



 **expedeon**  
*protein discovery & analysis*

**VersaWave**

**Microvolume  
Spectrophotometer**

**User Manual**



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## CE DECLARATION OF CONFORMITY

**Product:** Optic Coupled Spectrometer

**Type:** SWIR, NIR, VIS/NIR, UV/VIS.

We declare that the above-specified systems are compliant with the regulations of the European Community when installed as a system.

The devices are compliant with the following standards:

- LV Directive 72/23/EC
- EMC Directive 89/336/EC

Note: This declaration of conformity becomes invalid if:

- The devices are installed, modified, complemented or changed in a manner which has not been permitted in the devices "User Manual".
- In case of improper usage.



Dr Heikki Lanckriet  
CEO  
**Expedeon Ltd.**

### **Expedeon Ltd**

Unit 12 Buckingway Business Park, Anderson Road  
Swavesey, Cambridge, CB24 4AE, United Kingdom  
Phone: +44 (0) 1223 873364 Fax: +44 (0) 1223 873371  
Email: info@expedeon.com

## ESSENTIAL SAFETY NOTES

All product and brand names used in this document are trademarks or registered trademarks of their respective holders.

Please read carefully before installing or operating this instrument – if in doubt seek advice from Expedeon.

1. This instrument is designed for operation by trained personnel who are aware of the principles and applications involved. For further help and advice please contact your distributor or visit [www.expedeon.com](http://www.expedeon.com)
2. This instrument is a sensitive optical and electronic instrument designed for use in a laboratory environment. Careful adherence to the installation instructions must be observed. If in doubt contact a relevant and competent authority before proceeding.
3. Operators of this instrument must be trained in (a) general laboratory safety practices, (b) the specific safety requirements of the instrument and any other equipment being used and (c) the risks and safe practices for the analysis being undertaken, including those associated with sample handling. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired and any warranty invalidated.
4. This instrument is designed for minimal maintenance, which must be carried out carefully following the procedures detailed in this manual. All safety instructions detailed in these procedures as well as those for the area or environment where the work is being carried out must be observed.
5. Other than for those items defined in the maintenance procedures there are no user serviceable items in this instrument. Removal of covers and attempted adjustment, service or modification by unqualified personnel will invalidate any warranty and incur additional charges for repair.
6. Reference should always be made to the health and safety data for any chemicals or reagents used. All advice and warnings on the handling storage, use and disposal of these must be carefully observed. When not available this data must be requested from the supplier before proceeding.
7. It is important that good laboratory practice is observed when handling samples, chemicals, reagents and ancillary equipment in order to carry out measurement and analysis with this instrument. Suitable personal protective equipment (PPE) must be used at all times and in all circumstances.
8. If it is suspected that safety protection has been impaired in any way, the instrument must be made inoperative and be secured against any intended operation, with clear warnings as to this state. The fault condition must be reported to the appropriate servicing authority as soon as possible. In all such reports the model name/number and serial number must be quoted.

## Potential Safety Hazards

### Electrical

- Standard electrical safety precautions should be observed.
- Ensure that the proper voltage is being supplied before turning the instrument on for the first time.
- The device must be connected to a grounded socket.
- Do not touch any switches or outlets with wet hands.
- Switch the instrument off at the mains supply before disconnecting the AC power cord.
- Unplug the instrument prior to maintenance, cleaning up any major liquid spills and prior to servicing any of the electrical or internal components.
- If in doubt about any aspect of the electrical installation consult a qualified electrician. Only qualified personnel should perform electrical servicing.

### Hazardous Substances

- Reference should always be made to the health and safety data for any chemicals or reagents used. All advice and warnings on the handling storage, use and disposal of these must be carefully observed. When not available this data must be requested from the supplier before proceeding in any way.
- The relevant safety regulations must be observed when handling pathogenic samples, radioactive materials or other substances hazardous to health. The correct and safe disposal of waste materials must be observed.
- Before returning any item for service, repair or calibration a Decontamination and Safety Clearance Declaration must be completed to ensure the duty-of-care can be maintained for those who will be handling equipment used for such purposes.

## Solvent Compatibility

### Instrument case and paintwork

#### Compatible Solvents

Acetic Acid (dilute)	Chloroform	Methanol
Acetone	Ethanol	N-Propanol
Acetonitrile	Ether	Sodium Hydroxide
Benzene	HCl (dilute)	Sodium Hypochlorite
Bleach	Hexane	Toluene
Butanol	HNO <sub>3</sub> (dilute)	
Carbon Tetrachloride	Isopropanol	

#### Incompatible Solvents

Hydrofluoric Acid and derivatives

### VersaTip

Please note that the VersaTip should not be used with concentrated solutions of Phenol and Chloroform.

Follow this link for the MSDS:

[http://www.topas.com/sites/default/files/tp01\\_v4.02\\_eu-en.pdf](http://www.topas.com/sites/default/files/tp01_v4.02_eu-en.pdf)

## INTRODUCTION

### General

This instrument is a low volume, short path length spectrophotometer that provides the user with either;

1. the facility to recover their sample after measurements have been taken using in-tip UV pipette technology, or,
2. the facility to use an industry standard micro-volume cuvette (VersaCell or VersaTray), a flow cell (VersaFlow) or an Immersion Probe (VersaProbe). These accessories are available with varying fixed path lengths

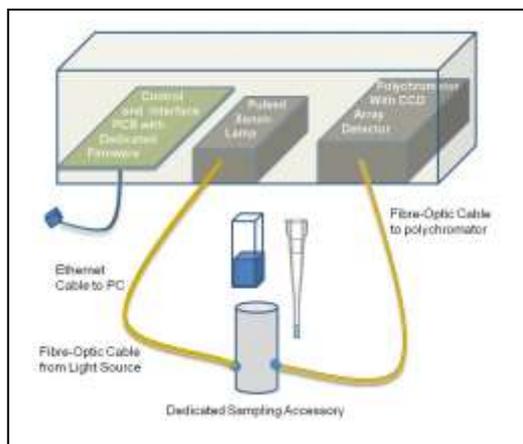
It is a full spectrum (190 ~ 1050nm) UV/Visible spectrophotometer which allows for measurements of many sample types commonly analysed in the laboratory. Because of its ultra-small sample requirement and ability to recover samples after measurement the product is ideally suited to life science applications including DNA, RNA and protein measurements. The VersaWave includes the appropriate sampling accessory and software dedicated to these applications.

### Principle of Operation

This instrument is one of a new type of spectrophotometer that integrates a high resolution CCD Array detector with fibre-optic coupled sampling accessories. Because there are no moving parts the inherent reliability and durability of these instruments far surpasses those of traditional design. Calibration is fixed in manufacture which eliminates the need for lengthy calibration procedures at switch on, while maintenance and service requirements are truly minimal.

The product uses a press-to-read system with a pulsed xenon lamp that is only powered up when a measurement is being made. This not only saves energy compared to spectrophotometers with lamps that are continuously on, but gives a much longer lamp life (in excess of 10 years) capable of far higher UV energy levels.

Light from the xenon lamp is transmitted to the sampling accessory via a fibre-optic cable. Here some wavelengths are absorbed by the sample, while the light transmitted through it is then returned to the polychromator and CCD Array detector by another fibre-optic cable on the other side of the accessory.



This enables the sampling accessory to be remote from the instrument and for dedicated, application specific sampling accessories to be designed without the restrictions of the traditional light-proof sample chamber being required. This also simplifies and speeds up operation as there is no need to keep opening and closing sample chamber lids for each measurement.

The VersaWave's configuration also isolates the lamp from the sensitive optics of the polychromator, so problems of heat and stray light from the light source become a thing of the past. The polychromator in the instrument is effectively a sealed-unit with a single fibre-optic cable as input and a single ribbon cable as output.

Compare this to traditional spectrophotometers where the lamp is usually an integral part of the monochromator, requiring forced cooling and so introducing dust and contamination into the optics, increasing maintenance, degrading performance and shortening its working life.

As well as removing all moving parts, another major advantage of the VersaWave polychromator in comparison to the monochromator used in traditional spectrophotometers is the speed of measurement. A full scan is completed in around 2 seconds while the 3648 element CCD Array ensures high resolution details are faithfully captured.

## Measurement Modes

This instrument has modes for measuring Nucleic Acids as double stranded DNA (dsDNA), single stranded DNA (ssDNA), and RNA. The concentration and purity ratio of the nucleic acid are reported along with a full wavelength scan, all in a measurement time of 2 seconds!

Microarray Dye Measurements report the labelling efficiency of reactions using the Ratio of Dye/Base along with the Absorbance and Dye Concentration. This can be done for two dyes simultaneously at two separate wavelengths. The nucleic acid concentration and purity ratio are also displayed in this mode.

Direct Protein measurements can be made against a general Absorbance reference as well as BSA, IgG, Lysozyme or user-entered reference. Indirect Protein measurements can be made using the Bradford or Lowry protocols as well as against user defined settings.

Cell culture measurements can be made to monitor bacterial cell growth at 600nm and provide a means of knowing the optimum point at which to harvest cells from a fermentation process.

Full wavelength scan is also available, with zoom and overlay facilities. A wavelength may be selected for normalization or background correction.

More information on applications is provided in the [Appendix](#).

## Principle of Measurement

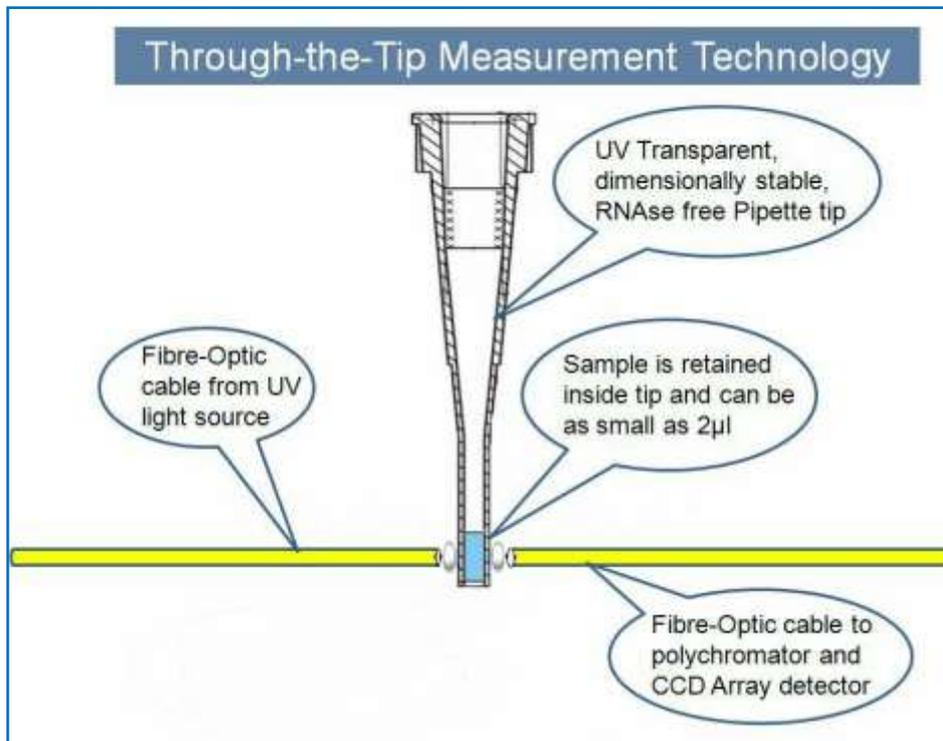
### VersaTip

Using a novel through-the-tip measurement principle samples as small as 2.5µl can be measured and still be retained for further processing and analysis. Using the specially designed micro-sampling accessory samples remain protected and contained within the special UV transmitting pipette tips where they are measured.

