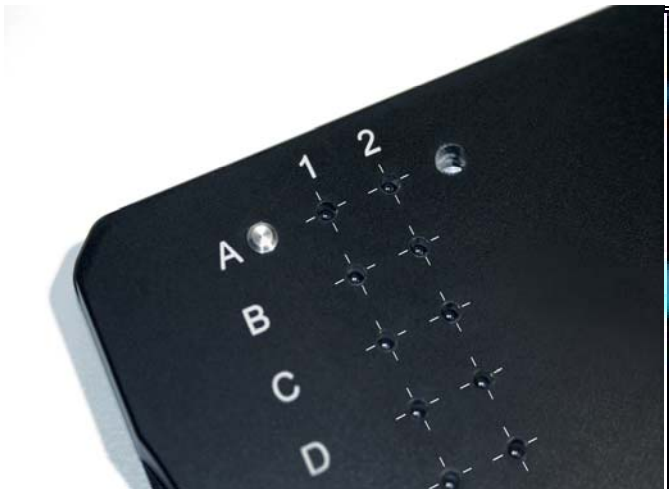
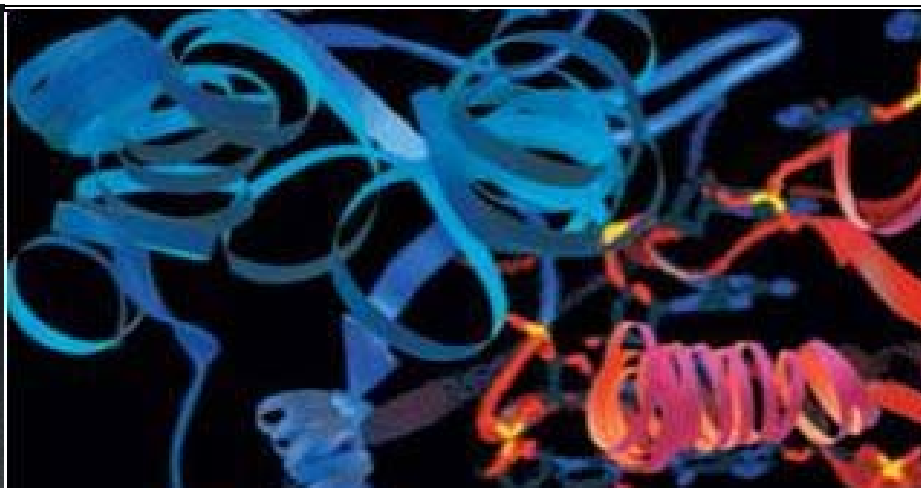
Protein Quantification in Small-Volume Samples

Absorbance-Based Protein Detection on Infinite® 200 NanoQuant





Introduction

Quantitative analyses of nucleic acids and proteins have been greatly facilitated by the availability of new tools that require only very small amounts of sample for accurate, reliable and reproducible quantification.

Among the devices suitable for measurements of small-volume samples Tecan's Infinite® 200 NanoQuant in combination with the NanoQuant Plate™ offers uncompromised performance for absorbance-based nucleic acid quantification and assessment of labeling efficiency and, in addition, can be upgraded at individual convenience with fluorescence and luminescence reading functions.
Tecan´s NanoQuant detection technology has been developed to facilitate the quantification of biomolecules in small-volume samples.

The present note describes the implementation of the NanoQuant Plate for protein measurements with regard to essential assay parameters such as linearity, uniformity, and reproducibility.

Assay principle

Absorbance-based quantification of proteins is common to many applications in basic science and clinical research. Proteins absorb ultraviolet light at 280 nm. This attribute may be utilized as a basis for fast and convenient photometric quantification of protein samples with unknown concentration. In contrast to nucleic acids which can be directly quantified by converting absorbance to concentration using extinction coefficients, the UV absorbance of protein samples is a function of the amino acid content of the analyzed protein.

Especially the fraction of tyrosin and tryptophan and, to a lesser extent, phenylalanine, strongly influences the absorbance at 280 nm by proteins. As a result, there may be significant protein-to-protein variations, and each purified protein would require a different extinction coefficient. Although there is a proportional relation between absorbance and concentration for a given protein dissolved in a given substance, the Lambert-Beer law is not accurate enough to be utilized over a broad concentration range with any type of protein sample. Thus, it is essential to include dilutions of standard proteins in protein quantifications with the NanoQuant Plate in order to be able to extrapolate the concentration of unknown samples.

