GE Healthcare
Life Sciences

Spectrophotometry

Handbook

Handbooks from GE Healthcare Life Sciences

Contents

Continued overleaf

Spectrophotometry basics

What is spectrophotometry?

Spectrophotometry is a scientific method based on the absorption of light by a substance, and takes advantage of the two laws of light absorption.

Definition

spec·tro·pho·tom·e·ter/ **spektrōfōˈtämitər/**

Noun: An apparatus for measuring intensity of light in a part of the spectrum, as transmitted or emitted by particular substances.
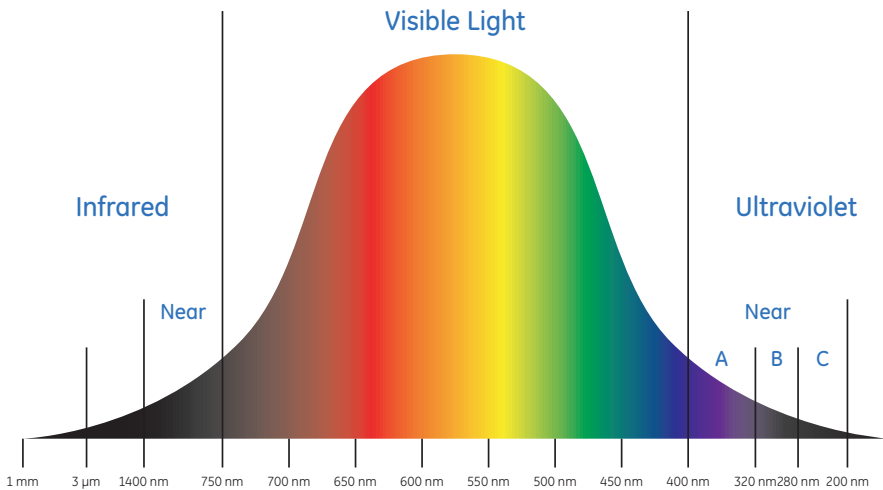
Fig 1. The electromagnetic spectrum. GE Healthcare Life Sciences offers a range of spectrophotometers which operate in the UV, visible and near infrared section of the electromagnetic spectrum.

Lambert's Law (1)

The proportion of light absorbed by a medium is independent of the intensity of incident light. A sample which absorbs 75% (25% transmittance) of the light will always absorb 75% of the light, no matter the strength of the light source.

Lambert's law is expressed as $I/I_o = T$

Where I = Intensity of transmitted light
I_o = Intensity of the incident light
T = Transmittance

This allows different spectrophotometers with different light sources to produce comparable absorption readings independent of the power of the light source.

Beer's Law (2)

The absorbance of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium. In Spectrophotometry the thickness of the medium is called the pathlength.

In normal cuvette-based instruments the pathlength is 10 mm. Beer's law allows us to measure samples of differing pathlength, and compare the results directly with each other. GE Healthcare offers a variety of instruments and accessories which allow measurement of pathlengths from 10 cm down to 0.2 mm.
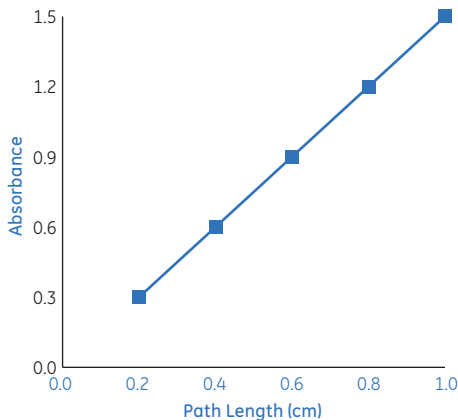
Fig 2. Beer's Law: The absorbance of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium.

Short pathlength instruments are used when the sample is of limited volume, scarce and maybe requiring recovery, or is very concentrated (e.g. > 50 µg DNA/ml), and the user wishes to avoid the need for dilution. Many samples would traditionally require dilution for two reasons:

1. So that there is enough volume to fill a 10 mm pathlength cuvette.
2. To lower the sample concentration enough to allow accurate measurement by the spectrophotometer.

Dilution introduces an aspect of human error and can also prevent the use of that sample in downstream applications.

To measure concentrated samples using a 10 mm pathlength would require a very powerful light source to give transmittance that is high enough to be detected reliably. A shorter pathlength reduces the absorbance – increasing the transmittance – hence reducing the incident light required to achieve a reliable result. This removes the need to dilute the sample, or to have a larger, more powerful or more expensive instrument.

When using short pathlengths (less than 10 mm), results are generally normalized to that of a 10 mm pathlength, e.g. In the case of a 0.2 mm pathlength, the absorbance results are multiplied by 50. However, at the same time any error from the system of absorption by the cuvette is also multiplied by 50, increasing the possible effect on the result.

In basic terms: Absorbance = Concentration × Pathlength
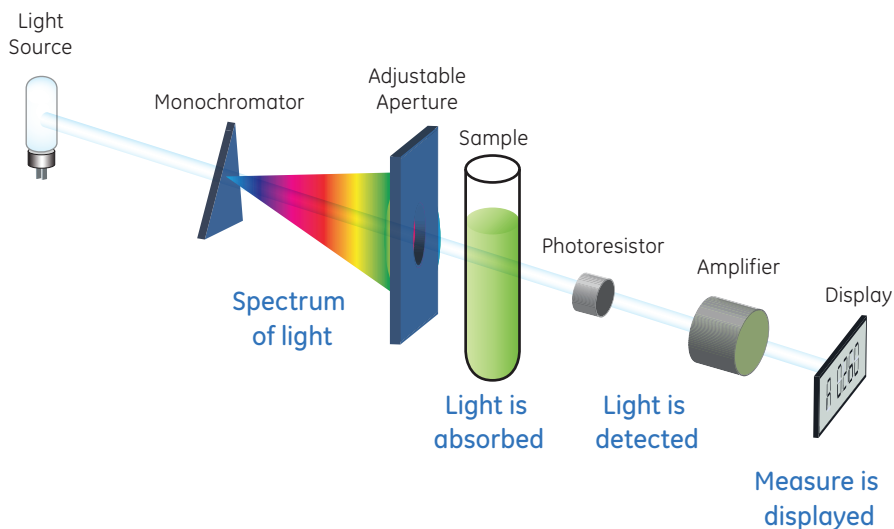
Fig 3. General schematic of a spectrophotometer.