They do not have to be dispensed from the tip so there is no risk of the cross contamination or carry-over that can occur with methods that require dispensing the sample onto a platform or pedestal. Additionally, there is no risk of concentration changes from evaporation of exposed solvent and no caps or covers to be placed over the sample, so measurement is quick and easy.

Precious samples can be handled within a sterile environment and are completely recoverable. Using individual disposable tips for each sample means there is no need to clean up after each measurement, removing any possibility of carry-over or contamination and making this the fastest and most efficient method of analysis.

A 2.5µl sample is drawn up directly into the VersaTip using the supplied pipette. The pipette is placed into the holder, which automatically positions the tip in a light beam, emitted from a fibre-optic cable connected to the holder. The light source is a pulsed xenon lamp housed in the instrument and the light path through the tip is 1mm.



The light passes through the sample, still retained in the tip, and the light transmitted through it is collected by a second fibre-optic cable on the opposite side of the holder. The transmitted light is then analysed in the polychromator-based spectrophotometer (also housed in the instrument) where the Absorbance, Concentration, Purity Ratio and other values are calculated. The instrument is

controlled using the supplied software run from the supplied laptop via an Ethernet connection. Software can also be installed on an existing computer.

The VersaTip pipettor, pipette tips and holder accessory are all critical components in making accurate and reproducible measurements of micro sample-volumes including DNA/RNA and Protein measurements. The tips are made from a special UV/visible transmitting optical grade polymer that is also significantly more dimensionally stable and reproducible than normal tips – do not use alternatives.

**ONLY USE THE PIPETTE AND TIPS SUPPLIED BY THE ORIGINAL EQUIPMENT MANUFACTURER – DO NOT USE ALTERNATIVES**

For applications where larger sample volumes are available a number of optional sampling devices can be used with this spectrophotometer.

### **Micro-volume cuvette**

Information on the light path through the VersaCell and the VersaTray is supplied with the appropriate cuvette.

### **Data Management**

For full traceability all results are stored with the date and time of measurement along with an incrementing number. This can be further enhanced by an alpha-numeric 'name' that can also be appended to any sample.

When making measurements, results can be viewed in real-time, or exported in a tab-delimited Microsoft Excel format for archiving or presentation in other programmes. All spectrum scans have deep levels of zoom available in both the x and y axis ensuring the required level of detail can be viewed. Graphical printouts can be made on standard PC printers and include the scan, results, settings and sample information, giving a concise hard-copy record when required.

## INSTALLATION

### Unpacking

Remove the instrument from the packaging and ensure the following items are included.

1. Spectrophotometer Unit
2. Touch Screen Laptop with VersaWave software preinstalled
2. Pipette with special adapter barrel fitted to it
3. Pipette Holder and Base
4. Box of 96 UV pipette tips
5. Ethernet Cable
6. Fibre-Optic Cables with Connectors (2)
7. Mains cable with Plug
8. 24V Power Supply (universal input voltage)
9. 1.5mm Allen Key (for maintenance)
10. Fibre Optic Connection Extractor Tool (for maintenance of pipette holder)

Any shortages or damage must be reported to the carrier and your local distributor as soon as possible

Keep all packaging materials in case the unit has to be re-shipped or stored at a later date; always place items in clean polythene bags before packing to avoid the ingress of fibres and dust from the cardboard.



### Positioning

The ideal installation environment for the instrument and its accessories will be clean, dry and dust free with the temperature and humidity controlled. All items should be sited away from windows where extreme and rapidly changing temperatures can be experienced even in a controlled environment. The spectrophotometer should be placed on a rigid, flat, clean surface. Make sure that the instrument is completely stable.

Where the ideal conditions cannot be met, the instrument and its accessories must be given additional protection both when in use and idle. Protection using dust covers is always recommended when equipment is not in use. Where conditions are less than ideal routine maintenance and cleaning may be required more frequently.

The instrument requires a bench area of just 20 x 20 cm and the micro-sample accessory just 78 cm<sup>2</sup>. Further consideration should be given to the space for, and location of, the PC required to drive the product, as well as other peripheral devices such as printers etc.

When choosing a location thought should also be given to the additional space required for sample preparation and the equipment and reagents that may be required for this, as well as to the proximity of a suitable and safe waste disposal system.

The instrument should be positioned within two meters of an easily accessible mains socket, preferably with an isolating switch. The Ethernet cable supplied is 1 metre long so the PC will need to be positioned so the Ethernet port can be reached by this cable.

Please ensure that the ventilation slots of the device remain clear and free to vent at all times. A space of at least 10cm should be left around the spectrophotometer. The space underneath the instrument must be kept clear of paper, loose material and dust build-up.

The ambient temperature should be between 10°C and 30°C, the humidity between 0% and 95% non-condensing.

## **Hardware installation (if not already pre-assembled)**

### **Assembling the VersaTip holder (for in-tip measurement)**



The VersaTip holder has been dismantled and disconnected for shipment. Fix the tubular pipette holder to its round base by simply screwing it on.

### **Important information about fibre optic cables**

- 1 Although they carry out a similar interconnection function to electrical cables, fibre optic cables are made of glass and CANNOT be handled in a similar manner to metal based conductors.
- 2 Do not exceed the maximum bend radius for the cable, in use or in storage. This is typically 300 times the cladding diameter, so for core diameters of 100µm to 600µm this approximates to a bend radius of 4cm (100µm) to 20cm (600µm), but is dependent on the detail of the construction of each cable type.

**The fibre optic cables on this instrument have a core of 100µm, so the bend radius is 4cm.**

- 3 Ensure all optical surfaces are clean and dust free before making any connections. Always replace dust-caps on fibre ends and instrument SMA905 connectors (screw in fibre optic couplings) when fibre optic cables are disconnected.
- 4 Do not use tools to tighten the screw locking ring on SMA905 connectors, but do ensure they are firmly hand-tight.
- 5 Do not pull on the cable to remove it when disconnecting, only use the connector screw locking ring.
- 6 Do not subject the cable to axial twisting in use, installation or storage. Spool or un-spool long lengths of cable in a figure-of-eight (as each part of the 'eight' puts an opposite twisting moment in the cable) turn the 'eight' over when carrying out the reverse process.
- 7 Do not subject the cable to pulling stress during installation or use. Fibres are usually stronger in direct tension relative to the cross section, but when fibres are small it is very easy to break them. Unfortunately each cable construction will have its own limits and it is difficult to give fixed rules for this, so minimizing pulling stress is important.
- 8 Try to restrict movement of cables during the period a measurement is being made.
- 9 Take extra care not to bend or crush cables at their exit point from VersaProbe heads, in use and storage, and do not support the weight of a VersaProbe by the cable.
- 10 Do not use any cable, cable assembly or VersaProbe beyond its specified performance limits or outside of its environmental operating and storage conditions. If in doubt consult your supplier, alternatives may be available, specials suitable for most situations can often be supplied or alternative procedures developed.

**Connecting the fibre optic cables to the instrument**



Connect the SMA to SMA launch cable from the lamp socket (top right) by pushing the connector into the socket and hand-tighten the knurled ring completely to hold the connector in place. Do not use tools to tighten the knurled ring. No polarity or orientation needs to be observed.

Connect the MTP to SMA collection cable to the detector (bottom left) by pushing into the slot with the white dot at the top; note that to remove it, the shroud should be pulled back.

**Connecting the fibre-optic cables to pipette holder or CH1 VersaVette Cuvette Holder**



Ensure that the launch cable from the lamp socket is connected to the pipette / VersaVette Cuvette Holder with the holder slot / opening facing forward; this will be on the right hand side of the holder.

Ensure that the collection cable to the detector is connected to the pipette / VersaVette Cuvette Holder with the holder slot / opening facing forward; this will be on the left hand side of the holder (see figure for the pipette holder configuration)

In both cases, push the connector into the socket and hand-tighten the knurled ring completely to hold the connector in place. Do not use tools to tighten the knurled ring.

## The completed assembly



Always use the instrument with the pipette / VersaVette Cuvette Holder facing towards you and without excessive twisting of the fibre optic cables. Note that the fibre optic cables on this instrument have a core of 100 $\mu$ m, so the bend radius is 4cm.

## Connection and loading the software to a PC

The instrument has an external switch-mode power supply that will accept any mains voltage from 100 to 240V AC at 47 to 63Hz. If the correct mains plug has not been supplied an alternative lead or plug may be fitted. If in doubt consult a qualified electrician.

Always ensure that the instrument switch is in the power off position (rocker down) before plugging in the mains lead.

Use only the power supply module supplied, other makes, although similar, may damage the unit or cause erratic performance. Connect the jack plug from the power supply module to the jack socket on the rear of the product. Then switch the instrument on.

The LED on the front of the unit should be illuminated. If it does not, check all connections are made correctly and that the supply is switched on at the mains switch; see also the [Troubleshooting section](#).

Connect the instrument to the provided laptop or a suitable PC on which the VersaWave software has been installed using the short Ethernet cable supplied.

### **Note:**

- The instrument should be connected directly to the Ethernet port, other configurations are not supported.
- It is important to set up your wired Ethernet connection as follows;

IP address: 10.0.0.127  
Subnet mask: 255.0.0.0.

The procedure for setting IP addresses is covered in the appendices at the end of this manual for Windows Vista, Windows XP, Windows 7 and Windows 8/10. [Please go to this section now.](#)

To install the software on a different pc visit [www.expedeon.com](http://www.expedeon.com) and navigate to Resources → Software Download. The most recent version of the software will be available here to download.

### **Minimum specification for the PC**

Microsoft® Windows® XP Service Pack 2, Vista® or Windows® 7/8/10  
512Mb RAM  
2.8GHz Pentium® 4 or 1.6GHz Core Solo or Core Duo  
Minimum screen resolution 1024 x 768  
200Mb of free disk space  
One Ethernet port

Please click on the 'Setup' file. The software will install automatically, leaving a shortcut on the desktop to the instrument interface.

If the installation has been completed successfully after double clicking the desktop icon the initial screen will be displayed.

## APPLICATION MODES

### Getting Started

- The provided laptop has a touch screen. Software can also be navigated with the trackpad or a mouse. In the following instructions “press” may be substituted with “click” depending on your preferred control method.
- Software is preinstalled on the provided laptop. If using a different computer, first install the Versawave software from [www.expedeon.com](http://www.expedeon.com) → Resources → Software. An icon will be visible on the desktop and there will be option from the Start button. Select this and the VersaWave home screen will appear:



- Before using the instrument for the first time, select Settings.

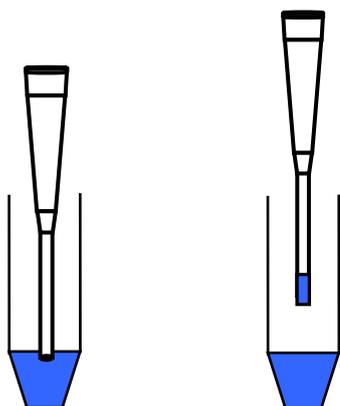


- High precision mode forces the xenon lamp to remain firing even if measurements aren't being taken. This aims to reduce any hysteresis from uneven lamp temperature changes during sample measurement. However, this will result in a constant audible noise from the VersaWave while plugged into mains power and will increase power consumption. Most users should only use “High Precision” mode when results between samples are incredibly close.
- Data Storage Path enables the user to define the default folder to which all data and results are to be saved on the PC (or on the lab network).
- Language selection mode allows users to select language.

