Measurement**
Display of numeric result unit (**A** is Absorbance Units; **%T** is % Transmission; **C** is concentration units).

d) **Mode of Operation**
Display of instrument enhancement mode :

FC and **SC** are factor and standard concentration modes;
DNA, RNA, OLI are DNA, RNA, oligonucleotide quantification modes;
PRO is protein concentration mode;
RAT is absorbance ratio mode;
KNR is display of absorbance with time;
KND is display of change of absorbance with time;
SCN is scan to chart recorder.

e) **Temperature Indicator**
Display of temperature status if a peltier heating accessory is in use.

°**C** is at temperature;
HI is over temperature or cooling down;
LO is under temperature or heating up.

f) **Sample Number**
Display of sample number.

OPERATION

Introduction

Your UV/Visible spectrophotometer is a simple to use, microprocessor controlled instrument.

It works on the basis of light from either of the lamp sources being directed by a motorised 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 which 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 a defocussing lens to a solid state detector unit. The resulting signal is then amplified and displayed.

Your spectrophotometer :

- ❒ measures standard absorbance, concentration and transmittance.
- ❒ has stored parameters for DNA, RNA and oligonucleotide quantification and purity checking, as well as for protein contamination measurement in nucleic acid solutions.
- ❒ measures absorbance ratios.
- ❒ measures change of absorbance with time and can either print the numerical data or output the absorbance time plot to a chart recorder (synchronised).
- ❒ outputs a wavelength scan to a chart recorder (asynchronous).
- ❒ can be connected to a standard centronics parallel printer for output of results.
- ❒ can be linked to a PC via its serial interface and used with the SWIFT range of software application programs operating in the Windows environment.

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

Measurement Modes

mode

The mode key gives access to the 6 operational modes; these are linked in a continuous loop. After pressing mode, use the arrow keys to move up/down/left/right in the matrix shown below. Each mode is described subsequently.

Select from the table below with mode < > ∧ ∨ enter

→ Absorbance ↔ Concentration ↔ Nucleic Acids ←

FACTOR CONCENTRATION
STANDARD CONC

DNA
RNA
OLIGO
PROTEIN IMPURITY
ABS RATIO

→ Reaction Rate ↔ Scan ↔ Transmission ←

RATE - RAW DATA
RATE - DELTA DATA

SCAN TO RECORDER

mode < > ∧ ∨ enter to choose required measurement mode.

By pressing the mode button within a measurement mode the user can return to the previous step of operation.

The instrument can be switched off at any stage of operation.
A flashing cursor indicates an option which is currently selected.

Absorbance

mode

Absorbance mode is the default after power on. It is used to perform simple absorbance measurements on samples, and it measures the amount of light that has been passed through a sample relative to a blank (this can be air).

The procedure is to select the appropriate wavelength, insert blank and set reference on it, remove the blank, and insert the sample(s). If **sample** is pressed and cell position 2 selected, the display will show the wavelength used and the absorbance and cell position of the sample in the light path. If **run** is pressed the sample absorbance is measured and the cell changer rotated sequentially to the next position; the display therefore shows the wavelength used, and the absorbance and cell position of the next sample, since it is now in the light path. The advantage of using run is that if a printer is connected and RUN PRINT in Setup selected, then there is automatic print out of the absorbance details of the sample just measured.

Using (sample) with 2 samples

mode	360 0.000 A 1		enter
wave	Wavelength = 546	Set λ	enter
☐ set ref	546 0.000 A 1	remove blank	
▒ ▒	546 1.200 A 1	Absorbance of sample 1	
sample	cell = 2	cell position ?	enter
	546 1.412 A 2	Absorbance of sample 2	

Using (run) with 2 samples

	360 0.000 A 1		enter
wave	Wavelength = 546	Set λ	enter
☐ set ref	546 0.000 A 1	remove blank	
▒ ▒	546 1.200 A 1	Absorbance of sample 1	run
	546 1.412 A 2	Absorbance of sample 2	run
	546 0.000A 3	cell position 3 empty	

(set ref) to set absorbance to 0.000 AU on a reference solution at all wavelengths in the mode selected. During a standard operating procedure, the user is prompted to insert a cell containing reference into cell holder, by the message [set reference]. This is displayed until the reference is inserted ☐ and set ref is pressed.