Nucleic acid applications

Spectrophotometry can be used to estimate DNA or RNA concentration and to analyze the purity of the preparation. Typical wavelengths for measurement are 260 nm and 280 nm. In addition measurements at 230 nm and 320 nm can provide further information.

Purines and pyrimidines in nucleic acids naturally absorb light at 260 nm. For pure samples it is well documented that for a pathlength of 10 mm, an absorption of 1A unit is equal to a concentration of 50 µg/ml DNA and 40 µg/ml for RNA. For oligonucleotides the concentration is around 33 µg/ml but this may vary with length and base sequence.

So for DNA: Concentration (µg/ml) = $Abs_{260} \times 50$.

These values are known as conversion factors.

A number of other substances which also absorb light at 260 nm could interfere with DNA values, artificially increasing the result calculated from the absorption readings. To compensate for this a selection of ratios and background corrections have been developed to help eliminate false readings.

X: 300
Y: 0.063
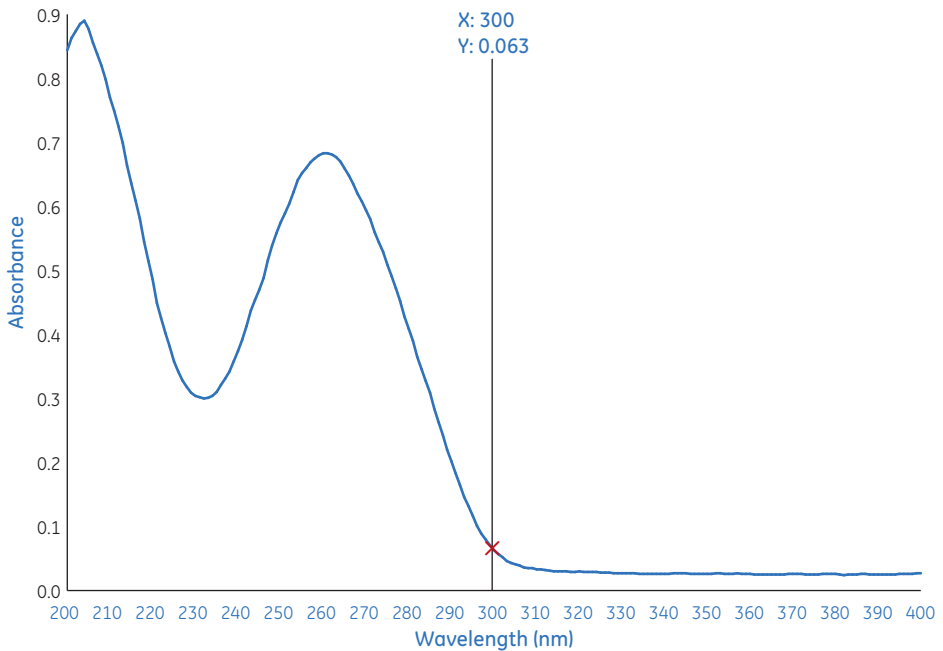
Fig 4. A typical wavelength scan for a pure DNA sample.

There is a wide absorbance peak around 260 nm preceded by a 'dip' at 230 nm. Therefore to measure the DNA absorption, the 260 nm DNA peak must be distinguishable from the 230 nm reading.

If the readings at 230 nm are too similar to those at 260 nm, DNA cannot be measured accurately. Higher 230 nm readings can indicate contaminants in the sample. There should also be a rapid tail-off from 260 nm down to 320 nm. For this reason, 320 nm is often used to measure background (see background correction).

Direct UV measurement

A_{260}/A_{280} Ratio

The most common purity check for DNA and RNA is the A_{260}/A_{280} ratio. Any protein contamination will have maximum absorption at 280 nm. Measurements are taken at both 260 nm and 280 nm and compared to give a ratio. For DNA the result of dividing the 260 nm absorption by the 280 nm needs to be greater or equal to 1.8 to indicate a good level of purity in the sample. For RNA samples this reading should be 2.0 or above. Results lower than this are indicative of impurities in the sample.

A_{260}/A_{230} Ratio

An increase in absorbance at 230 nm can also indicate contamination, which may in turn affect the 260 nm reading for DNA and RNA. A number of substances absorb at 230 nm, as this is the region of absorbance of peptide bonds and aromatic side chains. Several buffer components exhibit strong absorption at 260 nm and therefore can alter the results of photometric quantification. One example of such a component is EDTA in concentrations above 10 mM. Contaminants in a sample, such as proteins, phenol, or urea, can result in absorption at 230 nm. Phenol contamination also increases a sample's absorption at 280 nm and therefore can be identified through a lower A_{260}/A_{280} ratio. An A_{260}/A_{230} ratio of 2 or above is indicative of a pure sample.

A_{320} Background correction

Background correction is a process whereby the absorption at a point on the spectrum unrelated to the sample being analyzed is also measured, and the reading subtracted from the peaks. Absorption at 320 nm may be due to light scatter caused by particles, or to a precipitate in the sample. Dirty or damaged cuvettes can cause absorption at 320 nm. Contaminations with chaotropic salts, such as NaI, can also lead to increased light scatter.

Measuring and correcting for the reading at 320 nm therefore removes any interference from light scatter, from the cuvette, or in cases where a blanking plate is used to target the light beam though the sample.

Background correction is particularly useful when using small volume cells or specialist small volume spectrophotometers.

Nucleic acid measurements with low volume instruments

To get meaningful results, as a rule of thumb, the following two criteria must be met:

- Abs_{260} > approx. twice Abs_{230} (A_{260}/A_{230} Ratio=2)
- Abs_{260} = 0.1 or more (indicative of a high enough concentration, ie solution not too dilute)

Typically the only measurements checked for DNA measurement are concentration and A_{260}/A_{280} ratios. Unexpected results can often be explained by looking at the underlying A_{230}, A_{260}, A_{280}, A_{320} values.