- Once these have been defined, press OK to save settings and return to the home screen. Note that once set, there is no need to go back to Settings unless there is a change of modus operandum.

### Correct pipette use and sample handling

- Vortex sample briefly (5-20secs) and then spin down samples briefly (10-15secs in a microfuge).
- Using the supplied VersaTip pipettor and UV VersaTip pipette tips, pipette up your sample. A minimum volume of 2.5ul is recommended. To minimise solution on the outside of the tip, avoid submerging the tip too far below the sample meniscus. If necessary, wipe off excess liquid from outside of tip with a dry lint-free tissue, this is particularly important if using viscous protein solutions. Be careful not to touch the bottom of the tip as the sample may be drawn out by the tissue.
- The detection point in the tip is 2.5mm from the end, so it is best procedure to try not to submerge the tip more than 2mm into your solutions, otherwise tip wiping may be necessary.
- Deeper insertion can leave residue on the tip and affect results
- Use the same tip for blank and sample** - this is similar to use of a conventional single beam spectrophotometer where the same cuvette is usually used in the same orientation
- Always place the pipette in the same relative position in the holder; have the pipette button or volume display facing out directly above the slot which is present in the holder**
- Do not drop or smash the pipette down into the holder; take care when placing it into the holder**
- We recommend that the pipette and pre-fitted adapter barrel is not used for other applications in the lab; use it with this product only.
  - The adapter barrel and holder have been specially designed to be used together
  - You should leave the adapter barrel supplied fitted to the pipette where it is
  - It is suggested that you do not use the optical grade pipette tips for other applications in your lab
- We also recommend that you do not use other makes of pipettes with these tips fitted for in-tip measurements on the instrument; other pipettes will not fit the holder accurately and may therefore give unreliable results**
- Do not place the UV VersaTip pipette tips or your sample too close to heaters or the fan of the PC as heating the tips or sample may result in a rapid contraction in volume once the tip is placed in the cooler pipette holder. This sample contraction will result in a space or bubble being visible at the bottom of the tip. This space may interfere with sample measurement if allowed to rise more than 2mm up the tip. It is preferable that the sample, tip and pipette holder are allowed to equilibrate to room temperature before commencing measurement.



WARNING – DO NOT LEAVE THE PIPETTE IN THE HOLDER FOR EXTENDED PERIODS AFTER MEASUREMENT HAS BEEN MADE.

Recommended pipetting procedure

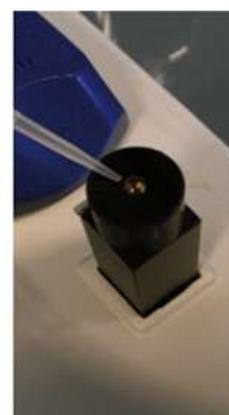
## Correct VersaCell and VersaTray use and sample handling

Instructions for use and cleaning are supplied with the appropriate cuvette.

VersaCell



VersaTray

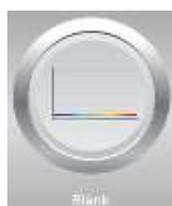


## Nucleic Acid Measurement

- General application information and background is described in the [Appendix](#).
- We recommend that the instrument is allowed to warm up for 10 minutes before use, so that an internal thermal equilibrium is achieved.
- Select the relevant nucleic acid measurement mode (dsDNA, ssDNA, RNA) from the "Nucleic Acids" section of the home screen.

The descriptions in the following section assume pipette tips are used; if this is not the case, the same sequence of actions is still applicable.

- Pipette 2.5µl of 'Blank' solution into a tip and place the tip and pipette adapter into the pipette holder (see [Correct pipette use and sample handling](#)).



- Press the button marked 'Blank'.
- The software will run a reference scan on the spectrophotometer and set the baseline to zero. For several seconds the LED on the front of the unit will turn orange; the buzzing noise is due to the xenon lamp.
  - If for any reason (including air bubbles or settling turbidity in the sample) a blank cannot be completed successfully a message stating that the reagent blank could

- not be captured will be displayed. If this occurs simply try blanking again, and if the procedure continues to fail, try cleaning the pipette holder and outside of tip.
- Once the instrument has been successfully 'Blanked' then the greyed out 'Measure' button to the right of the blank button will change to a coloured button signifying that you can commence measuring your samples.
- Insert the pipette with sample and press the 'Measure' button.
- After a few seconds the concentration (in ng/μl) will appear in the table in the bottom left of the window, the spectrum will be plotted and details displayed below and to the left of the graph.
- It is not necessary to 'save' the results as all results are done so automatically.



Example of results using ssDNA mode

- Subsequent measurements are positioned below the previous one. Several sets of data can be selected and are then overlaid automatically (use Clear Overlays to remove this)
- To delete a set of data, highlight by pressing in the blank "select" box and then select Delete.
- To run another reference or Blank, press the 'Blank' button as before and note that to see the reference spectrum it is necessary to run it as a sample by pressing the 'Measure' button after the Blank button.

### Export of data to Excel

Data can be easily exported to Excel for subsequent analysis and normalisation for inclusion in reports etc. To do this:

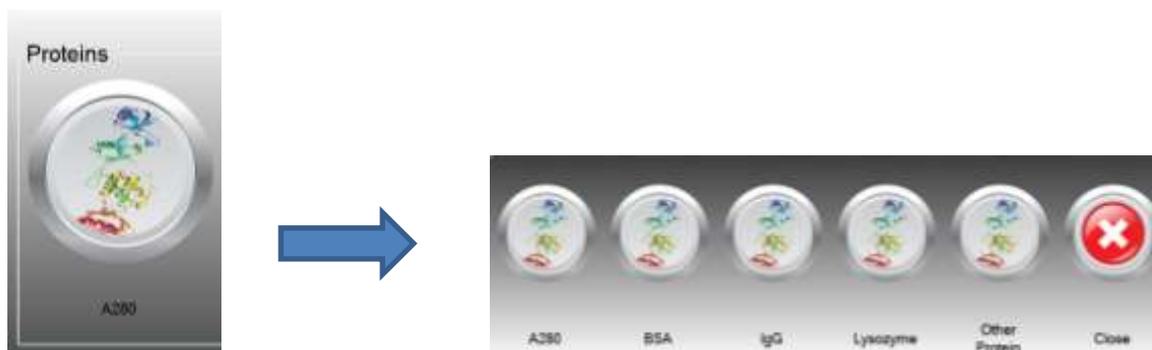
- Press the blank square in the "select" column of the results of interest
- Select "High Resolution?" to export readings of every 0.1nm and leave unselected to export every 1nm. (1nm should normally be sufficient otherwise the data files will be extremely large)
- Select the "Horizontal recording?" to export horizontal data (Vertical is usually more manageable)

- Select "results table" to export data with text headers for columns.
- Press "file" then navigate to a folder to which the data is to be saved and enter a file name
- Open Excel and navigate to the file in question; note that data is formatted as a text file.
- Open the file; it is a text delimited file, with tab separation.
- Manipulate that data as appropriate and save in the required format.

## Direct Protein Measurement

- General application information and background is described in the [Appendix](#).
- We recommend that the instrument is allowed to warm up for 10 minutes before use, so that an internal thermal equilibrium is achieved.
- Select A280 from the "Proteins" section of the home screen.

The descriptions in the following section assume pipette tips are used; if this is not the case, the same sequence of actions is still applicable.



- The options listed are detailed below:
  - A280 - setting based on a 0.1% (1 mg/ml) protein solution producing an Absorbance at 280 nm of 1.0 A (where the path length is 10 mm).
  - BSA (Bovine Serum Albumin) - unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/ml) BSA solution.
  - IgG - unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution.
  - Lysozyme - unknown (sample) protein concentrations are calculated using the mass extinction coefficient of 26.4 at 280 nm for a 1% (10 mg/ml) Lysozyme solution.
  - Other Protein - user-defined either enter the mass extinction coefficient (L gm<sup>-1</sup>cm<sup>-1</sup>) for a 10 mg/ml (1%) solution of the respective reference protein or provide the Molar Extinction Coefficient (AU/Mol/ml) and Molecular Mass (Da) then use the calculate button to produce the E1% value. These user-defined values can be stored for convenient later re-use.
- Pipette 2.5µl of 'Blank' buffer solution into a tip and place the tip and pipette adapter into the pipette holder (see [Correct pipette use and sample handling](#)).
- Press the button marked 'Blank'. The software will attempt to blank the spectrophotometer and set the baseline to zero; the buzzing noise is due to the xenon lamp.

- If for any reason (including air bubbles or settling turbidity in the sample) a blank cannot be completed successfully a message stating this will be displayed. If this occurs simply try blanking again, and if the procedure continues to fail, try following the protocols detailed in the Trouble Shooting Section.
- Once the instrument has been successfully 'Blanked' then the 'Measure' button will become selectable signifying that you can commence measuring your samples.
- Insert the pipette with sample and press the 'Measure' button.
- Note: When using viscous samples, it is important to ensure that the sample does not adhere to the outside of the tip. Simply wiping the outside of the tip with a piece of clean dry tissue will normally avoid this potential problem. Ensure not to leech sample from the opening of the tip by touching it with absorbent material.
- After a few seconds results will appear, the spectrum will be plotted and details displayed in the table at the bottom left of the screen
- The graph can be pressed on to display absorbance at specific wavelength values.
- Press and hold until a rectangle appears, then release to zoom in on a specific area of the graph.
- The arrows either side of "Cursor Wavelength" allow wavelength adjustments of 0.1nm.
- It is not necessary to 'save' the results as all results are done so automatically.



*Example of results from Direct Protein measurement*

### Export of data to Excel

Data can be easily exported to Excel for subsequent analysis and normalisation for inclusion in reports etc. To do this:

- Press the blank square in the "select" column of the results of interest
- Select "High Resolution?" to export readings of every 0.1nm and leave unselected to export every 1nm. (1nm should normally be sufficient otherwise the data files will be extremely large)

- Select the "Horizontal recording?" to export horizontal data (Vertical is usually more manageable)
- Select "results table" to export data with text headers for columns.
- Press "file" then navigate to a folder to which the data is to be saved and enter a file name
- Open Excel and navigate to the file in question; note that data is formatted as a text file.
- Open the file; it is a text delimited file, with tab separation.
- Manipulate that data as appropriate and save in the required format.

## Indirect Protein Measurement

- General application information and background is described in the [Appendix](#).
- We recommend that the instrument is allowed to warm up for 10 minutes before use, so that an internal thermal equilibrium is achieved.
- Select the 'Bradford', 'Lowry', 'BCA' or 'A660', from the home screen.

The descriptions in the following section assume pipette tips are used; if this is not the case, the same sequence of actions is still applicable.

- From the next dialogue box, use Create to start a new method or select a previously stored method from the list. To select press the blank "select" box to highlight the method, then press select.



- If a new method is being created for the chosen technique press "Create" and enter a name, units, normalisation and measurement wavelengths. Then press next. Press the currently selected accessory to change to a different one. If using a previously created method ensure the parameters look correct and the right accessory is selected, then press next.