Material and Methods

Instrument and plates

- Infinite M200 NanoQuant Quad4 monochromators™ microplate reader (Tecan Austria)
- NanoQuant Plate with 16 individual sample positions with integrated quartz optics (Tecan Austria)

Reagents and additional materials

- BSA, bovine serum albumin (BSA; 50 mg/ml)
- ddH_2O
- 70% ethanol
- laboratory paper towels

Dilution series of standard protein

BSA which was used as a standard protein was serially diluted 1+1 in ddH_2O as summarized in table 1:

Dilution	final conc. [mg/ml]	pipetted
A	50	200 µl undiluted stock
B	25	100 µl A + 100 µl H_2O
C	12.5	100 µl B + 100 µl H_2O
D	6.25	100 µl C + 100 µl H_2O
E	3.13	100 µl D + 100 µl H_2O
F	1.56	100 µl E + 100 µl H_2O
G	0.78	100 µl F + 100 µl H_2O
H	0.39	100 µl G + 100 µl H_2O
I	0.20	100 µl H + 100 µl H_2O
J	0.10	100 µl I + 100 µl H_2O
K	0 (blank)	100 µl H_2O

Table 1: Dilution series of BSA

Measurement parameters and settings

For all measurements, the NanoQuant Plate was used in standard i-control™ mode. All measurements were performed with two replicates; blanking was done with ddH_2O.
Prior to all measurements, the NanoQuant Plate was cleaned according to the corresponding Quick Guide instructions [1], using an ultrasonic bath and high-pressure compressed air.
A measurement script was set up in i-control V1.5 according to the parameters listed in table 2 with a measurement wavelength at 280 nm and a reference wavelength at 310 nm for correction.

Measurements on Infinite M200	
Parameter	**Setting**
Plate	Tecan 16 Flat Black [NanoQuant Plate]
Part of the plate	A1-H2
Well	
Absorbance	280 nm
Bandwidth	5 nm
Flashes	25
Absorbance	310 nm
Bandwidth	5 nm
Flashes	25

Table 2: Measurement parameters and instrument settings on Infinite M200 NanoQuant with Tecan i-control™ software

Results

The NanoQuant Plate was tested for its ability to measure the protein content of small-volume samples. Bovine serum albumin (BSA) was used as a standard protein to assess the measurement performance of the NanoQuant Plate in terms of linearity, uniformity and reproducibility.

Calculations

All results were evaluated using the following calculations.

Each OD_{280} value, including the blank values, was corrected by the corresponding OD value at 310 nm:

$$OD_{280\_corr} = OD_{280} - OD_{310}$$

All protein-containing samples were additionally corrected for the 310 nm-corrected OD values of the blanks:

$$OD_{280\_blanked} = OD_{280\_corr} - average\ blank$$

Average results, standard deviations and coefficients of variation (CV) were calculated using Microsoft Excel.

Linearity

Blank- and 310 nm-corrected OD_{280} values were plotted in a line diagram and a trend line was added. Slope and coefficient of determination (R^2) were calculated.
Figure 1 shows good linearity values for BSA dilutions ranging from 50 to 0.1 mg/ml, resulting in an R^2 value of 0.9976 (*see figure 1*).

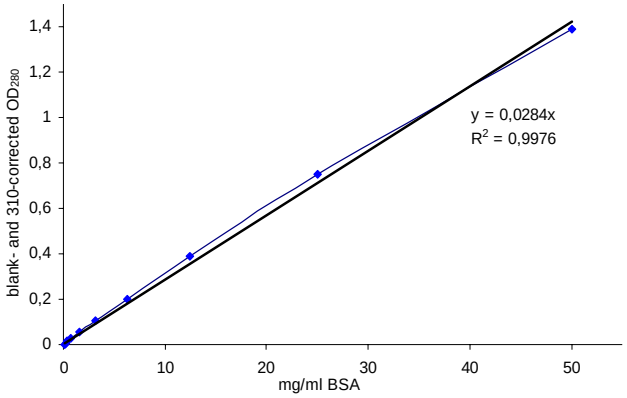


Figure 1: Absorbance linearity of standard dilution series (50 – 0.1 mg/ml BSA) at 280 nm after blank- and 310 nm-correction.

In addition, a set of protein samples was prepared and the resulting OD_{280} values were corrected for blanks and 310 nm absorbance in the same way as the standards used for the dilution series. The effective concentrations (x) were determined by inserting the measured OD values (y) into the ascending slope formula of the standard dilutions (*figure 1*).

$$effective\ sample\ concentration = \frac{OD_{280\_blanked}}{F}$$

Note: The factor *F* for calculation of the protein concentration depends on the kind of protein, the solvent and standards used.

The results as well as the deviations to the originally pipetted concentrations are summarized in table 3.