As an example:

Sample 1

Concentration 10.7 µg/ml

A_{230}	A_{260}	A_{280}	A_{320}
9.27	0.244	0.129	0.030

A_{260}/A_{280}		A_{260}/A_{230}
2.162		0.023

Sample 2

Concentration 11.1 µg/ml

A_{230}	A_{260}	A_{280}	A_{320}
9.33	0.323	0.206	0.101

A_{260}/A_{280}		A_{260}/A_{230}
2.114		0.024

Sample 3

Concentration 11.0 µg/ml

A_{230}	A_{260}	A_{280}	A_{320}
9.32	0.326	0.211	0.107

A_{260}/A_{280}		A_{260}/A_{230}
2.106		0.024

Sample 4

Concentration 10.5 µg/ml

A_{230}	A_{260}	A_{280}	A_{320}
9.30	0.303	0.192	0.094

A_{260}/A_{280}		A_{260}/A_{230}
2.133		0.023

In this example, the instrument is giving concentration values which are very close together (<2% variation). So the reproducibility for concentration values is within the expected values. However, looking at the underlying absorbance readings shows that the 230 nm reading is very high compared to other readings making the ratio A_{260}/A_{230} very low. A value < 1.8 indicates potential contamination.

Note that in this example

- The background at A_{320} nm is provided for information & must be subtracted from the A_{260} nm reading to arrive at the absorbance
- For information: Not all low volume instruments display the A_{320} nm subtraction in the calculation.

Protein applications

As with DNA, proteins absorb light at a specific wavelength, allowing direct measurement using a spectrophotometer. The amino acids Tyrosine and Tryptophan have a very specific absorption at 280 nm, allowing direct A_{280} measurement of protein concentration. Direct UV measurement at 280 nm has many advantages, since the protein solution alone is used, without the addition of reagents, and it is not modified or inactivated during the process. No incubation period is required, so measurements are quick and highly reproducible.

The chemical composition of the protein will affect the absorption: the number as well as the type of amino acids will cause variation. How much a protein absorbs at 280 nm is dependent on the amount of the amino acids Tyrosine and especially Tryptophan: the aromatic ring of Phenylalanine absorbs well at 260 nm, but not 280 nm. So proteins of similar molecular weight can have quite different absorbances, since they can have completely different Tryptophan and Tyrosine content. UV absorbance of aromatic side chains is also affected by protein structure. Therefore conditions which affect structure, such as temperature, pH, ionic strength, or the presence of detergents, can affect the ability of aromatic residues to absorb light at 280 nm, and change the value of the protein's extinction coefficient.

As with nucleic acids each protein has its own conversion factor. The common standard protein bovine serum albumin (BSA) has a factor of 1.551.

Concentration (µg/ml) = Abs$_{280}$ × Factor

The A_{260}/A_{280} ratio can be used as a guide to the purity of the sample.

Some instruments also contain factors for other common proteins such as BSA or IgG which allow users to choose the protein closest in type to their sample, if the factor for the sample of protein is unknown.
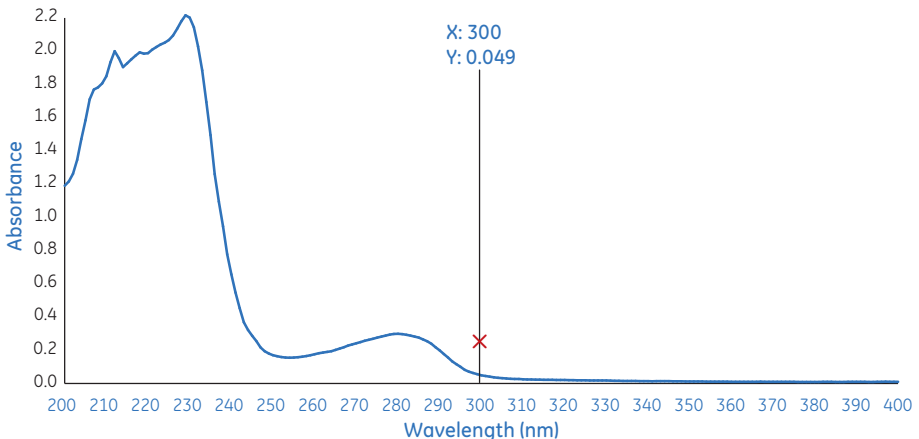

Fig 5. A typical wavelength scan for a protein sample.

Protein concentration calculations

The protein concentration, c, in mg/ml is calculated by:

$$c = A_{280}/(E_{280,1 \text{ mg/ml}}) \times l)$$

The absorbance coefficient ($E_{280,1 \text{ mg/ml}}$) corresponds to the A_{280} of a 1 mg/ml solution of the protein and varies between proteins.

$E_{280,1 \text{ mg/ml}}$ can be determined:

1. by measuring the absorbance of the protein in a solution of known concentration; or
2. by the theoretical calculation

$$E_{280, 1 \text{ mg/ml}} = (5500n_{Trp} + 1490n_{Tyr} + 125n_{S\text{-}S})/M$$

where n_{Trp}, n_{Tyr} and $n_{S\text{-}S}$ are the number of Trp and Tyr residues, $n_{S\text{-}S}$ is the number of disulfide bonds (S-S bonds) in the protein sequence, and M is the molecular weight of the protein. Coenzymes and cofactors may also contribute. Examples of values for E_{280}, 1 mg/ml include 0.67 for BSA, 1.37 for IgG, and 2.64 for lysozyme.

Light scattering correction of the A_{280} value can be made by:

$$A_{280} = A_{280} \text{ (measured)} - 1.929 \times A_{330} \text{ (measured)}$$

Christian and Warburg (3)

Nucleic acids have absorbance at 280 nm (maximum at 260 nm). If the presence of nucleic acids is suspected, the protein concentration can be estimated (with less accuracy) according to Christian, W. and Warburg, O. (3):

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

The constants 1.55 and 0.76 refer to a specific protein used by Christian and Warburg. For best accuracy, the factors should be determined for the target protein at hand. Refer to the *NanoVue™ Plus User Manual, 28-9574-75* from GE Healthcare.

Colorimetric methods

A wide variety of colorimetic methods for protein concentration measurements are available, they all work in a similar way.

The reagents contain a dye which binds specifically to the proteins in a solution. The absorption of a known set of standards is measured and a standard curve produced. Absorption measurements of unknown samples can then be compared to the curve to establish their concentration.

It is important to check the working ranges of the commercial product being used, as these can vary.