- Press Next to go the define standards dialogue box



- Pipette 2.5µl of 'Blank' buffer solution into a tip and place the tip and pipette adapter into the pipette holder (see [Correct pipette use and sample handling](#)).
- Press the button marked 'Blank'. The software will attempt to blank the spectrophotometer and set the baseline to zero; the buzzing noise is due to the xenon lamp.
  - If for any reason (including air bubbles or settling turbidity in the sample) a blank cannot be completed successfully a message pop up will indicate this. If this occurs simply try blanking again, and if the procedure continues to fail, try following the cleaning protocol detailed in the Trouble Shooting Section.
- Once the instrument has been successfully 'Blanked' then the greyed out 'Measure' button will change to coloured signifying that you can commence measuring your standards and replicates.
- To begin recording the concentrations of your known standards
  - Choose 'Add' to get the Measure standards dialogue box. Enter the known concentration of your standard, place the pipette tip containing your standard into the instrument.
  - Press Replicate 1 to measure the absorbance of the standard. Up to 5 replicates of the same standard can be measured and the average value displayed.
  - Press Next when the replicates for this standard are complete

## Capturing reference standards

Measuring absorbance for the standard 25.0ng/ul

Average Absorbance = 0.68A      CV% = 1.65%

0.688A    0.685A    0.681A    0.670A    0.661A

Replicate 1    Replicate 2    Replicate 3    Replicate 4    Replicate 5

Press to measure absorbance reference replicates

Close    Re-blank    Next

- Repeat this step for each standard until the standard curve is complete. This standard curve can be exported by selecting "Export" when completed.
  - Select the curve fit required using the "Fit type" dropdown list under the graph display area. (linear regression, quadratic, cubic, quartic)
  - Note that a minimum of 2 standards are necessary to draw a linear regression standard line/curve.

### Method Builder

Name: Uracil

Measurement wavelength: 258.0nm    Normalization wavelength: 320.0nm

Date Created: 14/08/2015 13:25    Date Modified: 14/08/2015 13:25

Concn	OD	OD%	OD	OD%	OD	OD%	OD	OD%	OD	OD%	OD	OD%	Result	Result
25.00	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	Yes	Resample
14.00	0.88	1.34	1.25	1.35	1.34	1.33	1.33	1.33	1.33	1.33	1.33	1.33	Yes	Resample
10.00	0.88	0.76	0.80	0.82	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	Yes	Resample
20.00	0.45	5.13	5.36	5.34	5.33	5.33	5.33	5.33	5.33	5.33	5.33	5.33	Yes	Resample
22.500	0.80	0.700	0.700	0.700	0.700	0.700	0.700	0.700	0.700	0.700	0.700	0.700	Yes	Resample

Calibration Curve

Y = 5.652 + 0.088x

R<sup>2</sup> = 1.000

Fit type: Linear

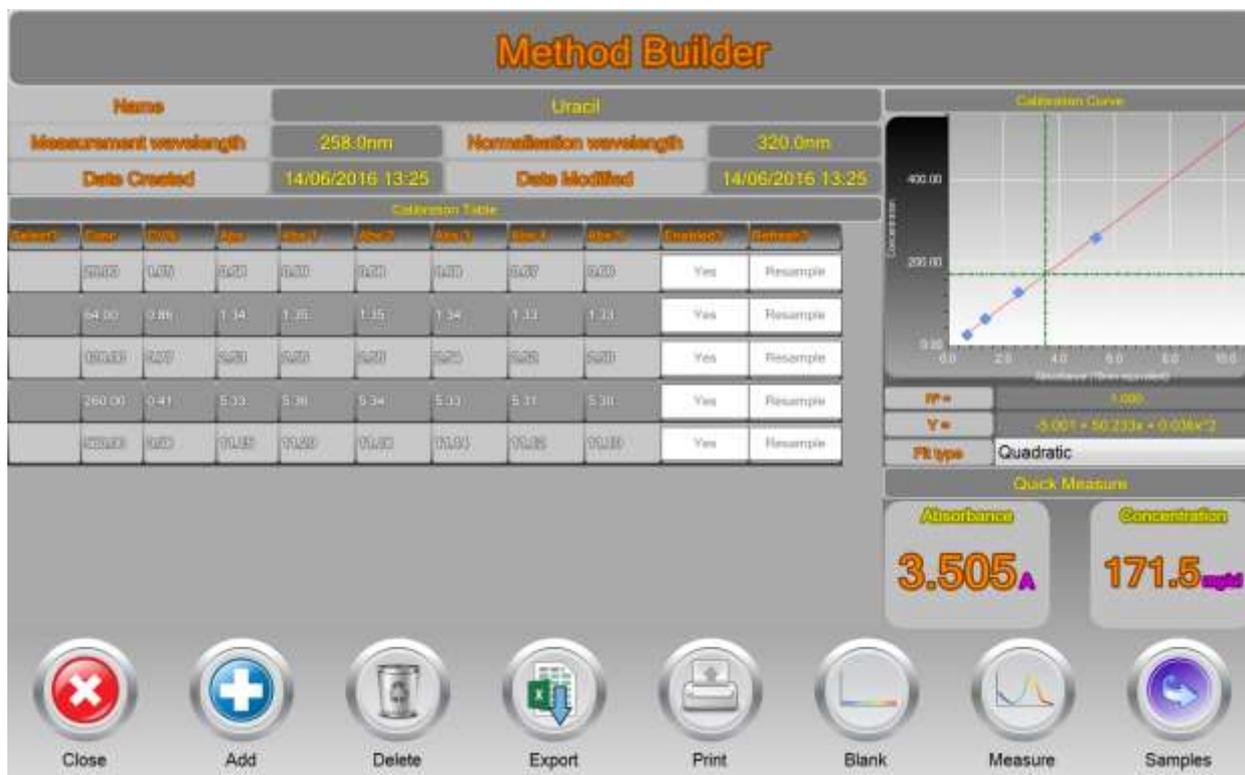
Quick Measure: Absorbance (A), Concentration (mg/ul)

Close    Add    Delete    Export    Print    Blank    Measure    Samples

### Display of calibration standards, linear regression

- To measure samples against this standard curve, place the pipette tip containing your protein into the instrument and press Measure; the absorbance result is extrapolated to the curve and the corresponding calculation result is displayed.

- This result should be recorded in a laboratory notebook.
- Indirect protein sample determination should always be done using freshly prepared standards and not using standard curves prepared previously. For this reason, results are not available for print-out or transfer to spreadsheet.



*Display of result measured against the standard curve*

## Cell Culture Measurement

- General application information and background is described in the [Appendix](#).
- We recommend that the instrument is allowed to warm up for 10 minutes before use, so that an internal thermal equilibrium is achieved.

DUE TO THE TURBID NATURE OF THE SAMPLE, WE RECOMMEND THE USE OF EITHER

1. A 10MM PATH LENGTH DISPOSABLE PLASTIC CELL / VERSAVETTE CUVETTE HOLDER COMBINATION

OR

2. A 10MM PATH LENGTH VERSAPROBE IMMERSION PROBE / BEAKER COMBINATION, FOR THE CONTAINMENT AND MEASUREMENT OF CELL CULTURE SOLUTIONS.

- Select the 'Cell Culture' mode from the dialogue box.

## Kinetics Method List

Select	ID	Standard name	Measurement	Cell factor	Instrument factor	Accessory	Date created
	10000	2450	OD	1.0000	1.0000	10mm Cuvette	10/1/2014 11:30
	10001	2 sec. 100	275	1.0000	1.0000	10mm Cuvette	10/1/2014 11:30
	10002	10 sec. 100	275	1.0000	1.0000	10mm Cuvette	10/1/2014 11:30

Close
 Add
 Delete
 Edit
 Use

- To start a new experiment, select Add. If you are carrying on with an ongoing experiment, press it to select and then press Continue.

### Kinetics Method Editor

**Method name** Chlorophyll

Sampling wavelength (nm) **650.0**   
 Number of samples **100**   
 Recording interval (s) **2.0**
Auto-sampling is enabled

**Instrument conversion factor** 1

**Cell number conversion factor** 1

**Notes** Chlorophyll readings for areas A1-B6

Cancel
 10mm Cuvette
 Next

- Enter wavelength (default 600nm, but can be set to any wavelength)
- Instrument conversion factor is used to compare results obtained from this instrument with those from another (see [Appendix](#)); if you are not doing this, leave as the default of 1.
- Cell number conversion factor will convert the measured OD to cell number (see [Appendix](#)); if you do not want to do this, leave as the default of 1.
- To pre-define a number of sample measurements at specific time intervals, as in a "kinetics" style experiment, enter the number required as Samples, the required time interval with unit and then select the "auto sample?" option.

- It is more likely that this will not be the case when you are sampling from a fermenter, so ensure that "auto sample?" is off. In this case the time interval does not apply
- Enter specific or relevant information for your record in the Notes section and press Next.



- Press on the Y Axis tab to select between the choice of
  - Optical Density
  - Log Optical Density
  - Cell Count (use if the cell number conversion factor has been applied)
- Press on the X Axis tab to select between the choice of
  - Absolute Time (use if measuring at specific time intervals)
  - Relative Time (use if measuring at non-specific or "random" time intervals)
- Press on the X-Axis (Units) tab to select between the choice of
  - Seconds
  - Minutes
  - Hours
- Press on the X-Axis (Scaling) tab to select between the choice of
  - Fine
  - Medium
  - Coarse
- Transfer the appropriate quantity of blank solution to the containment combination of choice.
- Press the button marked 'Blank'. The software will blank the spectrophotometer and set the baseline to zero at normalization wavelength (if set); the buzzing noise is due to the xenon lamp.
  - If for any reason (including air bubbles or settling turbidity in the sample) a blank cannot be completed successfully a message indicating this will be displayed. If this occurs simply try blanking again, and if the procedure continues to fail, try following the cleaning protocol detailed in the Trouble Shooting Section.
- Once the instrument has been successfully 'Blanked', the button will change to Measure signifying that you can commence measuring your samples.

- Transfer the appropriate quantity of sample to the containment combination of choice and press the 'Measure' button.
- After a few seconds the results will be displayed; note that the graph axes will not be shown as labelled until several measurements have been taken.
- It is not necessary to 'save' the results as all results are done so automatically.
- If you are constructing a growth curve rather than a one-off measurement, subsequent results will be appended to this result and the time axis updated with the time that has lapsed since the previous measurement.

### Export of data to Excel

Data can be easily exported to Excel for subsequent analysis and normalisation for inclusion in reports etc. To do this:

- Press the blank square in the "select" column of the results of interest
- Select "High Resolution?" to export readings of every 0.1nm and leave unselected to export every 1nm. (1nm should normally be sufficient otherwise the data files will be extremely large)
- Select the "Horizontal recording?" to export horizontal data (Vertical is usually more manageable)
- Select "results table" to export data with text headers for columns.
- Press "file" then navigate to a folder to which the data is to be saved and enter a file name
- Open Excel and navigate to the file in question; note that data is formatted as a text file.
- Open the file; it is a text delimited file, with tab separation.
- Manipulate that data as appropriate and save in the required format.

### Full Scan

- Use this mode to obtain a spectrum of a sample over the full wavelength range of the instrument (220-1050nm), to examine and compare spectra and to normalise one wavelength relative to another (background correction).
- We recommend that the instrument is allowed to warm up for 10 minutes before use, so that an internal thermal equilibrium is achieved.
- Select the Full Scan mode from the dialogue box.

The descriptions in the following section assume pipette tips are used; if this is not the case, the same sequence of actions is still applicable.

