BioDrop™ Application Note

Using a dual beam spectrophotometer to measure the Protein concentration of low volume samples

The determination of protein concentration is one of the longest established methods in life science research and is frequently used within virtually all biochemical research laboratories. Scientists can choose from many different methods to measure their protein concentration with methods varying in the sensitivity of the procedure as well as convenience with which they can be performed. Additionally, interference by other substances present in the solution becomes an important consideration in method selection.

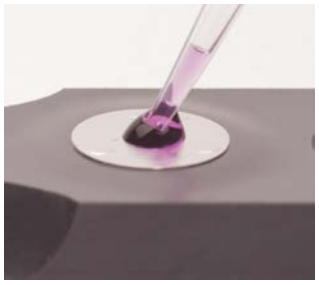
One of the most convenient methods is to measure protein concentration in purified protein samples by direct UV measurement, often referred to as an A_{280} measurement. The Absorbance of the protein solution is measured at 280nm and the protein concentration in mg/ml is calculated. The Absorbance at 280nm is due to the Absorbance of UV light by the aromatic amino acids phenylalanine, tryptophan and tyrosine and by disulphide bonds in cysteine in the protein solution. No additional reagents are required for this method and it is simple and quick for the user to carry out. However care must be taken with this method as some substances that are commonly present in protein samples, e.g. nucleic acids, surfactants and insoluble cell lysates also have an Absorbance at 280nm which can influence the result.

In this application note, we describe direct UV protein measurement using BioDrop, an innovative micro-volume cuvette. We investigate BioDrop's performance and accuracy by measuring a range of Bovine Serum Albumin (BSA) concentrations using Biochrom's S60 dual beam spectrophotometer.

Biochrom S60 dual beam spectrophotometer

BioDrop Design

BioDrop is manufactured in two precisely machined halves which are held together using magnets. When they are mounted together, they have the same dimensions as a standard cuvette so that they can be used in a standard spectrophotometer.



Light shines through the device and the optical path length of the measurement area is defined by a precisely machined spacer ring which is mounted on a thin membrane.

As the two halves are brought together, the thin membrane provides enough pressure to overcome the surface tension of the sample and ensure that it fills the sample gap but that any excess liquid is forced out. This design ensures that the actual optical path length is accurate to within a few microns. The high energy throughput achieved with this simple optical layout helps to ensure that highly accurate measurements can be made.

BioDrop is available with various path lengths. In this application note, testing was carried out on a BioDrop 500 (0.5mm path length) and BioDrop 125 (0.125mm path length).

Measurement Technique

Bovine Albumin (Sigma-Aldrich Item# A4378-1G) was measured using BioDrop and the S60 Spectrophotometer. The 0.5mm and 0.125mm path length devices were both tested in the same way.

The S60 was set to the stated path length of the BioDrop and 5 repeat measurements of each standard taken and the averaged calculated. Standards were progressively diluted and the measurement repeated for each concentration.

The measured concentration was plotted against the dilution factor.

The device was then tested for repeatability by measuring 10 replicates of 1mg/ml and 10mg/ml samples calculating the peak:peak and RMS variation between measurements. Detection limit was tested by performing a series of measurements on ultra pure water and recording the reported concentrations.