BCA (4)

This method relies on the reaction of cupric ions (Cu2+) and peptide bonds. This forms Cuprous ions (Cu+) which are then detected with bicinchoninic acid (BCA). This gives an absorbance peak maximum at 562 nm. Most commercially available kits state that this method is designed to quantify 125 to 2000 µg/ml.

Biuret (5)

Like BCA, the Biuret method takes advantage of the reaction between cupric ions and peptide bonds in alkali solution. In this case no additional development step is used. Absorption is measured at 546 nm. Most commercially available kits state that this method is designed for the quantification of 1000 to 15 000 µg/ml.

Bradford (6)

This method uses a dye, Coomassie Brilliant Blue, which binds to the protein. The protein solution shows an increase in absorbance at 595 nm which is proportional to the amount of bound dye. Bradford is very resistant to interference making it a very common method. Most commercially available kits state that the useful range of this method is 1 to 1500 µg/ml.

Lowry (7)

Lowry takes advantage of the reaction of Folin-Ciocalteu's phenol reagent with tyrosyl residues of an unknown protein. The absorption at 750 nm can then be compared to a standard curve of a known protein, normally BSA. Most commercially available kits state that this method is useful to quantify 1 to 1500 µg/ml.

2-D Quant Kit

The 2-D Quant Kit (80-6483-56) is designed to determine protein concentration in samples to be analyzed by high resolution electrophoresis techniques such as 2-D electrophoresis, SDS-PAGE or IEF. Many reagents used in preparing such samples, including detergents, reductants, chaotropes and carrier ampholytes, are incompatible with other protein assays. The procedure quantitatively precipitates proteins, leaving interfering substances in solution. The assay is based on the specific binding of copper ions to protein. Precipitated proteins are resuspended in a copper-containing solution and unbound copper is measured with a colorimetric agent. The color density is inversely related to the protein concentration. The assay is linear in the range of 0–50 µg protein. The procedure is compatible with 2% SDS, 1% DTT, 8 M urea, 2 M thiourea, 4% CHAPS, 2% Pharmalyte™ and 2% IPG Buffer. Absorbance is read at 480 nm.

See the following pages for a summary you can use for choosing your protein assay method.

Please refer to the kit manufacturer's product data sheets for the exact ranges for your specific kit.

Choosing your protein assay

A$_{280}$ Direct UV

Working Range	100 μg/ml to approx 100,000 μg/ml This range is valid for low volume spectrophotometers such as NanoVue (linearity in NanoVue: 100-100,000 μg/ml ± 100 μg/ml [based on 2*SD, 20 replicates] >10000 μg/ml ± 1% [20 replicates, %CV]) For a standard instrument working range is 0.05 to 2000 μg/ml
Advantages	Simple, direct UV measure minimizes need for dilution Suited to identifying protein on column fractions Wide working range
Disadvantages	Higher order structure in the proteins will influence the absorption Detection can be influenced by nucleic acids and other UV-absorbing contaminants, and by light scattering from particles in the sample

Bradford

Working Range 'Kits'	1 to 1500 μg/ml
Advantages	Dye-reagent stable for ~1 hour More stable than A$_{280}$ (2.5-25 micro assay)
Disadvantages	Absorbance spectra of 2 species partially overlap (bound v. unbound) Stains labware on contact Susceptible to interference by high detergent concentrations in solution Precipitates can form High protein-to-protein signal variability

Lowry

Working Range 'Kits'	1 to 1500 μg/ml
Advantages	Sensitive over wide working range Commonly referenced procedure for protein determination Reaches a stable end-point Performed at room temperature (RT)
Disadvantages	Susceptible to interference from wide range of substances Reagent time-consuming to prepare Reagent must be prepared fresh each time Photosensitive assay Amount of color varies with different proteins Precipitate can form in the presence of detergents Interference from carbohydrates and some buffers

Micro BCA

Working Range 'Kits'	1 to 40 µg/ml (standard BCA range 125 to 2000 µg/ml)
Advantages	Very sensitive Not as susceptible to interference from common buffer substances (eg. many detergents) Low protein-to-protein variability
Disadvantages	Concentrated protein samples need diluting Sensitive to interference by strong reducing agents Requires longer incubation (e.g. 1 hr) at higher temperatures (e.g. 37°C-60°C) to minimize protein-protein variation effects and improve assay sensitivity

Biuret

Working Range 'Kits'	1000 to 15 000 µg/ml
Advantages	Wide working range Single reagent, single incubation, performed at RT – easy to use
Disadvantages	Relatively low sensitivity Relatively large amounts of protein sample needed for accurate analysis Amino buffer (e.g. Tris) used in pH range 8-10 can interfere with reaction Sensitive to interference by strong reducing agents

Measuring proteins in a low volume instruments

NanoVue Plus Spectrophotometer can be used to determine the concentration of protein samples by a variety of methods including Bradford, BCA, Lowry, Biuret, and direct UV methods with a choice of line fit and the ability to run up to 27 standards (including replicates). These calibration curves can be viewed on the graphical display, printed or stored as a method for future use.

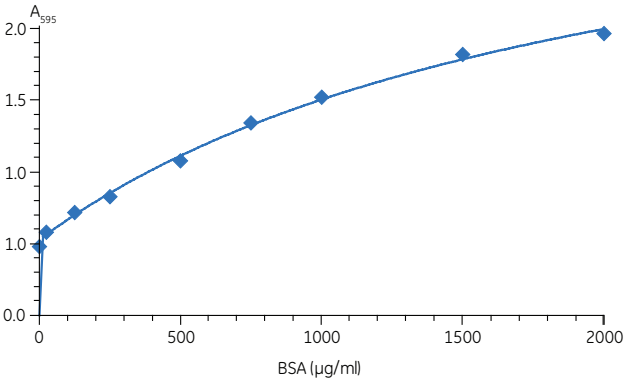**Fig 6.** Bradford protein assay curve generated from samples measured on NanoVue Plus (n = 3 replicates; Mean +/- 1SD). The sample volume was 4 µl.
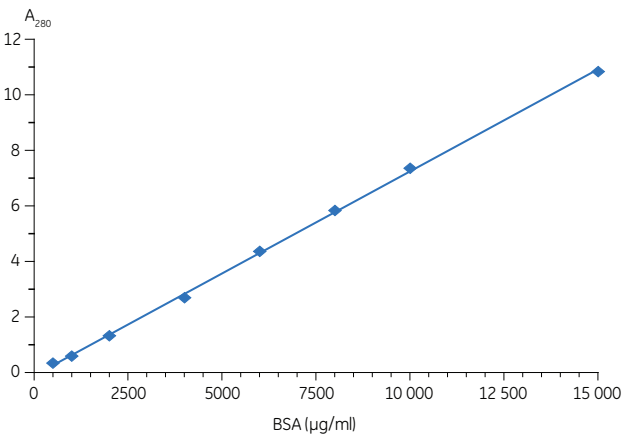

Fig 7. Protein concentration curve generated from 2 µl BSA samples measured at 280 nm using NanoVue Plus (n = 3 replicates; Mean +/- 1SD).