- Pipette 2.5µl of 'Blank' solution into a tip and place the tip and pipette adapter into the pipette holder (see [Correct pipette use and sample handling](#)).
- Press the button marked 'Blank'. The software will blank the spectrophotometer and set the baseline to zero at normalization wavelength (if set); the buzzing noise is due to the xenon lamp.
  - If for any reason (including air bubbles or settling turbidity in the sample) a blank cannot be completed successfully a message requesting that you're-blank' will be displayed. If this occurs simply try blanking again, and if the procedure continues to fail, try following the cleaning protocol detailed in the Trouble Shooting Section.
- Once the instrument has been successfully 'Blanked' then the greyed out 'Measure' button will become coloured signifying that you can commence measuring your samples.
- Insert the pipette with sample and press the 'Measure' button.

- Use the arrow keys either side of "cursor wavelength" enter a particular wavelength for which an absorbance value is required.



*An example of a full wavelength scan (potassium dichromate solution)*

### Zoom

To zoom in to an area of interest press and hold a position on the graph then release once a square is shown. The red lines indicate cursor wavelength and Absorbance. These lines can be moved to show values at specific points in the graph by pressing and quickly releasing.

If using a mouse then left click to move only the wavelength ruler or right click to move both the wavelength and absorbance rulers at the same time. Hold down right click and drag to zoom in on the selected area.

Any zoom adjustments can be reset by pressing the "Reset Zoom" button to the bottom right of the graph.

### Graph overlay

It is possible to superimpose graphs for up to 7 samples onto the same graph window by simply selecting the "Overlay?" box displayed to the right of the sample. Overlays can be removed by pressing 'Clear overlays', shown at the bottom of the measurement window.



*An example of zooming in on a multiple overlays*

### Normalisation of wavelength (background correction)

A normalisation wavelength to correct for its absorbance value over the whole spectrum (and therefore background correction at a specific wavelength) can be applied if required. This is done by pressing the Enable option and entering the normalisation into the box or changing with the arrow keys either side; the spectrum will need to be run again.

### Export of data to Excel

Data can be easily exported to Excel for subsequent analysis and normalisation for inclusion in reports etc. To do this:

- Press the blank square in the "select" column of the results of interest
- Select "High Resolution?" to export readings of every 0.1nm and leave unselected to export every 1nm. (1nm should normally be sufficient otherwise the data files will be extremely large)
- Select the "Horizontal recording?" to export horizontal data (Vertical is usually more manageable)
- Select "results table" to export data with text headers for columns.
- Press "file" then navigate to a folder to which the data is to be saved and enter a file name
- Open Excel and navigate to the file in question; note that data is formatted as a text file.
- Open the file; it is a text delimited file, with tab separation.
- Manipulate that data as appropriate and save in the required format.

## Partial Scan (Quick Measure)

For measurement in a hurry with the minimum amount of setup, use the partial scan option from the home screen. All that is required is the wavelength of interest, from which, a range of +/- 5, 10, 20, 50 or 100 (depending on wavelength maximum and minimum) can be chosen by pressing the corresponding on screen button.

Choose your wavelength of interest;



The Quick Measure screen appears, insert your blank and press the Blank button. Now insert your sample and press the Measure button. To zoom into an area of interest press and hold anywhere on the chart, then release (use right click and drag if using a mouse/touchpad). Press and quickly release to move the Wavelength and Absorbance rulers. For finer accuracy of wavelength, use the numeric control to either nudge wavelength up and down, or type in a wavelength that is in range.



The OD 600 is as above except the wavelength is pre-set to 600nm.

## VersaTip Pipette tips

ONLY USE THE PIPETTE AND TIPS SUPPLIED BY THE ORIGINAL EQUIPMENT MANUFACTURER – DO NOT USE ALTERNATIVES

The pipette included with the instrument requires a supply of disposable UV tips. These tips are made from a special UV/visible transmitting optical grade polymer that is also significantly more dimensionally stable and reproducible than normal tips – do not use alternatives.

These VersaTip pipette tips are available in two forms, one which is RNase free and must be used where the sample is to be retained; the other which is bulk packed can be used where RNase free handling is not required. Order codes and descriptions are as follows...

Order Code	Pack Quantity	Total Tips	Description
<a href="#">VTR5000</a>	50 boxes x 96 tips	4800	RNase free tips in easy dispense rack
<a href="#">VTR1000</a>	10 boxes x 96 tips	960	RNase free tips in easy dispense rack

## Micro-volume cuvettes

VersaCell is available with a path length of 0.5, 0.2 and 0.125mm and VersaTray is available with a path length of either 1 or 0.2mm; contact us for further information.

## Other

CH1	Standard path length cell holder, 10mm.  For use with other cuvettes (0.5mm, 1mm, 5mm and 10mm path lengths can be accommodated)	
VSP3010	VersaProbe Immersion Probe with 3.17mm stainless steel barrel; includes 10mm path-length tip and connecting UV grade fibre optic cables (patch cords not required).	

## MAINTENANCE

### Cleaning:

This spectrophotometer is designed to require a minimum amount of maintenance by the user. They can be cleaned using water or a mild laboratory-cleaning agent e.g. ethanol or general lab cleaner

The instrument should not come into contact with aggressive solutions. Ensure that no liquid enters the spectrophotometers. For safety reasons, the device must be switched off and disconnected from the power supply prior to cleaning.

### Quick Clean' procedure for sample holder:

In the event that sample leaks from a pipette tip or dust reduces the light transfer through the pipette holder simply unscrew the fibre-optic cables from each side of the pipette holder (no tools required – silver screws should be only hand tight). Unscrew the circular base from the tube section. Either soak the holder in hot water with detergent for 30mins and air or drip dry or alternatively simply wash with ethanol or a similar solvent. Reassemble and re-test instrument.

If this quick-clean procedure does not improve the results please follow the "[sample holder cleaning procedure](#)" described below.

## Troubleshooting

### Power indicator does not light

- Check that the external power supply unit is plugged into a mains outlet and that this is switched on, if an extension socket is being used trace this back to the original wall socket and ensure this is switched on and working. Check power is available at each socket by substituting a known working device and ensure this is OK. If power is still not available contact a trained and qualified electrician to identify the reason for loss of power at the socket.
- Check the jack plug is securely connected to the rear panel connector. If the power indicator still does not light check that 12V DC is available on the jack plug using a digital voltmeter, take care not to short-circuit the outer and inner contacts of the jack plug. The centre contact should be +12V with respect to the outer. If this voltage cannot be measured contact the manufacturer or your local distributor for support.

### Sample absorbance is negative

- When the sample absorbance is negative this indicates that the blank is absorbing more than the sample in that specific region of the spectrum; look very carefully at the spectrum of the sample buffer and the de-ionised water.

- De-ionised water can often contain a high level of organic material because the process of de-ionisation mainly removes inorganic materials. The presence of any organic matter will cause an increased absorbance in the UV.
  - Prepare a small volume of the reagent buffer without any RNA present.
  - Select the RNA measurement mode
  - Use this buffer as the blank, and set zero in RNA mode
  - Then measure the de-ionised water (containing no RNA) as an RNA sample against the buffer.
- If this result is positive, then the de-ionised water is the cause of the problem. In this case the buffer should be used to set the blank.

### **Inconsistent result**

- Switch the instrument on and leave it for 10 minutes to come to thermal equilibrium.
- If you should obtain an unexpected erroneous result you should remove the pipette from the holder and immediately inspect the tip to check that sufficient sample remains in the tip. The liquid column should be at least 2mm in height and should be continuous from the end of the tip - i.e. no air gap at the bottom\*. Leakage from the tip can occur if the tip is not securely attached to the pipette or if the end of the pipette tip is touched against the side of the pipette holder when placing the tip in the location hole. Faulty and poorly maintained pipettes may also not retain the sample and should be verified by the following procedure.
- Re-sample and check the liquid column in the tip both before and after sampling. View the tip in the vertical position over a few minutes and check that there is no change in the liquid column in the tip and specifically that there is no formation of a meniscus outside the end of the tip, as this indicates that the seal between tip and pipette or in the pipette itself is damaged.
- If this test proves satisfactory and the result is still not good, increase the sample volume to say 3µl and do the visual checks before and after measurement.
- If, having completed this, results are still not satisfactory follow the 'Quick Clean' procedure detailed on the previous page.
- If the results are still not good, then the following cleaning procedure should be adopted as detailed below:

### **Sample holder cleaning procedure**

- Detach the optical fibres by unscrewing from each side of the pipette holder.
- Use the '1.5mm Allen' key (as supplied) to loosen one of the two grub screws in front of the bottom of the pipette holder (not applicable for CH1 VersaVette Cuvette Holder)
- Then screw the Fibre optic connection removal tool (as supplied) a few turns onto the SMA905 connector, nearest the loosened screw, from which the fibre optic cable was removed. Repeat this procedure for the other connector assembly, carefully noting which position each came from.
- Check whether the connectors are wet or blocked. This may happen if excess sample is picked up from the outside of the pipette tips.
  - Wash each connector with pure water and dry with lint free tissue.
  - Use a cotton tip soaked in acetone to clean and dry the connectors.
  - Remove the round base from the holder by unscrewing and check that the sample holder tube is clean and dry. If necessary wash and use an air-duster to ensure all moisture is removed from the centre of the tube, connector ports and screw holes before re-assembling.

- Re-assemble the unit in the reverse order to the above ensuring the connector assembly is pushed fully home before tightening the recessed Allen key screws. Inspect the inside of the tube to ensure no dust or swarf is present, blow out or clean as necessary and then re-test.
- To avoid future problems, monitor pipette performance; take care not to immerse the tips too deeply in sample when picking it up and wipe the outside before inserting in the holder.

### Connection Lost message

This message tells the user that the spectrometer has crashed. Disconnecting the power from the spectrometer and reconnecting it should solve the problem. There is no need to shut down or restart the software and your data should remain intact.

### Communications Error message

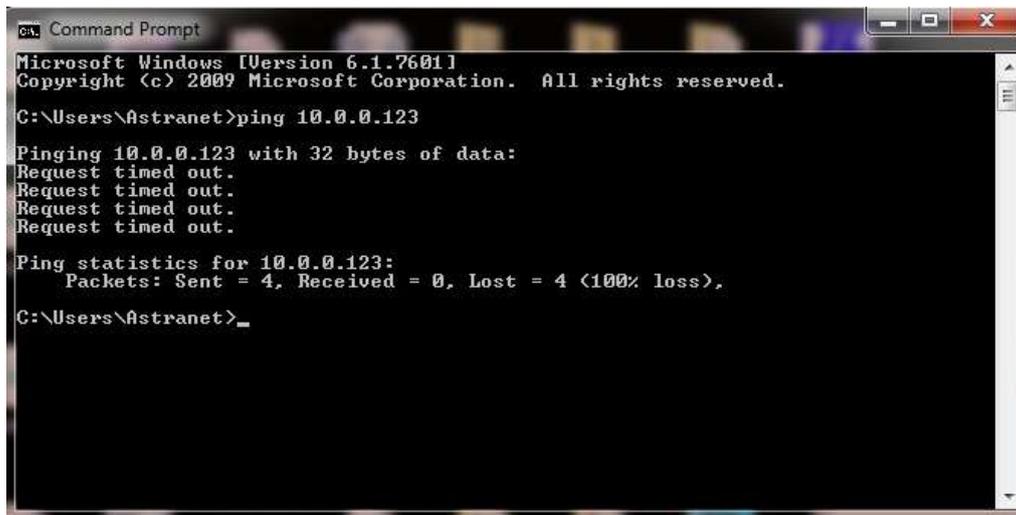
In the event of a software hang-up or communications glitch, a message will be displayed. Click/press on Details to view the information there; this should be copied, pasted into an email and sent to [info@expedion.com](mailto:info@expedion.com) with the subject line "VersaWave Error Log" and a description of how the error occurred.