Sample	$OD_{280\_blanked}$	effective [c] [mg/ml]	theoretical [c] [mg/ml]	% deviation
1	1,3910	48,98	50	2,04
2	0,8696	30,62	30	2,07
3	0,7452	26,24	25	4,97
4	0,4433	15,61	15	4,08
5	0,2881	10,14	10	1,44
6	0,2453	8,64	8	7,98
7	0,1789	6,30	6	5,03
8	0,1463	5,15	5	3,04
9	0,0891	3,14	3	4,51
10	0,0287	1,01	1	1,20
11	0,0236	0,83	0,8	4,17
12	0,0141	0,50	0,5	0,23

Table 3: Accuracy of protein sample quantification with various BSA concentrations

In a concentration range of 50 to 0.5 mg/ml, the analyzed samples exhibit deviations of less than 10% to the theoretical protein concentrations, i.e. the known dilutions of the BSA standard. Samples below 0.5 mg/ml can not be reliably quantified because of significant results variation (CV > 10%).

Uniformity

The uniformity of protein sample quantification on the NanoQuant Plate was evaluated by applying a representative protein sample onto all 16 sample positions. Absorbance was read at 280 nm and 310 nm, respectively, and the results were blanked and 310 nm-corrected as described previously. Average, standard deviation and CV were calculated and summarized in table 4.

280 nm	1	2
A	0,3677	0,3672
B	0,3702	0,3804
C	0,3624	0,3791
D	0,3667	0,3628
E	0,3619	0,3640
F	0,3610	0,3572
G	0,3703	0,3673
H	0,3749	0,3679

310 nm	1	2
A	0,0992	0,0991
B	0,1003	0,1163
C	0,0969	0,1127
D	0,1000	0,0984
E	0,0984	0,1010
F	0,0970	0,0962
G	0,1068	0,1009
H	0,1079	0,1019

310 nm-corr. OD_{280}:		blank-corr. OD_{280}:	
0,2685	0,2681	0,2629	0,2625
0,2699	0,2641	0,2643	0,2585
0,2655	0,2664	0,2599	0,2608
0,2667	0,2644	0,2611	0,2588
0,2635	0,2630	0,2579	0,2574
0,2640	0,2610	0,2584	0,2554
0,2635	0,2664	0,2579	0,2608
0,2670	0,2660	0,2614	0,2604

average	**0,2599**
stdev	**0,0023**
CV [%]	**0,89**

Table 4: Measurement uniformity over 16 samples on the NQP

The NanoQuant Plate possesses a very good measurement uniformity for protein samples, as shown by the exceptionally low results variation of 0,89% (CV).

Reproducibility

In an additional set of experiments the reproducibility of protein quantification measurements on the NanoQuant Plate, a protein sample with 10 mg/ml was used in five consecutive measurements, each of which was composed of 4 independent sample replicates. The blank- and 310 nm-corrected average OD_{280} values are plotted in figure 2.

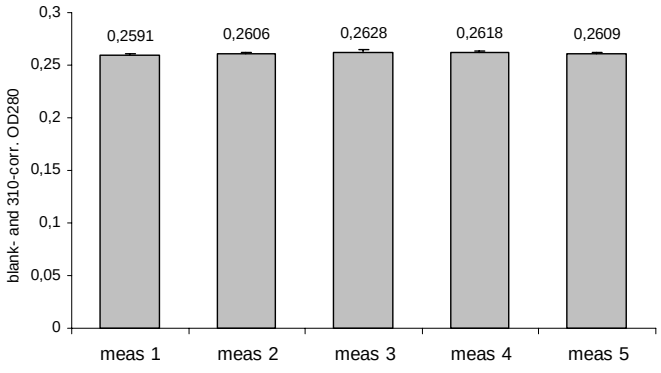


Figure 2: Measurement reproducibility of 5 individual samples on the NanoQuant Plate

As shown in figure 2, the variation between the single measurements was negligible, resulting in a CV of 0.78%. Thus, the NanoQuant Plate exhibits excellent reproducibility in terms of protein quantification.

Conclusion

Tecan's NanoQuant Plate has become a reliable and valuable tool for the quantification of nucleic acids out of small-volume samples. In addition, the NanoQuant Plate has now been tested for its capacity to measure protein samples.

As shown by the presented results, the NanoQuant Plate is able to quantify proteins via their absorbance at 280 nm. The results show that the NanoQuant Plate is able to quantify protein samples without the need for dilutions and produces excellent results in terms of measurement linearity, uniformity and reproducibility.
For reliable and reproducible analysis it is recommended to generate a standard curve of a protein with known concentration in order to accurately quantify samples of interest.

Taken together, there is a broad field of applications for the NanoQuant Plate, from quantification of nucleic acids and labeling efficiency measurements to quantitative analysis of protein samples.

Abbreviations

BSA	bovine serum albumin
CV	coefficient of variation
NQP	NanoQuant Plate
OD	optical density
stdev	standard deviation

Literature

[1] Quick Guide NanoQuant Plate™, 2008 (Tecan Austria)

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