More information can be found in *Use of NanoVue spectrophotometer to measure protein concentrations Application Note 28-9468-37*.

Other applications

Fluorescent dyes

Many spectrophotometers come with pre-programmed methods for analysis of fluorescent dyes for DNA or protein quantification. DNA yield can be measured at 260 nm while the incorporation of fluorescein, Cy™3, Cy5 and other dyes are measured at their respective absorption peaks. This method is also useful for measuring the yields and brightness of fluorescently labeled in-situ hybridization probes.

Cell culture

Specialist cell culture spectrophotometers are available for measuring cell density, which can be used to measure the growth curve of a cultured population to allow harvesting of cells (typically bacteria) at the optimum point.

The basic method of measurement is to analyze light scatter at 600 nm: the greater the number of cells, the larger the amount of light scattered as it passes through the cuvette, and if more light is scattered, less light reaches the detector.

Not all spectrophotometers can measure bacterial cell culture at 600 nm: the instruments need to be designed to restrict the amount of scattered light that can reach the detectors. More advanced spectrophotometers also offer background correction at 800 nm and algorithms to give more consistent results.

Cell culture measurements are not quantitative but are used to gauge the current point of the cell culture process and identify the peak concentration for most effective harvesting. As the bacterial population approaches its maximum the growth rate will slow and plateau before beginning to drop off as the cells begin to die due to lack of nutrients and increase in toxic waste products from the growth process.

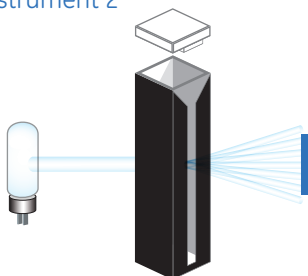The numerical results from different spectrophometer designs are not directly comparable, but the same pattern of increase, level and decrease will be seen on all instruments.

Instrument 1
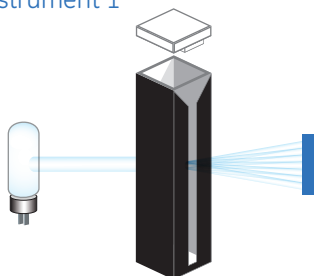
Instrument 2

All scattered light hits the detector

Not all scattered light hits detector therefore a higher absorbance reading is obtained

Fig 8. Different spectrophotometers will give different readings when such readings are related to light scatter e.g. for bacterial culture measurement. Depending on the exact set-up of an individual spectrophotometer, the light scatter, and thus the absorbance reading, are likely to be different, as seen here.

Features of the instrument design will affect the way in which scattered light can reach the detector and hence the numerical results e.g. The distance from the sample holder to the detector lens.

Other molecules

As well as biomolecules such as Proteins and DNA, there are a wide range of light absorbing substances which it is important to quantify. Spectrophotometers are used in a wide range of industries from waste water analysis, to pharmaceutical quality control and food analysis. One such example is the grading of olive oil.

The European regulations ECC/2568/91 and ECC/2472/97 set out the characteristics for grading of olive oil (extra virgin, virgin etc.) One section looks at the absorption values for 232 nm and 270 nm: this is a measurement of oxidation. To be classed as extra virgin olive oil A_{232} has to be <2.5 and A_{270} <0.22. For virgin olive oil these values are slightly higher at 2.6 and 0.25. A spectrophotometer is essential for the grading of olive oil in line with European regulations.

The absorbance spectrum for the molecule to be measured may vary from very narrow (e.g. <1 nm) to broad (e.g. >3 nm). If extreme accuracy is required, for example in the quality control of small molecules, it may therefore be necessary to alter the bandwidth of the instrument accordingly. Instruments such as the Ultrospec 9000 have variable bandwidth to enable more precise measurements in method development and small molecule measurements. (For more information, see Bandwidth in glossary).

Spectrophotometry hints and tips

Choosing a cuvette

Beam heights

The height at which the light path of the spectrophotometer hits the cuvette is known as the beam or Z height. Most common beam heights are 8.5 mm, 15 mm, and 20 mm. It is important to use cuvettes which are designed for the correct beam height, firstly to ensure they are optically clear at the point the light hits the surface, and secondly because the stated pathlength will be at this exact point in the cuvette – and may differ at other heights on the cell. Some manufacturers offer packing pieces to allow the use of cuvettes designed for lower beam heights on their systems.

Choice of cuvettes

The first decision when choosing a cuvette type is whether you wish to use disposable cells or cuvettes designed to be cleaned and reused. Disposable cuvettes remove the need for a cleaning process within the laboratory, removing the chance of carry over, but they add an on-going cost to running the analyzer, have higher native absorbencies and may not be produced to the same precise standards as reusable items.

Reusable cuvettes are much more expensive than their disposable counterparts, and when using a dual beam spectrophotometer the cuvettes need to be matched to ensure accurate blank readings.

The second decision is the choice of cuvette material. Cuvettes are generally split into use for visible only and UV/vis. Normal plastics and glass naturally absorb UV light, so a glass cuvette would not be suitable for DNA analyses but would be fine for cell culture measurements. UV cuvettes are more expensive than their visible alternatives as they need to be made from specialist plastics or quartz glass.

Buffer Compatibility

There are many commonly used processes and methods in use for biological sample preparation and purification and these often require the use or a detergent or buffer.

Many buffers and detergents absorb light themselves, interfering with the spectrophotometric analysis of the sample. It is important to take this into account when designing the sample preparation phase and choose a non-absorbing buffer or one which will not interfere with the sample analysis.