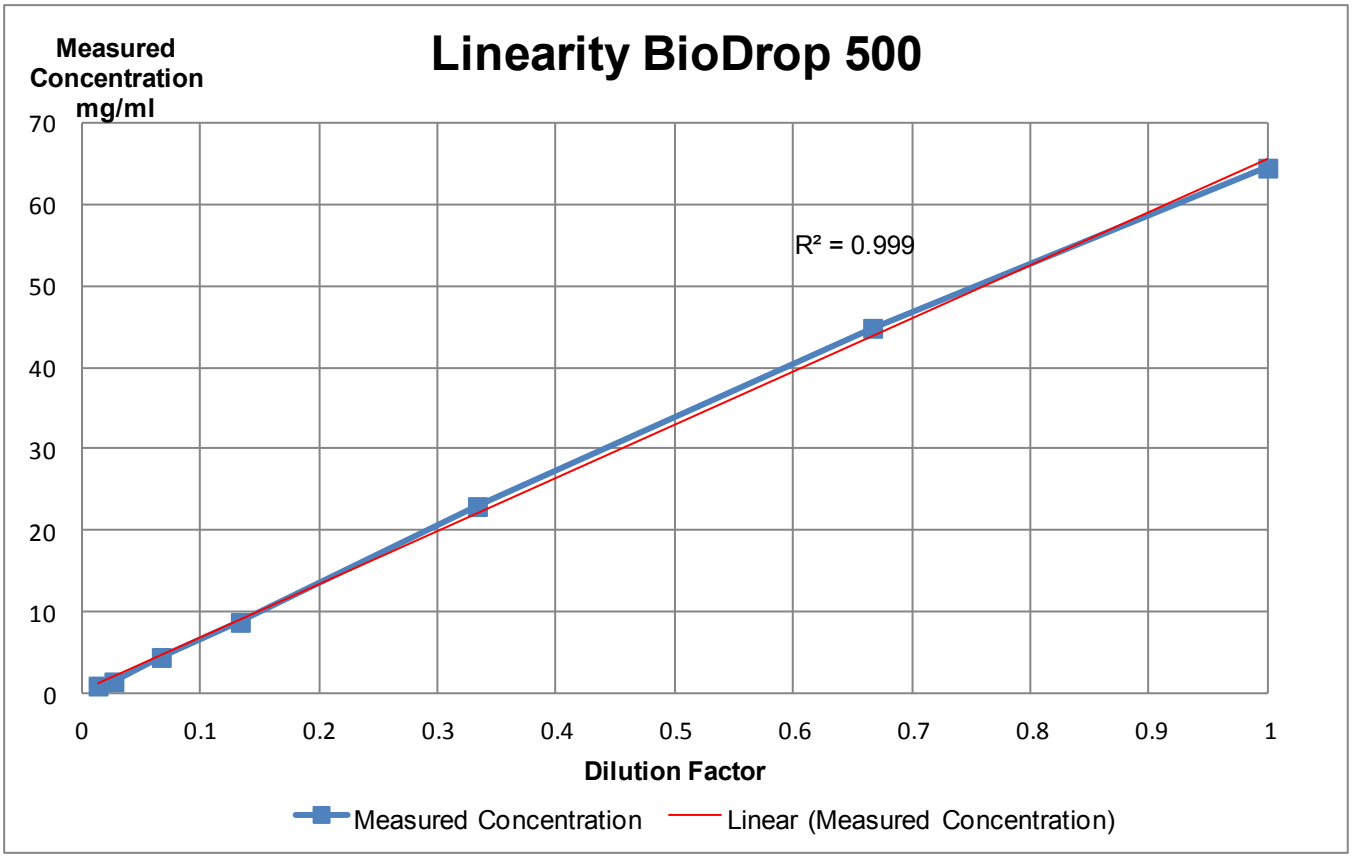
Finally sample carry over was assessed by alternating measurements of ultra pure water and concentrated protein samples to evaluate the effectiveness of cleaning between samples.

Summary

The tests completed on both BioDrop 500 and BioDrop 125 confirm their excellent measurement performance over a wide dynamic range when used with an S60 class machine. Unlike other methods, it is also likely that in many “real world” experiments, only one path length would be required. This simplifies experimental procedures considerably.

BioDrop 500 being used in an S60 Spectrophotometer

Measured Performance BioDrop 500



The graph shows the measured concentration against dilution factor. A linear least squares fit has been applied and it can be seen that the device exhibits excellent linearity with a correlation of 0.999. At higher concentrations, the performance will be increasingly dominated by the stray light performance of the instrument.

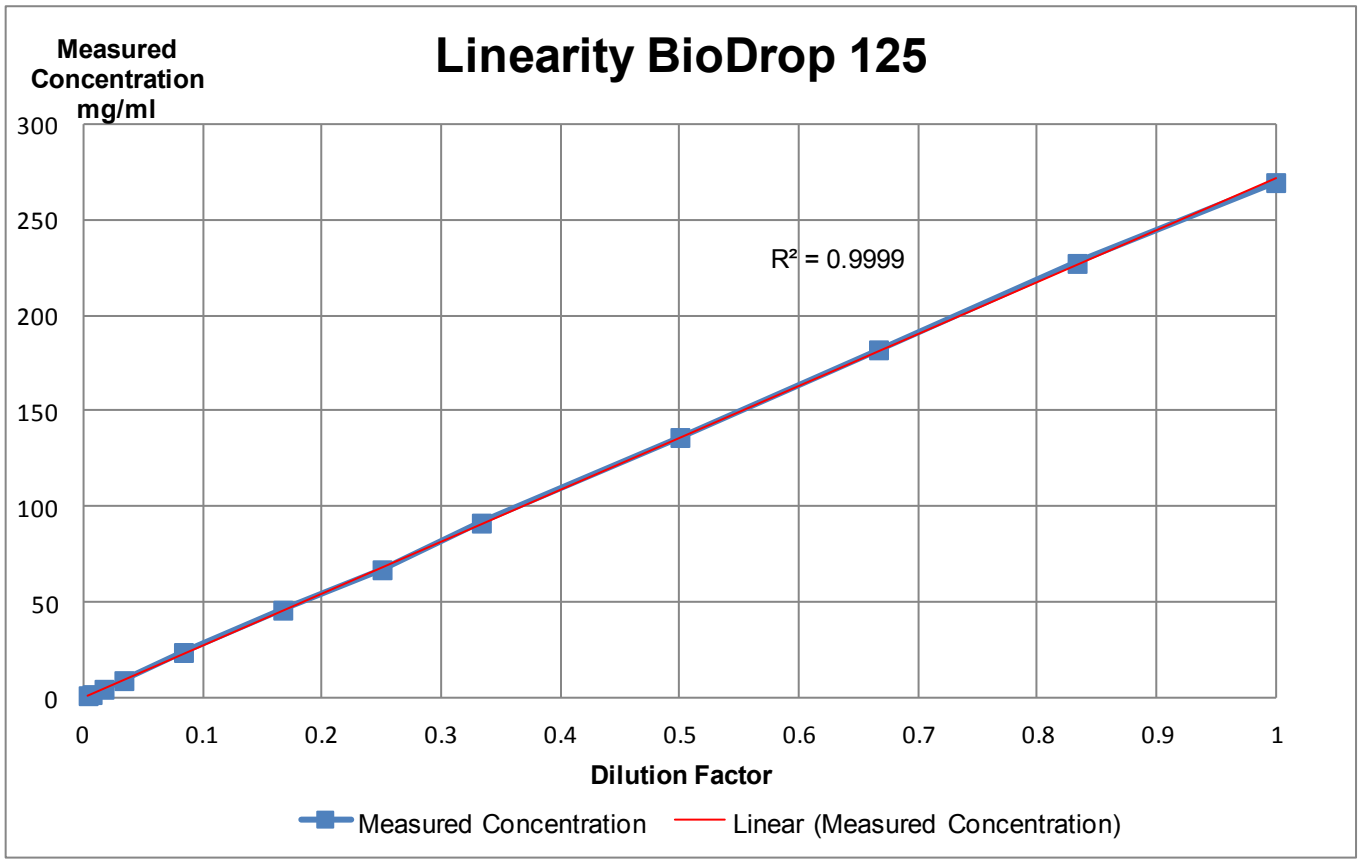
Reproducibility is shown in the table to the right.