### Ethernet Connection failed

- To check the connection between instrument and the PC, open a "command prompt" by pressing the Start button in the bottom left corner of the screen and then select "All Programs" and then select "Accessories" and then select "Command Prompt" once the Command Prompt window has appeared type;
  - ping 10.0.0.123
  - and then press return:



- It is critical that there are no lost packets, the "Lost" value must read 0%. If the loss is above 0% then the network adapter isn't configured correctly or is disabled or another network adapter is still enabled and responding to requests to 10.0.0.123. Go back over the steps and ensure each action has been followed.
- An example of the result of the ping command where incorrect network settings are in use;



```
ca. Command Prompt
Microsoft Windows [Version 6.1.7601]
Copyright (c) 2009 Microsoft Corporation. All rights reserved.

C:\Users\Astranet>ping 10.0.0.123

Pinging 10.0.0.123 with 32 bytes of data:
Request timed out.
Request timed out.
Request timed out.
Request timed out.

Ping statistics for 10.0.0.123:
    Packets: Sent = 4, Received = 0, Lost = 4 (100% loss),

C:\Users\Astranet>_
```

- If this test is not successful re-check that the correct Ethernet cable has been used and is connected correctly. Also ensure that the settings detailed in the installation procedure have been set correctly and that any conflicting network settings have been disabled.
- Other Networks can be disabled by selecting the Networks option in the Control Panel, clicking on one of the other networks to highlight it, then by right clicking the highlighted network and selecting 'Disable', carry out the same procedure with each unwanted network. These can be re-enabled by carrying out the same procedure and selecting the 'Enable' option.

### Returning instrument to the manufacturer

In the unlikely event that an instrument has to be returned, even after discussing the matter with your supplier, then the form on the next page should be printed out, completed and packed with the instrument before return (typically via your supplier). Note that this **cannot** be done until the relevant RMA (Return to Manufacturer Authorisation) number has been obtained via the supplier in advance.

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Title: Decontamination Form	Issue No. 1	

## EXPEDEON VERSAWAVE SPECTROMETER DECONTAMINATION DECLARATION

<b>Company:</b>	<b>Contact:</b>
<b>Address:</b>	<b>Department:</b>
	<b>Tel:</b>
	<b>Fax:</b>
	<b>E-mail:</b>

<b>Invoice No:</b> .....
<b>RMA No:</b> .....

<b>Description of Spectrometer(s) (Make / Model):</b> Please include serial numbers
---

<b>Nature of contaminant:</b>
<b>Method of decontamination:</b>
Decontaminated by:      Date decontaminated:

<b>I confirm that the above information is accurate, and that the spectrometer(s) detailed is/are safe to handle</b>
<i>(please PRINT)</i>

Name: .....
Signed.....
Position .....
Date.....

**Expedeon Ltd**

Newton Hall, Newton, Cambridge, CB22 7ZE, UK  
Phone: +44 (0) 1223 872197 Fax: +44 (0) 1480 330017  
Email: sales@Expedeonsystems.com

Reg in England No: 4215505



## SPECIFICATIONS

Parameter	Specification
Path length with tips	Nominally 1mm
Path length using optional VersaVette Cuvette Holder	Path length depends on the VersaCell or VersaTray used 10mm, 5mm, 1mm depending on other cuvettes used
Sample volume: <ul style="list-style-type: none"> <li>• Micro-volume samples</li> <li>• Other samples</li> </ul>	<ul style="list-style-type: none"> <li>• 2.5µl using tips (from microplate well or Eppendorf)</li> <li>• Volume depends on the VersaCell or VersaTray used</li> <li>• Up to and over 2.5ml (if VersaVette Cuvette Holder or VersaProbe immersion probe accessories are used)</li> </ul>
Light Source	Pulsed Xenon Lamp (powered for reading only)
Detector	3648 pixel CCD Array (UV enhanced)
Photometric Linearity	Better than 1%
Photometric Range	-0.2 to 2.5A
Wavelength Range	220 to 1050nm
Wavelength Accuracy	±0.5nm
Spectral Bandwidth	< 2nm
Absorbance Precision	0.003A
Detection Limit (DNA)	3ng/µl using tips Detection limit depends on the VersaCell or VersaTray used
Detection Limit (BSA)	0.1mg/ml using tips Detection limit depends on the VersaCell or VersaTray used
Read Time	2 seconds (includes full wavelength scan)
PC Communication	Ethernet (cable supplied)
Power	24 DC from supplied adapter with input 100 - 240V AC
Dimensions	18 x 18 x 17 cm
Weight	3.5kg

These specifications reflect the performance that can be achieved under normal conditions in a laboratory environment. Our products are subject to continuous review and may be changed or up-dated without notice.

## APPENDIX 1 - APPLICATIONS

### Measuring Nucleic Acid Concentration and Purity

The concentration of a sample can be calculated from its measured absorbance at the wavelength of maximum absorbance ( $\lambda_{max}$ ). All nucleic acids have a  $\lambda_{max}$  at 260nm in the UV region of the electromagnetic spectrum.

The natural bandwidth of this peak is around 47nm, so in line with good practice a spectrophotometer with a spectral bandwidth at least ten times less (around 4-5nm) must be used for measuring these samples.

From the Beer-Lambert law  $A = \epsilon cl$  (where A is the absorbance,  $\epsilon$  is the molar extinction coefficient, c the concentration and l is the path length).

Where a 1cm path length cuvette is used, solving the equation for concentration gives:  $c = A/\epsilon$ . In practice the measured absorbance is multiplied by a 'factor' that is the reciprocal of the molar extinction coefficient as defined in the following table.

Nucleic Acid	Molar Extinction Coefficient ( $\epsilon$ )	Factor ( $1/\epsilon$ )	Resulting Units
dsDNA	20 $g^{-1}cm^{-1}l$	50	$\mu g/ml$ or $ng/\mu l$
ssDNA	30 $g^{-1}cm^{-1}l$	33	$\mu g/ml$ or $ng/\mu l$
RNA	25 $g^{-1}cm^{-1}l$	40	$\mu g/ml$ or $ng/\mu l$
Oligonucleotide	33 $g^{-1}cm^{-1}l$	30	$\mu g/ml$ or $ng/\mu l$

NOTE: Where the nucleic acid has been diluted, correction for the dilution ratio must be included. All measurements are made at 25°C and at a neutral to slightly alkaline pH. The instrument automatically corrects for the reduced path length of the *VersaTip*.

The factor can also be determined from the slope of a linear calibration plot of absorbance versus concentration for the pure nucleic acid under consideration. This also enables us to define a simple range of stock calibration and QC standards as follows...

A solution of 50  $\mu g/ml$  or 50  $ng/\mu l$  dsDNA will give a reading of 1.00A at 260nm

A solution of 33  $\mu g/ml$  or 33  $ng/\mu l$  ssDNA will give a reading of 1.00A at 260nm

A solution of 40  $\mu g/ml$  or 40  $ng/\mu l$  RNA will give a reading of 1.00A at 260nm

The instrument compensates for samples where particles in suspension or background turbidity may be a problem by subtracting the absorbance at 320nm.

The Purity Ratio determines the level of contamination from proteins and is based on the absorbance of these at 280nm. For pure DNA the 260/280 ratio should be 1.8, for RNA it should be 2.0, lower values indicate contamination by proteins.

### Microarray Measurements (cDNA)

## Calculating the Labelling Efficiency and Concentration of Nucleic Acid for Microarray samples

The relative efficiency of a labelling reaction can be evaluated by calculating the approximate ratio of bases to dye molecules. This ratio can be determined, as described below, by measuring the absorbance of the nucleic acid at 260 nm and the absorbance of the dye at its absorbance maximum ( $\lambda_{max}$ ). The calculations are based on the Beer-Lambert law:

$$A = \epsilon \times \text{path length (cm)} \times \text{concentration (M)}$$

where  $\epsilon$  is the extinction coefficient in  $\text{cm}^{-1}\text{M}^{-1}$ . The absorbance measurements can also be used to determine the concentration of nucleic acid in the sample. Optimal labelling for hybridization of microarrays is 1 dye for every  $\sim 12$ –20 bases. Higher levels of labelling interfere with hybridization.

### Measuring the Base : Dye Ratio

Measure the absorbance of the nucleic acid–dye conjugate at 260 nm ( $A_{260}$ ) and at the  $\lambda_{max}$  for the dye ( $A_{dye}$ ). Measure the background absorbance at 260 nm and  $\lambda_{max}$ , using buffer alone, and subtract these numbers from the raw absorbance values for the sample.

To perform these measurements, the nucleic acid–dye conjugate should be at a concentration of at least  $5\mu\text{g/ml}$ . Depending on the dye used and the degree of labelling, a higher concentration may be required.

### Measuring the Concentration of Nucleic Acid in labelled samples

The absorbance values  $A_{260}$  and  $A_{dye}$  may also be used to measure the concentration of nucleic acid in the sample. In order to obtain an accurate measurement for a dye-labelled nucleic acid, a dye-corrected absorbance value ( $A_{base}$ ) must be used. In addition, for concentration measurements, the path length (in cm) is required. The path length of the VersaTip is 0.1cm but all values are multiplied by 10 to display values equivalent to a standard 1cm cuvette.

$$\text{Nucleic Acid (mg/ml)} = (A_{base} \times MW_{base}) / (\epsilon_{base} \times \text{path length})$$

### Correct for the contribution of the dye to the $A_{260}$ reading

Most fluorescent dyes absorb light at 260 nm as well as at their  $\lambda_{max}$ . To obtain an accurate absorbance measurement for the nucleic acid, it is therefore necessary to account for the dye absorbance using a correction factor provided by the manufacturer ( $CF_{260}$ ) and the following equation:

$$A_{base} = A_{260} - (A_{dye} \times CF_{260})$$

Calculate the ratio of bases to dye molecules. Use the following equation:

$$\text{base:dye} = (A_{base} \times E_{dye}) / (A_{dye} \times E_{base})$$

where  $E_{\lambda}$  is the extinction coefficient for the fluorescent dye and  $E_{base}$  is the average extinction coefficient for a base in double stranded Nucleic acid (dsDNA or ssDNA or RNA). Note that since the calculation is a ratio, the path length has been cancelled out of the equation.

Nucleic Acid	Ebase	MWbase
dsDNA	6600	330
ssDNA	8919	330
RNA	8250	340

## Protein Measurements

Proteins can be measured directly at their  $\lambda_{max}$  of 280nm, or indirectly by adding reagents to the sample to produce a colour proportional to the protein concentration and then measuring at the wavelength of maximum absorbance ( $\lambda_{max}$ ) for that colour.

### Indirect measurement

This product is pre-programmed with the Bradford and Lowry indirect methods where analysis is at 595nm and 750nm respectively. The choice of which method to use will depend on the expected range of the samples, solvent and extraction reagent compatibility, preparation time restrictions and the ease-of-use required. Please refer to your reagent kit supplier and the pack insert for full details of limitations and restrictions, sample preparation procedures and respective analytical performance.

Indirect methods have been developed to ensure all proteins in a mixed sample are measured correctly.

### Direct measurement

The direct method requires little or no sample pre-treatment so has a lower cost and is much quicker and easier to perform than the indirect methods, but is less accurate when measuring mixtures of proteins.