Studies have found that some detergents have more of an effect on direct UV results than others. Using detergents such at Brij 35, CHAPS and Tween 20 may give a better results than Igepal or Triton x-100, but this will vary greatly on the concentration of detergent present in the sample.

Low volume spectrophotometers

Choice of volume

For low volume spectrophotometers, the pathlength is generally much smaller than for cuvette-based instruments (e.g. 0.1 mm to 1 mm), and may rely on the sample itself to form the "cuvette". Therefore contact with both the upper and lower sample plates/heads is critical. For best results when using volumes below 2 µl with NanoVue, select the 0.2 mm pathlength. For volumes of 2 µl or above select the 0.5 mm pathlength.

Background correction

If your low volume instrument has the possibility to apply background correction, as with NanoVue, it should always always be switched on because it can:

- Correct for drop misplacement
- Correct for sample background value of sample. eg. DNA should have zero absorbance at 320 nm

Any reading at 320 nm can indicate a contaminant or faulty drop placement.

As a rough guide, the expected 320 nm value should be approximately $\frac{1}{10}^{th}$ of the 260 nm reading.

Background correction is very important when using low volume spectrophotometers. A reading is taken at a point in the spectrum away from the wavelength of interest and this reading is subtracted from the main result. This removes the likelihood of any interference.

Sources of interference may include:

- Poor drop placement: This causes incomplete light transition though the sample. If the light path intersects with the end of the sample, light is refracted, preventing it from reaching the return optics, so it could be counted as absorption.
- Air bubbles in sample: As with poor drop placement, air drops cause refraction of the light from its normal path, which can in turn cause some unabsorbed light not to reach the detector and thus increasing apparent absorption.
- Particles in sample: Particle contamination in a sample causes light scatter, preventing unabsorbed light reaching the detector. Again this will increase the apparent absorption.

Which spectrophotometer?

Some general features and questions to consider when choosing your next instrument are:

How does your data need to be recorded or stored? Consider whether standard PC software is suitable or whether electronic records and audit trails are required.

How many users will there be, and with what level of experience? Pre-programmed methods and password control of your own methods may be useful.

What range of sample volumes do you need to measure?

What level of sensitivity do you need?

Do you want to measure multiple samples at the same time?

What applications are routinely run in your laboratory? Could this change in the near future?

Do you need Pharmacopoeia compatibility?

Consideration of system specification is a fine balancing act between bandwidth, absorbance range, system noise and cost. Ideally, this would be a narrow bandwidth instrument with a wide absorbance range, low noise and low cost. However, reducing the bandwidth reduces the available light, in turn reducing the absorbance range available. To overcome this one could use a more powerful light source and/or more sensitive detectors, which will increase system noise, increasing the lower limit of detection. Higher specification electrical systems and detectors will help reduce noise – but generally at a much higher cost than most laboratories can afford.

GE Healthcare has a range of spectrophotometers for a broad range of scientific needs.

Spectrophotometer range

Ultrospec™ 10 for cells

Novaspec™ basic vis absorption/ transmission/kinetics

Ultrospec 9000 variable band-width, advanced applications and method development

Ultrospec 8000 Pharmacopoeia compatible, dual-beam analysis

GeneQuant™ flexible for DNA, RNA, protein, cultures

Ultrospec 7000 economical dual-beam functionality

Ultrospec 2100 lab standard, DNA/RNA, protein, kinetics - up to 8 samples

NanoVue™ micro-volume: drop-measure-done simplicity

Spectrophotometer selection guide

Model	Main features	DNA/RNA quantitation	PCR/sequencing/hybridization Primer quantitation and design	Protein determination (Bradford, Biuret, BCA etc)	Cell culture OD$_{600}$	Enzyme activity kinetics at 340 nm	Fluorescent probe quantitation, cDNA probes for microarrays, PCR probes	Pharma method development
Ultrospec 9000	High-performance dual-beam, variable bandwidth UV-Visible spectrophotometer. Wavelength range 190-1100 nm. European Pharmacopeia compatible. Optional Life Science or CFR Datrys software upgrade available.	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended
Ultrospec 8000	High-performance dual-beam, 1 nm bandwidth UV-Visible spectrophotometer. Wavelength range 190-1100 nm. European Pharmacopeia compatible. Optional Life Science or CFR Datrys software upgrade available.	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended	Not suitable
Ultrospec 7000	High-performance dual-beam, 2 nm bandwidth UV-Visible spectrophotometer. Wavelength range 190-1100 nm. Optional Life Science or CFR Datrys software upgrade available.	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended	Not suitable
Ultrospec 2100 *pro*	3 nm bandwidth instrument, wavelength range 190 to 900 nm with 8-position cell changer and options for temperature control.	Recommended	Suitable	Recommended	Recommended	Suitable	Suitable	Not suitable
NanoVue Plus	Novel sample plate. No cuvettes needed. PC control now available. Low-volume measurements (0.5 to 5 µl).	Recommended	Recommended	Recommended	Suitable	Not suitable	Recommended	Not suitable
GeneQuant 1300	As GeneQuant 100, with built-in enzyme kinetics, and CyDye™ applications plus Bluetooth™ data output options.	Recommended	Recommended	Recommended	Suitable	Recommended	Recommended	Not suitable
GeneQuant 100	5 nm bandwidth instrument with built in applications for nucleic acids, proteins and cell density.	Recommended	Suitable	Recommended	Recommended	Not suitable	Suitable	Not suitable
Novaspec III	7 nm bandwidth visible spectrophotometer, 330 to 800 nm.	Not suitable	Not suitable	Not suitable	Not suitable	Recommended	Not suitable	Not suitable
Novaspec Plus	As Novaspec III, with stored protein methods, easy to read graphical display, and temperature control option.	Not suitable	Not suitable	Recommended	Recommended	Recommended	Not suitable	Not suitable
Ultrospec 10	Battery-powered, portable cell density meter.	Not suitable	Not suitable	Not suitable	Recommended	Not suitable	Not suitable	Not suitable

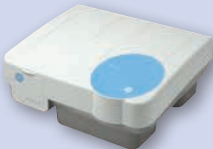Key: ■ Recommended   ■ Suitable   □ Not suitable
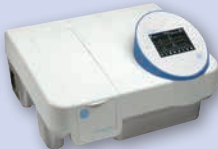
GE Healthcare spectrophotometer range