A detection limit of 0.06 mg/ml was measured.

Carry over testing was carried out with the results being similar to the detection limit indicating that even with the simple cleaning protocol used, there is no significant carry over. For measuring highly concentrated protein samples (>75mg/ml) a wet tissues is recommended for cleaning BioDrop between samples.

Reproducibility BioDrop 500		
Concentration	**Peak to peak**	**Standard Deviation**
1mg/ml	**0.014**	**0.011**
10mg/ml	**0.132**	**0.085**

Measured Performance BioDrop 125



The graph shows the measured concentration against dilution factor. A linear least squares fit has been applied and it can be seen that the device exhibits excellent linearity with a correlation of 0.9999. At higher concentrations, the performance is increasingly dominated by the stray light performance of the instrument.

Reproducibility is shown in the table to the right.

A detection limit of 0.29 mg/ml was measured.

Carry over testing was carried out with the results being similar to the detection limit indicating that even with the simple cleaning protocol used, there is no significant carry over. For measuring highly concentrated protein samples(>75mg/ml) a wet tissues is recommended for cleaning BioDrop between samples.

Reproducibility BioDrop 125		
Concentration	**Peak to peak**	**Standard Deviation**
1mg/ml	**0.069**	**0.048**
10mg/ml	**0.166**	**0.130**

Ease of Use

Micro-volume cuvettes often require careful installation in the spectrophotometer to ensure that the light beam is correctly coupled into the device. It is common for special adapters to be used that include lenses to focus the light beam from the instrument and screw adjustments to move the cuvette into a precise position.

Because of its simple optical design, BioDrop can be removed and replaced into the spectrophotometer with little concern about exact positioning. During the repeatability testing, BioDrop was removed from the instrument between each measurement. The results obtained demonstrate that the exact positioning of BioDrop in the S60 does not significantly impact measurement results.

There is no need to use a special cell holder

Contamination

During testing, care was taken to ensure that no bubbles or dust particles were present in the samples. This is of particular concern in low volume measurements as even a tiny particle can have a major impact on the measured result.

Due to the very large optical window, it is easy to see any physical contamination in the sample. In addition, BioDrop is provided with a special viewer to allow the user to inspect the sample.

Cleaning

When making measurements on biological samples, cleanliness is of paramount importance. Contamination of a sample with the previous one will give inaccurate and misleading results. If it is planned to reuse the sample, then it can have even more serious consequences.

Cleaning BioDrop between protein measurements

Fortunately, BioDrop is very easy to clean and will normally only require a quick wipe with a lint free cloth. The carry over tests completed in this application note show that the level of contamination from one sample to the other is negligible. However, due to the robust nature of BioDrop, it is possible to use solvents and detergents to clean the device further. This is of particular importance when testing proteins which tend to be far more sticky than DNA..

BioDrop 500 in the bubble viewer

One last thing…

BioDrop is supplied in an elegant carry case that contains up to two separate path length devices, a quick start guide, the bubble viewer and a USB stick with the complete operations manual.

One of the challenges facing scientists performing low volume measurements is pipetting very small volumes accurately. Since BioDrop is magnetic, a ferrous plate can be used to stabilise it during pipetting. The supplied carry case has such a plate on its lid and this provides a convenient and stable way to secure the BioDrop when it is being loaded with the sample.

Distributors worldwide

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BioDrop's intelligent design delivers accurate, affordable low volume spectroscopy – from just 1µl