

## High sensitivity DNA and protein analysis with Thermo Scientific Multiskan Spectrum and Thermo Scientific Microtiter UV Microplates

**This paper shows how Multiskan Spectrum® microplate spectrophotometer together with Microtiter® UV Microplates can be used for DNA and protein analysis with excellent sensitivity. The detection limit for DNA quantitation in 384-well UV Microplates is 60 ng/ml and spectral analysis of DNA in 96-well UV Microplates can be performed with samples clearly smaller than 2 µg of DNA. For BSA quantitation in 96-well UV Microplates, assay detection limit is 7 µg/ml using results as such, but can be improved to 4 µg/ml by using pathlength correction function of the instrument. The stable and low background of both instrument and microplates enables to measure small sample concentrations with low volumes and so to save valuable sample material.**

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### Introduction

Nucleic acid and protein quantitation with high sensitivity is needed in a wide variety of biological applications. Nucleic acid concentration is commonly measured by determining UV absorbance at 260 nm. The absorption of nucleic acid bases adenine and guanine have an absorbance maximum slightly below 260 nm and thymine and cytosine slightly above 260 nm. Therefore absorbance peak of nucleic acids depends on the base composition. For concentration determination of protein solution, UV absorbance at 280 nm is measured. The absorption of proteins at 280 nm is mainly due to the aromatic amino acids tyrosine and tryptophan and disulfide bonds of cystine (1). The relationship of absorbance to nucleic acid or protein concentration is linear, following the Lambert-Beer law:  $A = \epsilon \times C \times l$ ,

where  $A$  = the absorbance,  $\epsilon$  = the solution's extinction coefficient and  $l$  = the pathlength of light. Absorbance measurements are fast and convenient, since no additional reagents or incubations are required. This measurement type has also an additional benefit that the sample is not consumed and may be used for further analysis. In this application note we present examples of DNA and protein measurements with Multiskan Spectrum UV/Vis spectrophotometer and Microtiter UV Microplates.

### Materials and methods

Quantitative DNA assay was performed in 384-well UV Microtiter Microplates (cat no. 8405) using purified DNA from calf thymus (Sigma D-3664). DNA was dissolved in distilled water and diluted to calibrator concentration series between 1.25 and 100 µg/ml. Measurement of DNA spectra were done with Thermo Scientific 96-well UV Microtiter Microplates (cat. no. 8404) using DNA concen-

trations 1.25 - 10 µg/ml.

Quantitative protein assay was performed in 96-well UV Microtiter Microplates. Protein solutions with concentrations between 25 and 1000 µg/ml were prepared from bovine serum albumin (BSA, Sigma A-7030) in distilled water.

DNA or protein calibrators and water blanks were added into the microplate using four replicates and using 50 µl and 200 µl well volume for 96-well and 384-well microplates respectively. All absorbance measurements were performed with Thermo Scientific Multiskan Spectrum (cat. no. 51118650).

## Results

A DNA standard curve for concentrations between 1.25 and 100 µg/ml is shown in Figure 1. The standard curve is linear at a broad range of concentrations enabling DNA quantitation of small sample volumes at low and high concentrations. The detection limit for DNA quantitation calculated according to the IUPAC standard 3SD method is 60 ng/ml indicating the sensitivity of this measurement.

Absorbance spectra of different DNA solutions are shown in Figure 2. The figure shows clearly that spectra can be measured from samples having clearly less than 2 µg of DNA. Figure 3 shows BSA standard curves for concentrations between 25 and 1000 µg/ml. This protein assay shows the same broad linear concentration range as the DNA assay above. Multiskan Spectrum SkanIt software has a possibility to correct for the differences in the light pathlength, making the results obtained with microplates directly comparable with the data from cuvette measurements. Without this correction the detection limit for BSA quantitation according to the IUPAC standard 3SD method is 7 µg/ml and using the pathlength correction the detection limit is 4 µg/ml. The pathlength correction function takes the differences in liquid levels into account and so minimizes the effect of pipetting variations. This explains the small improvement in the detection limit.

## References

1. Pace, C.N. et al. 1995. How to measure and predict the molar absorption coefficient of a protein. *Protein Sci* 4, 2411-2423.

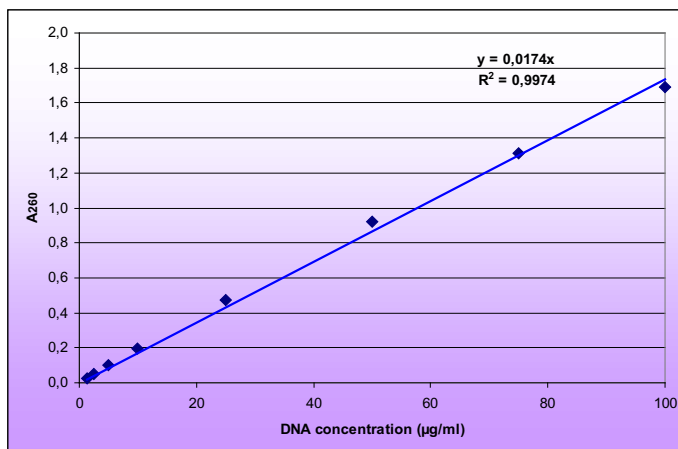


Figure 1. DNA standard curve in 384-well UV transparent Microplates.

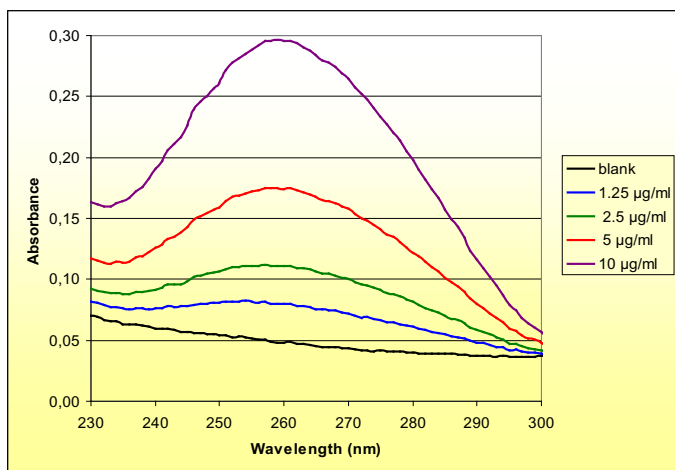


Figure 2. UV spectra of pure DNA at different concentrations.

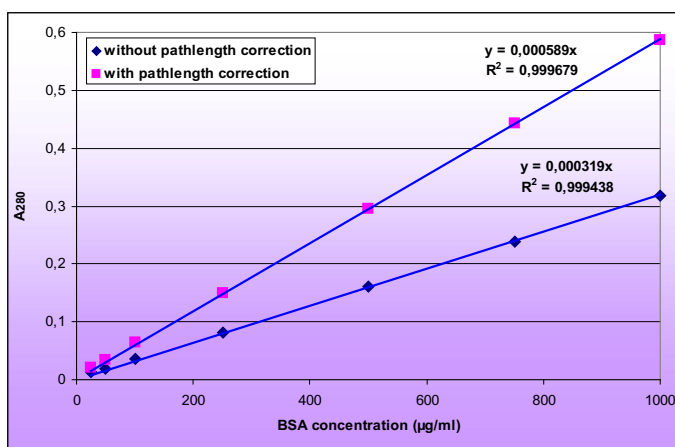


Figure 3. BSA standard curves in 96-well UV transparent Microplates. The graph shows the results as such and after applying pathlength correction.

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