Although all proteins have a  $\lambda_{max}$  at 280nm their molar extinction coefficients vary, so the correct factor or extinction coefficient has to be chosen for different sample types to ensure they are measured accurately. The following table gives details of these extinction coefficients...

Sample Type	Concentration at 1.0 Absorbance		Extinction Coefficient
	mg/ml	%	
General (Abs)	1	0.1	
BSA	10	1	6.7
IgG	10	1	13.7
Lysozyme	10	1	26.4
Other	10	1	User Entered

## Molar Extinction Coefficients vs. Absorbance for 1% Solutions

Using the molar extinction coefficient in the calculation of protein concentration gives a result in terms of molarity. We know from the Beer-Lambert law that absorbance  $A = \epsilon bc$  and solving this equation for concentration using 1cm path length cuvettes gives  $c = A/\epsilon$ , then:

$$A / \epsilon_{\text{molar}} = \text{molar concentration}$$

However, many sources do not provide molar extinction coefficients. Instead, they provide absorbance,  $A_{280\text{nm}}$ , values for 1% solutions (which are 1 g/100 ml) measured in a 1 cm cuvette. These values can be understood as percent extinction coefficients ( $\epsilon_{\text{percent}}$ ) with units of (g/100 ml) $^{-1}$  cm $^{-1}$  instead of M $^{-1}$ cm $^{-1}$ .

Consequently, when these values are applied as extinction coefficients in the general formula, the units for concentration,  $c$ , are percent (i.e., 1% = 1 g/100 ml = 10 mg/ml).

$$A / \epsilon_{\text{percent}} = \text{percent concentration}$$

To report concentration in terms of mg/ml, then an adjustment factor of 10 must be made when using these percent extinction coefficients (i.e., convert from 10 mg/ml to 1 mg/ml).

$$(A / \epsilon_{\text{percent}}) 10 = \text{concentration in mg/ml}$$

The relationship between molar extinction coefficient ( $\epsilon_{\text{molar}}$ ) and percent extinction coefficient ( $\epsilon_{\text{percent}}$ ) is as follows:

$$(\epsilon_{\text{molar}}) 10 = (\epsilon_{\text{percent}}) \times (\text{molecular weight of protein})$$

Still other sources provide protein absorbance values for 0.1% (= mg/ml) solutions, as this unit of measure is more convenient and common for protein work than percent solution. This variation in reporting style underscores the importance of carefully reading stated values to be sure that the unit of measure is understood and applied correctly.

## Measuring a Protein or Protein Mixture with Unknown Extinction Coefficients

Most published protein extinction coefficients range from 4.0 to 24.0 ( $\epsilon_{\text{percent}}$ ); however the distribution is skewed in favour of the lower values giving a mode of 10. If no extinction coefficient information exists for a protein or protein mixture of interest and a rough estimate of protein concentration is required for a solution that has no other interfering substances, common practice is to assume an  $\epsilon_{\text{percent}}$  value of 10. Therefore, although any given protein can vary significantly from this approximation, the average for a mixture of many different proteins will most likely be approximately 10.

Note: the software assumes the user has entered the  $\epsilon_{\text{percent}}$  value as defined above.

The software converts Absorbance at 280nm into mg/ml of protein using the following formula...

Protein Conc. in mg/ml = (A280nm corrected to 10mm path length/ $\epsilon$ percent) x 10.

## Cell Culture Measurements

The stage of growth of a bacterial culture is monitored to ensure that the cells are harvested at an optimum point for the greatest density of healthy, live cells; this is usually done towards the end of the so-called log phase of growth. Discrete samples may be taken from an ongoing fermentation, and typically measured in 10mm path length disposable plastic cuvettes at various times in order to construct the growth curve (glycerol is sometimes added to the sample in the cuvette to stop the culture settling too quickly and to give a more stable result).

Since such samples are cloudy or turbid, they scatter light and the solution absorbance, *per se*, is not measured. Thus whereas different colorimeter or spectrophotometer types will give different results due to their different optical geometries, comparison between totally different instrument techniques has to be done via construction of a calibration curve for each.

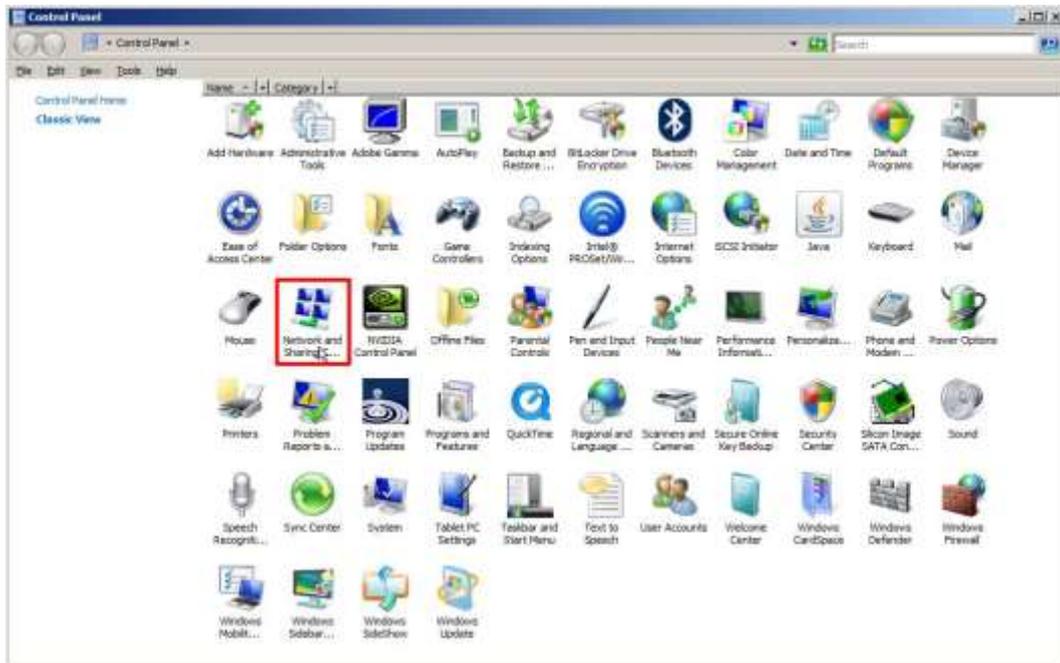
A factor to convert the result to that of another, or preferred, instrument type can be then be calculated, if required, using the instrument conversion factor. This is effectively an offset number so that the user can directly compare results from this instrument with those another instrument in the lab or one that has been used previously. Thus this instrument may read 0.4 OD whereas the other product reads 0.6 OD due to different optical geometry; in this case the factor would convert our result of 0.4 to the other results of 0.6.

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  $1 \text{ OD}_{600} = 8 \times 10^8 \text{ cells/ml}$  for E. Coli. Alternatively this factor can be applied to the measured OD using this "cell number conversion factor" to estimate the number of cells per ml in the sample.

## APPENDIX 2 – SETTING IP ADDRESSES

### Setting IP addresses in a Windows Vista system

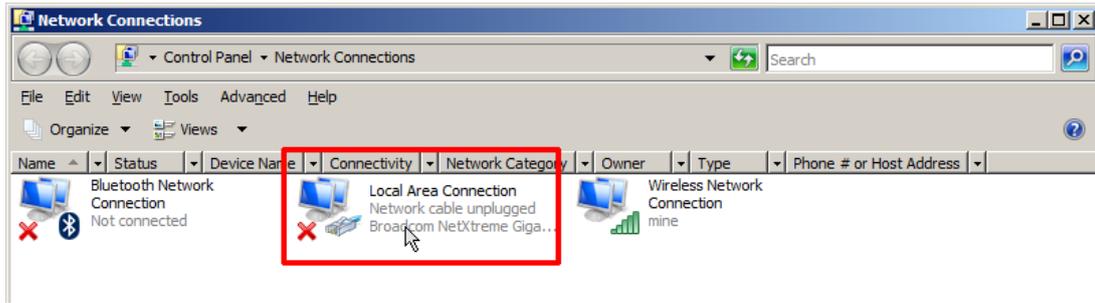
Open the Control Panel by pressing the Start button and then selecting Control Panel



Now select "Network and Sharing" then select "manage network connections" from the menu on the left, you may need to consult the manual for your computer system to find out which controller to use.

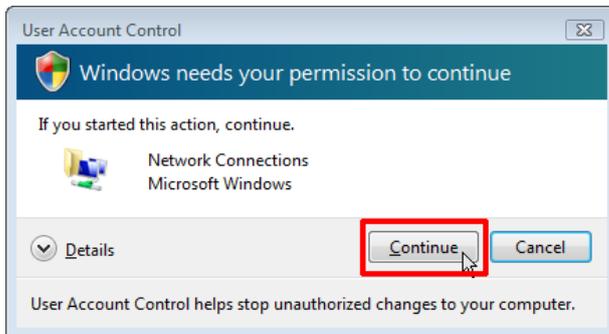


Double click on the wired Ethernet connection shown in the 'Local Area Connection'

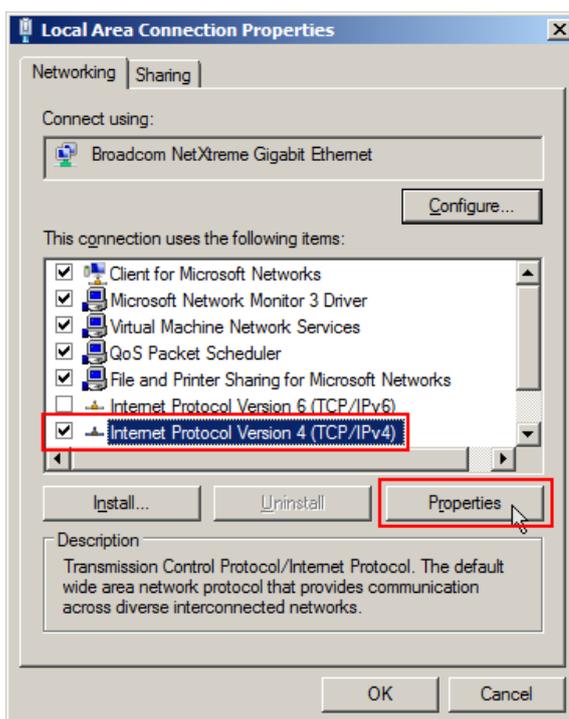


You will then usually be asked to confirm a restricted system action by the Windows UAC (User Access Control), then Select "Continue" to allow configuration of the network adapter.

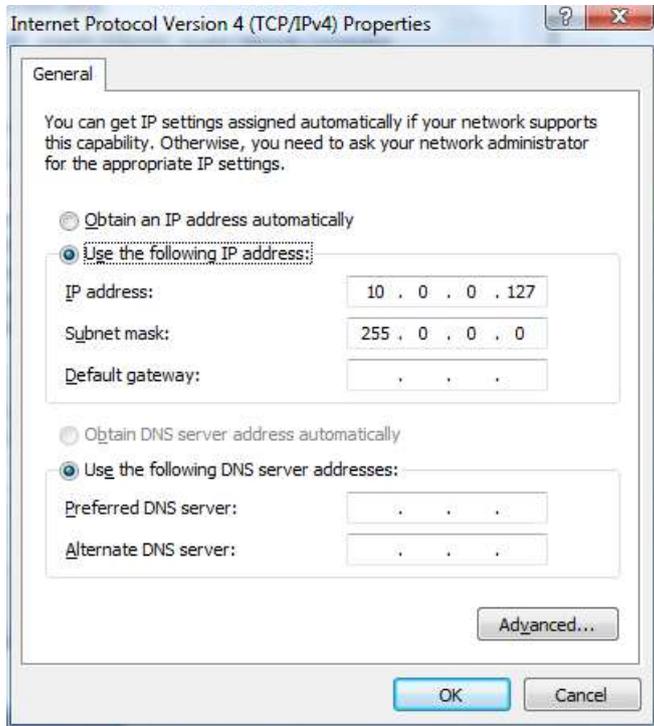
NOTE: It may be necessary to disable other networks if there is an IP address conflict – this can easily be done in the screen above by right-clicking on each network in turn and selecting the 'Disable' option. Any disabled network can be 'switched back on' by carrying out the same procedure and selecting the 'Enable' option.