NanoVue Plus	GeneQuant 1300	Novaspec III & Plus	Ultrospec 10
Small and simple UV/Visible spectrophotometer for measuring small volumes between 0.5 and 5 µl Pre-stored methods for DNA and proteins, no cuvettes required Just Drop, Measure, Done	Good all round UV/Visible instrument with pre-stored methods for DNA and protein analysis	Small and simple protein measurement	Portable battery operated cell density meter for bacterial cell culture, measuring OD_{600}
For most molecular biology and biochemistry labs	For research and teaching labs	Ideal for teaching labs and some research labs	For cell culture labs
Versatile UV/Visible spectro that quickly and accurately quantifies nucleic acids and protein samples, preferably within seconds. If a PC-piloted instrument is needed, Datrys™ Life Science software is available.	Flexible, economical solution, multivolume, perhaps multiple instruments, UV needed for DNA/RNA measures. GeneQuant does most applications and capillary cell can go down to 3 µl volumes	Economical solution, perhaps multiple instruments, where UV is not needed. Note: Novaspec does not do UV, so if UV is needed (if measuring DNA or RNA), prefer GeneQuant.	A simple solution for measuring bacteria cell cultures, avoiding contamination

Datrys PC control software

An external PC with appropriate software allows the ultimate in flexibility to control and manipulate spectrophotometry data. Whether looking for small differences in multiple spectral overlays, or carrying out post-run manipulations on large numbers of samples, Datrys software from GE Healthcare has the flexibility to work in the way you want. Available in four options: Datrys Lite, Datrys Standard, Datrys Life Science and Datrys CFR and compatible with Windows™ XP, Windows Vista™ and Windows 7 and 8 operating systems. Data export options include Microsoft Word™ and Excel™ plus Adobe® PDF formats.

Datrys software allows PC control of Ultrospec 7000, 8000 and 9000, and also PC control a number of other GE Healthcare spectrophotometers including the NanoVue and GeneQuant.

Ultrospec 2100	*NEW* Ultrospec 7000	*NEW* Ultrospec 8000	*NEW* Ultrospec 9000
UV/Visible 3 nm bandwidth instrument, wavelength range 190 to 900 nm, with an 8-position cell changer and options for temperature control	Entry level dual-beam instrument, with a xenon light source, Touchscreen, USB data storage and PC control option, 2 nm bandwidth	Dual beam, Deuterium tungsten light sources, Touchscreen, USB data storage and PC control, Pharmacopeia compatible, 1 nm bandwidth	Dual beam, Deuterium tungsten light sources, Touchscreen, USB data storage and PC control, Pharmacopeia compatible, Advanced method development, variable bandwidth (0.5, 1, 2 and 4 nm)
For research labs, core facilities	For some pharma, some research labs, core facilities	For pharma, some research labs	For pharma, high specification labs
Diverse applications UV/Visible Flexibility for measuring multiple samples, including for temperature-controlled enzyme kinetics	Multiple applications, dual beam instrument	Multiple applications, extreme accuracy, Pharmacopeia compatibility	Method development, extreme accuracy, Pharmacopeia compatibility

Accessories overview

Different accessories are available to expand the capabilities of GE Healthcare spectrophotometers. These include multiple cell changers, temperature-regulated cells, sipper system for automated sample aspiration, test tube holders etc.

A range of cuvettes are available, from capillaries (>3 µl working volume), ultra-microcells (7 µl volume) to standard quartz cells (up to 2500 µl).

The full range of accessories can be found at www.gelifesciences.com/spectros

Glossary

Absorption

Absorption is the amount of light that a substance takes in and does not allow to pass through it. Spectrophotometers actually measure transmission, the amount of light that passes through a sample, but this is converted into absorption by comparing the bulb output to the light that has passed through the sample.

Bandwidth

Bandwidth describes the property of the light emitted from the monochromator. When the light is analyzed, the peak may be at its maximum at the specified wavelength, but light is also emitted to a lesser extent at the wavelengths either side of the one selected. The Spectral Bandwidth is a measurement of the width of a peak at half-light intensity (Fig 9).
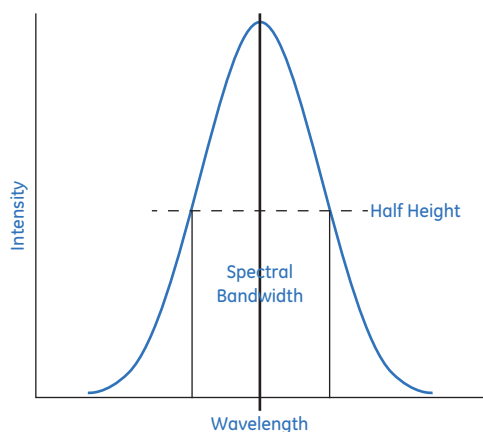
Fig 9. The Bandwidth is a measurement of the width of a peak at half-light intensity.

To obtain maximum sensitivity, the bandwidth of the instrument would cover the entire bandwidth of the substance being measured. When reducing bandwidth, the amount of available light for absorption is also reduced, so more sensitive detectors are needed to obtain the same working range.

Increasing bandwidth reduces resolution, and measurement at too broad a bandwidth could also possibly merge two absorption peaks into one larger one, giving an artificially high reading.

Low bandwidth instruments are very important in highly regulated environments such as pharmaceutical and Pharmacopoeia laboratories where resolution of 1 nm is often required, whereas a broader bandwidth is helpful for sensitive DNA quantification where absorption is strong, and only one, broad, 5 nm peak is being measured.

Beam technology

Single beam

Single beam refers to the fact that the light just passes through the sample holder straight to the detector. An initial reference is required to standardize the instrument before analysis can begin. Single beam instruments are generally very simple and economical to purchase. Examples of such instruments are Novaspec III & Plus.

Split Beam

The light from the source is split into two paths, with approximately 30% of the energy being diverted from the main path into a feedback detector. 70% is then passed through a monochromator, through the sample compartment to a detector. The feedback detector is used to correct variations in the energy emitted by the lamp. A reference sample is required at the start of each run to correct for cuvette and solvent absorption.

