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Low-volume protein measurements (280nm): validating the LVis Plate over many concentrations

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- **Different instrumentation was used to measure UV protein absorbance at 280 nm**
- **Low and high range protein concentrations were successfully measured in 2 µl with the LVis plate on a microplate reader from BMG LABTECH**

Introduction

Measurement at the working concentration of an experiment in a high throughput manner is useful especially when dilution of the protein may affect the solubility characteristics. This would also be advantageous where accurate protein concentration is required on numerous samples (for example, column elution fractions), where dilution would be time consuming and potentially introduce pipetting errors. This coupled with the ability of being able to analyse the samples at low volume would also offer an added advantage when quantities of sample are limited.

The LVis Plate is a proprietary absorbance microplate from BMG LABTECH and is perfectly suited for low volume protein quantitation. It consists of sixteen microdrop well sites for 2 µL samples and adheres to the 96-well microplate format definition. The microdrop well sites are also easily accessible to wipe clean for further measurements.

The aim was to demonstrate that the LVis Plate was able to measure protein concentration at two different concentration ranges (0.2 to 18 mg/mL) reliably as compared to alternative methods, a UV/Vis spectrophotometer (manual cuvette sampling), and an alternative high throughput technique (NanoDrop).

The LVis Plate was used with a microplate reader (BMG LABTECH), which has an ultra-fast UV/Vis spectrometer. This technology captures an entire spectrum (220 - 1000 nm) in <1 sec/well. The LVis Plate is compatible with the SPECTROstar® *Nano*, Omega series, PHERAstar® *FS*, and CLARIOstar® microplate readers from BMG LABTECH.

Materials & Methods

- Sample Protein Stock Solution 30 mg/mL in 20 mM sodium acetate, 100 mM sodium chloride, pH 5.0
- Working buffer: 50 mM sodium acetate, 100 mM sodium chloride, pH 5.0 [filtered (0.22µm)]
- LVis Plate (BMG LABTECH)
- Cary Bio 50, UV/Vis Spectrophotometer
- NanoDrop, Thermo Scientific, UK

Experiment 1

To compare the reproducibility at a low concentration range (up to 1 mg/mL). The sample protein is a full length monoclonal antibody.

Serial dilutions of the protein stock solution were made ranging from 0.2 mg/mL to 1.0 mg/mL in buffer (detailed above). The absorbance at 280 nm was measured using the LVis plate (BMG LABTECH) and compared with results obtained from alternative spectrophotometers, a UV/Vis Spectrophotometer and the NanoDrop. Measurements were made in triplicate for each method.

Aliquots of 2 µL were used for the LVis Plate and the NanoDrop, whereas 100 µL was used for the UV/Vis spectrophotometer. In each case the samples were blanked against the buffer (detailed above).

The mean and the standard deviation of the absorbance readings at 280 nm at each concentration for each method was derived and then plotted against nominal concentration (Figures 1 - 4).

Experiment 2

To compare reproducibility at a higher concentration range (up to 18 mg/mL).

This experiment only compared the LVis Plate and NanoDrop since the linearity of the UV/Vis spectrophotometer is known to be unreliable at the higher concentration range and hence would not provide a fair test. Dilutions of the protein stock were made from 18 mg/mL to 1 mg/mL in buffer (detailed above). Triplicate measurements were made and the data plotted as in Experiment 1 (Figure 5).

Results & Discussion

Experiment 1

At concentrations ranging from 0.2 mg/mL and 1.0 mg/mL, the mean absorbance at 280nm (OD) was plotted against the concentration and the linear regression fit was calculated.

A high R squared value (R^2) in excess of 0.99 was obtained for the UV/Vis Spectrophotometer (Figure 1), LVis Plate (Figure 2) and the NanoDrop (Figure 3) .

An overlay of the data obtained from each measurement technique (Figure 4) showed that there was good correlation between the three methods.

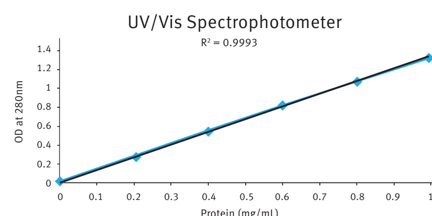

Fig. 1: Results obtained through Cary 50 Bio Spectrophotometer.

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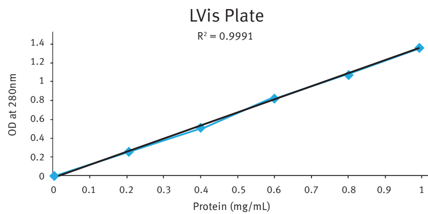

Fig. 2: Result obtained from LVis Plate from BMG LABTECH, UK.

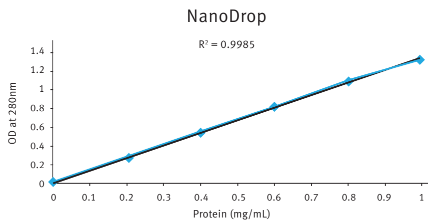

Fig. 3: Results obtained from NanoDrop from Thermo Scientific, UK.

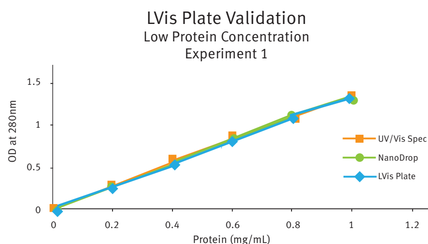

Fig. 4: Overlapped profiles of all three measurements.

Experiment 2

This experiment was performed to check the reliability of the LVis Plate to measure the absorbance at higher concentrations (up to 18 mg/mL) compared with the Nano Drop only. The mean absorbance values at different concentrations were plotted against the back-calculated concentration to show linearity.
The R squared values (R^2) were in excess of 0.99 for both the LVis Plate and NanoDrop and good correlation was observed between the two methods as illustrated by the overlaid results (Figure 5).

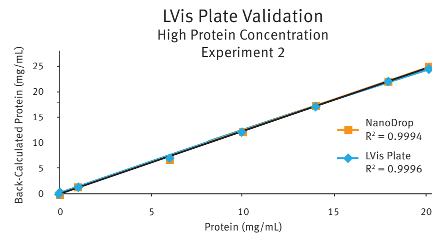

Fig. 5: Overlapped profiles of results obtained from LVis Plate and NanoDrop.

Conclusion

All three instruments for the measurement of concentrations up to 1 mg/mL showed equivalent results. At concentrations up to 18 mg/mL, the LVis Plate and the Nano Drop (an alternative high throughput method) showed comparable results.
Overall, the LVis Plate proved to be a reliable, low volume high throughput method for measurement of protein concentrations from 0.2 mg/mL to 18 mg/mL. This was validated against existing alternative spectrophotometric techniques.