Select "Internet Protocol Version 4 (TCP/IPv4)" ensuring that the checkbox to the left of the item remains checked. Now click on "Properties".



Enter the values as below and click OK to save changes. Make sure that there are no other network adapters active, you may need to consult your system's user manual to achieve this.

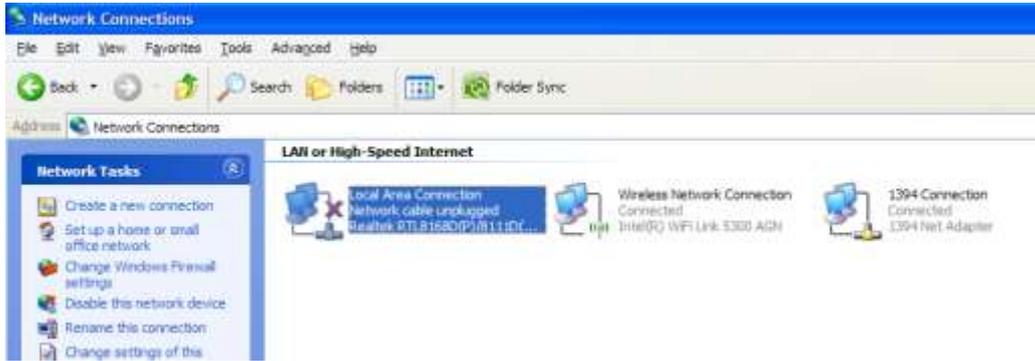


## Setting IP addresses in a Windows XP system

Open the Control Panel by pressing the Start button and then selecting Control Panel



Now double click "Network Connections" then "Local Area Connection"

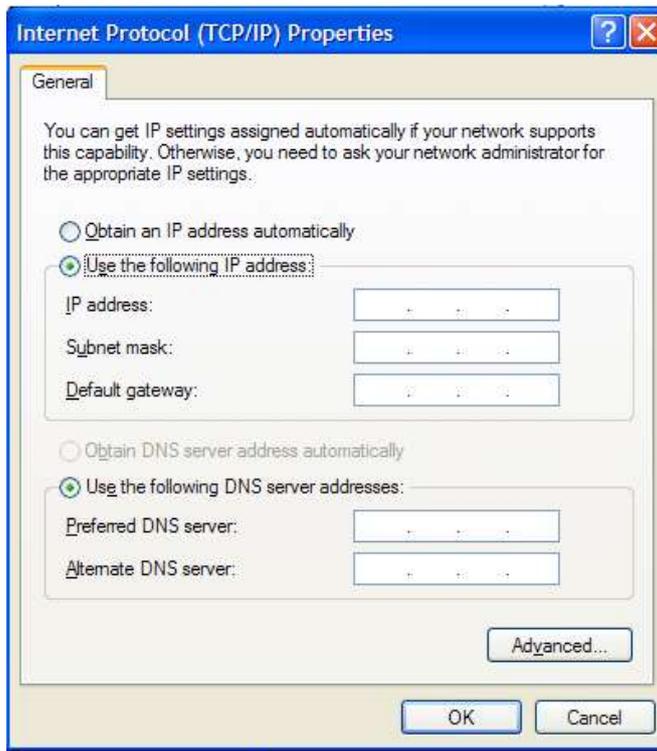


NOTE: It may be necessary to disable other networks if there is an IP address conflict – this can easily be done in this screen by right-clicking on each network in turn and selecting the 'Disable' option. Any disabled network can be 'switched back on' by carrying out the same procedure and selecting the 'Enable' option.

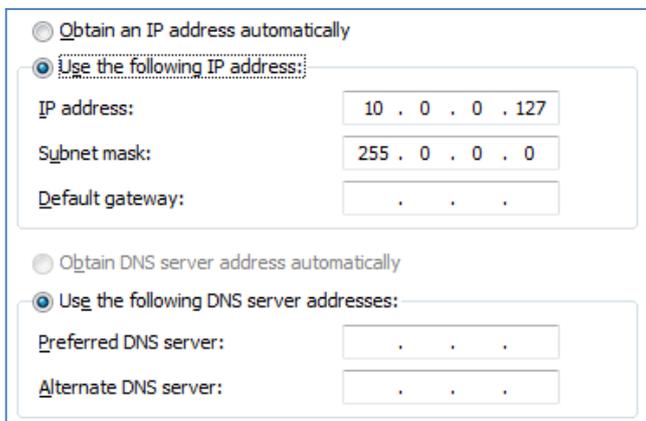
Select "Internet Protocol (TCP/IP)" from the following screen ensuring that the checkbox to the left of the item remains checked. Now click on "Properties".



Select the radio button next to "Use the following IP address"

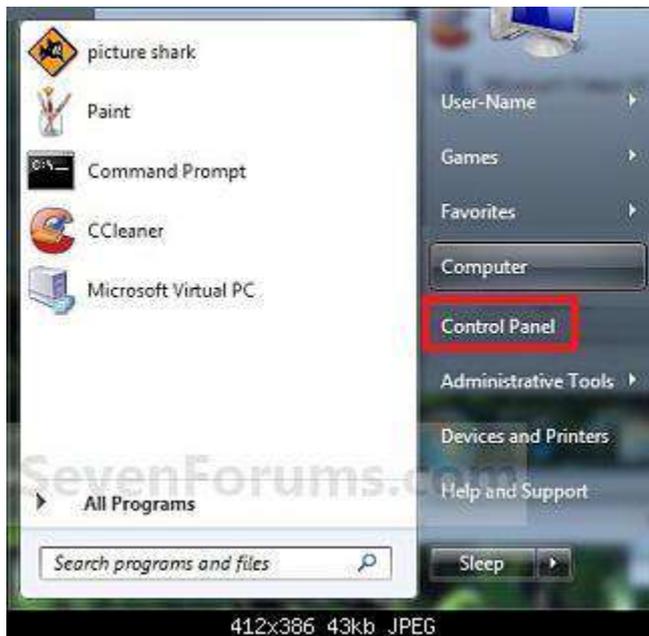


Enter the values as below and click OK to save changes. Make sure that there are no other network adapters active, you may need to consult your system's user manual to achieve this.



## Setting IP addresses in a Windows 7 / 8 /10 system

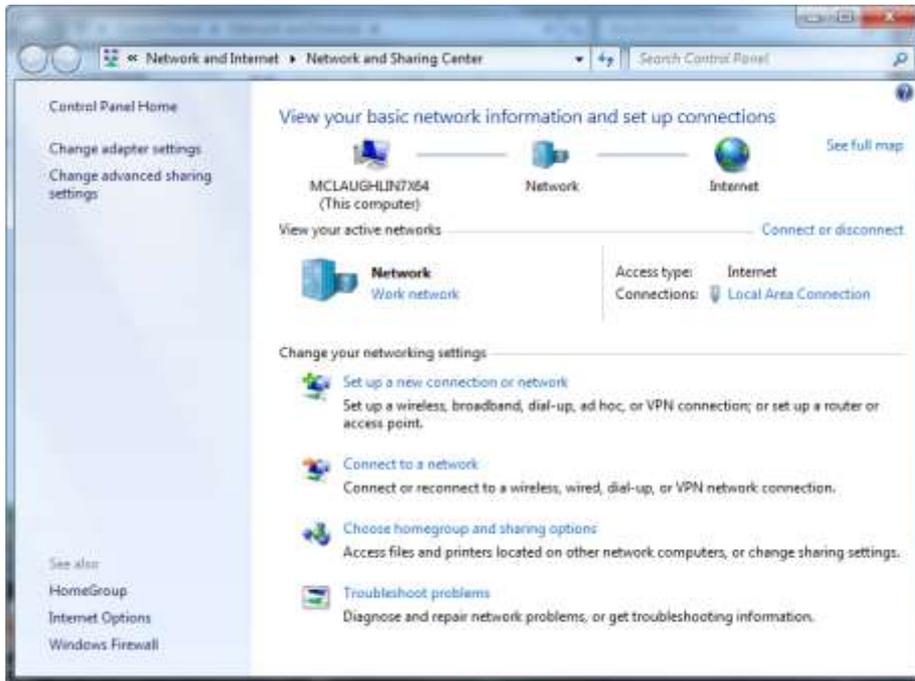
Open the Control Panel by pressing the Start button (Microsoft flag/globe) in the bottom left corner of the screen and then selecting Control Panel option in the menu to the right hand side of the start screen below.



From the control panel screen select the 'Network and Internet' option as shown below (ensure that View by Category is selected).

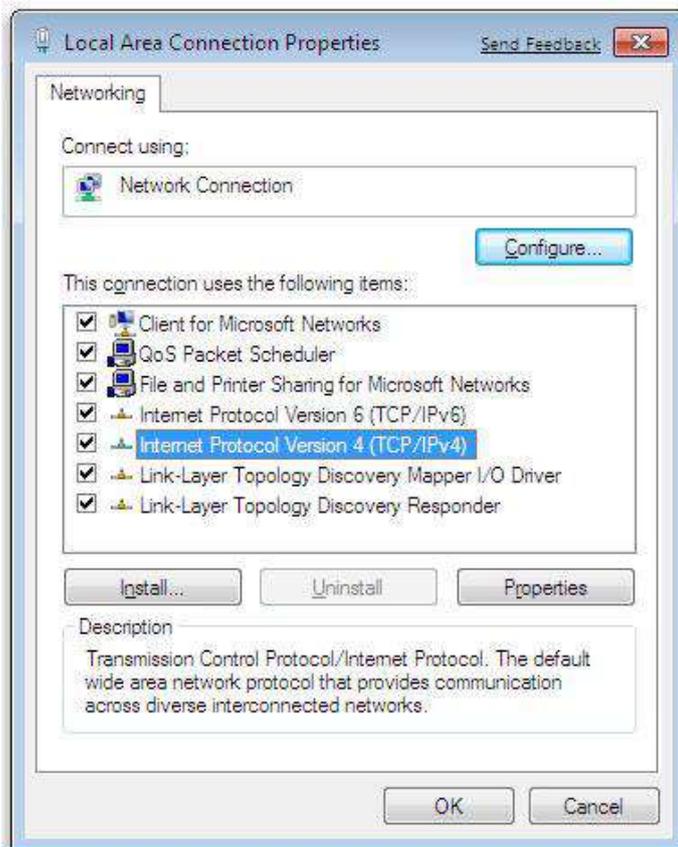


From the Network and Sharing Centre on the next screen, select Change Adapter Settings in the upper left corner of the screen.



Double click on Local Area Connection.

Highlight Internet Protocol version 4 and ensure it remains ticked. Then select the Properties button.



Select the radio button next to "Use the following IP address" and enter values shown below and click OK to save changes. Make sure that there are no other network adapters active, you may need to consult your system's user manual to achieve this.