Dual Beam

Sometimes known as double beam technology. The light is split into two paths, each of which passes through a cell holder onto its own detector. One cell holder is for the sample and the second is for a reference. The reference should contain the same cuvette type and solvent as the sample. The absorption of the reference path is subtracted from that of the sample path. Every measurement has its own reference, giving more reliable results. GE Healthcare Ultrospec 7000, 8000 and 9000 are all Dual beam instruments.

Light source

Deuterium

Deuterum arc lamps provide powerful and stable light in the UV region, 190-370 nm (Fig 10). In higher specification spectrophotometers a special design of deuterium lamp is used to maximize life without reducing output, called "press to read" (Pulse) technology. This allows the lamp to go into a low power mode, where the lamp is kept warm but is not actually on. This can increase the lamp life up to 3 times that of a conventional deuterium lamp. The deuterium lamps of the GE Healthcare Ultrospec 8000 and 9000 can be changed by the researcher in the lab. Deuterium lamps are paired with tungsten lamps to allow coverage of the full UV and visible spectrum.

Tungsten

Tungsten lamps give stable light in the visible and near infra-red areas of the spectrum, covering 320-1100 nm (Fig 10). GE Healthcare tungsten lamps also employ press to read (Pulse) technology to extend lamp life, and the lamps in the Ultrospec 8000 and 9000 can also be changed by the researcher in the lab.
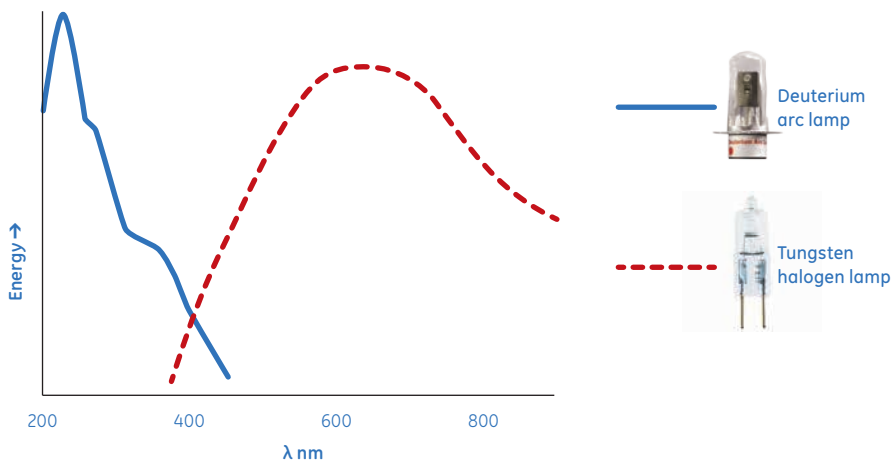
Fig 10. Both Deuterium and Tungsten lamps are required in order to cover the complete wavelength range from 190 nm to 1100 nm.

Xenon

Xenon flash lamps are a high energy light source emitting light across the UV, Visible and near IR spectrum. They are known as flash lamps as they are not on constantly but flash, up to 80 times a second. Xenon lamps have a very long life span, but don't provide the optical stability of the deuterium/tungsten set up in the higher specification systems. Xenon lamps must be changed by a service engineer. The entry-level dual beam Ultrospec 7000 instrument has a xenon lamp.

LED

Used for single wavelength applications, such as cell culture measurement in the Ultrospec 10. LEDs are stable, low cost and offer a long life.

Methods of measurement

Single wavelength

A simple method which allows measurement of transmittance (t%) or absorbance of a substance at a single fixed wavelength. E.g. 260 nm for DNA.

Wavelength scan

Measurements for a range of wavelengths are taken, allowing the system to plot absorbance against wavelength and produce a graph. Particularly useful for analysing an unknown sample. The start and finish wavelengths can be set to target the scan to a region or the available spectrum.

Kinetics

A single wavelength measurement is repeated at set intervals for a set number of times. The absorption is then plotted against time. This can be used to measure reaction progress, or for enzyme activity studies when combined with a temperature-controlled cell holder.

Quantitative analysis

A single wavelength measurement of a number of known standards can be taken, from which the instrument creates a standard curve, to which future measurements of unknown samples can be compared to establish their concentration.

Cell Density

Light scatter from bacterial cell cultures can be used as an indication of cell concentration and when plotted over time can be used to see if the culture is ready to be harvested. Measurements are taken at 600 nm.

Monochromator

In a UV/Vis spectrophotometer, a monochromator is the optical device that transmits a mechanically selected band of wavelengths of light from a wider range of wavelengths available from the light source. From the Greek *mono* (single) and *chroma* (color).

Pathlength

Pathlength is the distance through the sample, through which the light has to pass, in order to reach the detector. Standard cuvettes have a pathlength of 10 mm (1 cm). GE Healthcare offers instruments covering pathlengths from 0.2 mm all the way to 100 mm.

Stray Light

Stray light is light that reaches the detector that is of a different wavelength to that the monochromator is selecting. This can be caused by the diffraction pattern produced by the monochromator or from light leaks from the outside environment. GE Healthcare systems are designed to reduce all possible sources of stray light.

UV/Vis

The electromagnetic spectrum is split into a number of different regions by wavelength. These include X-ray (0.01-10 nm) Visible light (380-760 nm) and Microwaves (1-10cm). GE Healthcare UV/Vis spectrophotometers all measure in the UV (10-400 nm) visible (380-760 nm) and near infra-red (750-2250 nm) regions covering a range of 190-1100 nm.

Wavelength

Represented by the lower case Greek letter lambda λ. Wavelength is a measurement of the distance between successive peaks in a waveform.
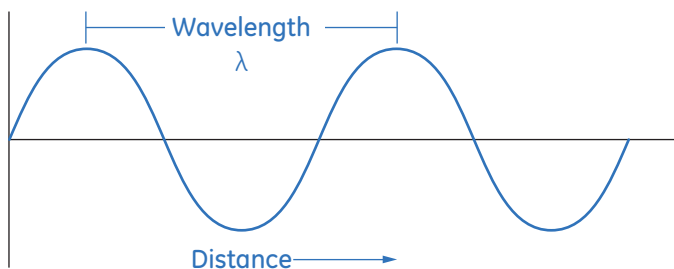
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Related products

GE Healthcare's spectrophotometer range and life sciences consumables are now available from your preferred supplier. Contact your local GE Healthcare representative for more details.

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