(run) to start making measurements; sample number (and cell position) are automatically incremented after measurement.

(stop) to stop making measurements or return to Absorbance mode.

(sample) [>] increments sample number (and cell position if cell changer is fitted).

The above and following sequences of instructions apply to the 6 cell changer which is supplied with the instrument. There are different self explanatory displays if a single cell holder or sipper is installed.

☐ and ▒ can be put in sequentially if a 6 cell changer is fitted.

Scan

An absorption spectrum can be obtained with your instrument by output to chart recorder. Ensure chart recorder is connected, as scan data is not visible without this. The Pharmacia Biotech REC 101 is recommended.

mode	SCAN TO RECORDER		enter
	Start Wave = 360	Start λ?	enter
	End Wave = 560	Finish λ?	enter
	Set Reference		set ref
run	Scanning		
	Recorder at 500nm	Chart position as scan is output to recorder AFTER scan has finished	
	560 0.209 A SCN 1		

Output to the Pharmacia Biotech REC 101 chart recorder is asynchronous (the chart paper drive is controlled on/off automatically). Chart recorder cable (80-2105-95) is required. Suggested chart recorder settings are 10mm/second, 200 mV full scale deflection (output is 100 mV for 1.000 Abs unit), recorder on, zero suppress on zero, set to internal.

If you require post run data manipulation routines, the SWIFT-SCAN software module is available for use on PC.

Transmission

In transmittance mode the instrument measures the amount of light at the specified wavelength that has passed through the sample and compares it with that which has passed through the reference. This is displayed as a percentage. The relationship between the concentration of the sample and its transmittance at any given wavelength is not linear, and hence transmittance mode is rarely used experimentally except for samples having very high absorbances (low transmittances)

mode	Transmission		enter
wave	Wavelength = 546	Set λ	enter
set ref	560 10.00 %T 1	remove blank	
	546 80.00 %T 1		

Messages

The spectrophotometer goes through a multi step calibration sequence. If for any reason the calibration is not completed satisfactorily, the messages displayed, if not self explanatory, are either as follows or relate to a fault condition which requires a service engineer from your local supplier. Other messages are displayed when an accessory is installed.

Error Messages

Display	Possible Causes	Remedy
GLP Calibration Fail		Refer to Appendix GLP Diagnostic Tests
1pt VIS calibrated	Visible region only calibrated	Check and replace UV Lamp if necessary
1pt UV calibrated	UV region only calibrated	Check and replace visible lamp if necessary
Abs Non - Linear (GLP on)	Instrument not at working temperature Misalignment of filter quadrant Dirty Filters	Let instrument reach working temperature Contact service engineer
Too much light	Too much light in sample compartment	Close lid properly and ensure baseplate plug is in place (if relevant)
Beam Blocked !	Not enough light getting to detector	Check light beam is not blocked and cell compartment empty If using a sipper : remove flowcell
Printer Error	RUN PRINT is ON, GLP PRINT OUT is ON, printer is off line or out of paper	Check printer state
No Printer	No active printer	Connect or switch on printer or deselect RUN PRINT and GLP PRINT OUT

Temperature failed	Heated accessory unplugged	Plug in and/or press (sample) (sample)
Call Service E---		Contact service engineer and advise diagnostic error code
Waiting for GLP	Instrument warming up	Refer to Appendix GLP Diagnostic Tests
< Min A	Abs value out of range (<-3.OAU)	Detector seeing too much light. Ensure baseplate plug is fitted Close lid properly
> Max A	Abs value out of range (>+3.OAU)	Sample too concentrated. Something blocking light path.
Vis mirror fail !	Detector did not see enough energy during calibration	Replace the visible lamp

Accessory Messages

After installation of accessory, press (sample) (sample)

4 cell changer	4 position cell changer
6 cell changer	All 6 position cell changers
Single Cell Holder	All single cell holders, except . . . .
Electric Cell	Electric Cell Holder
Thermostat holder	Peltier Cell Holder