

Rapid and simple quantification of DNA and protein using the VICTOR Nivo multimode plate reader.

Authors

Maria Kuzikov
Roman Kanke

Fraunhofer IME ScreeningPort
Hamburg, Germany

Introduction

Daily laboratory work in both research and drug screening applications requires the measurement of nucleic acid and protein concentration and purity in many samples, often with very low volumes, and a variety of microplate-based assays are available for this purpose.

In this application note we describe the detection of several commonly-used DNA and protein quantification assays, in both 96- and 384-well microplate formats, using the VICTOR Nivo™ multimode plate reader.

The VICTOR Nivo reader is a flexible, compact benchtop system with a dynamic filter wheel system for measurement of single fluorescent wavelengths and the option of either filters or a spectrometer for absorbance measurements. It is therefore well-suited to the determination of DNA and protein concentration and purity using methods such as those described below.

The most simple and widely used method is the measurement of absorbance at 260 nm for DNA or 280 nm for protein concentration. This also allows sample purity to be determined by calculation of the 260:280 nm ratio, thereby indicating contamination of the DNA sample by peptides or proteins. Purines and pyrimidines in nucleic acids naturally absorb at 260 nm whereas the amino acids tyrosine, tryptophan and cysteine contribute to 280 nm absorbance of proteins.

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In addition, absorbance at 230 and 320 nm indicate contamination of solvents, such as by phenolic solutions or carbohydrates, or light scattering due to precipitates. As the measurement of DNA or protein OD is influenced by a variety of factors such as different amounts of optically active molecules in DNA and protein, pH, temperature and salt concentration, alternative assays have been developed to reduce the impact of these factors.

For determination of DNA concentration, there are many fluorescent dyes available. An example is Quant-iT™ PicoGreen®, a dsDNA-specific fluorescent stain, which enables the detection and quantification of small amounts of dsDNA in the presence of salts, urea, ethanol, chloroform, or detergents.

For determination of protein concentration, the classical method uses Bradford Reagent in which a complex forms between the dye (Brilliant Blue G) and the protein. This results in a shift of the absorption maximum from 465 to 595 nm. The Qubit® Protein Assay by Life Technologies is another simple method for protein quantification based on a fluorescent stain with low protein-to-protein variation and high tolerance to reducing reagents, salts or free nucleotides.

Material and methods

DNA quantification using UV-Absorption

DNA samples (Deoxyribonucleic acid sodium salt from calf thymus, #D1501-100 MG, Sigma Aldrich) were diluted in dH₂O and 50 or 200 µL/well were transferred into a UV compatible microplate (UV-Star® Microplates, 384-well #781801, 96-well #655801, Greiner Bio-One) respectively. Absorption was detected using a pre-set protocol of the VICTOR Nivo reader (λ: 220-1000 nm, energy: 80, flashes: 50). OD₂₆₀ and OD₂₈₀ were used for data analysis.

DNA quantification using Quant-iT™ PicoGreen®

A dilution series of calf thymus DNA (Deoxyribonucleic acid sodium salt from calf thymus, #D1501-100MG, Sigma Aldrich) was prepared in TE-buffer, pH 7.5. The PicoGreen reagent was diluted 1:200 according to the manufacturer's protocol in TE-buffer, pH 7.5 and combined 1:1 with the DNA samples. After transferring 50 µL into black, non-binding 384-well microplates (OptiPlate-384 Black, #6007270,

Revvity) the fluorescence signal was detected using 485 Ex - 535 Em filters in the VICTOR Nivo reader. The assay plate was incubated for 12 min at room temperature inside the reader before the start of the measurement.

Protein quantification using bradford reagent

A serial dilution of a BSA protein standard (200 mg/mL, #P5369-10ML, Sigma Aldrich) was prepared in Dulbecco's phosphate-buffered saline buffer and combined 1:50 with the Bradford Reagent (#B6916, Sigma Aldrich). 100 µL or 250 µL were transferred into a clear 384- or 96-well microplate (UV-Star® Microplates, #781801 (384-well), 655801 (96-well), Greiner Bio-One). Absorption was detected using the pre-set protocol of the VICTOR Nivo reader (λ: 220-1000 nm, energy: 80, flashes: 50). The assay plate was incubated for 10 min at room temperature inside the reader before the start of the measurement. OD595 was used for data analysis.

Protein Quantification Using Qubit® Protein Assay Kit

A serial dilution of a BSA protein standard (200 mg/mL, #P5369-10ML, Sigma Aldrich) was prepared in Dulbecco's phosphate-buffered saline buffer. The standard was diluted 1:20 in Qubit® working solution (Component A 1:200 in Component B), mixed and incubated for 15 min at room temperature. 100 µL/well were transferred into the 384-well microplate. Fluorescence signal was detected using the 485 Ex - 616 Em filter set in the VICTOR Nivo reader.

"In all methods, unless otherwise mentioned, four replicates were measured per concentration."

Results

DNA quantification using UV-Absorption

When using a microplate for detecting the OD of a solution, the measurement is performed vertically through the solution. Therefore, the pathlength (l) is directly dependent on the volume in the well and can be determined using the standard formula (1). Well dimensions can be used to calculate the distance (2) or the pathlength can be determined empirically by using the specific absorbance of water near infrared at 977 nm. The detected absorbance can then be compared with the OD₉₇₇ of a measurement in a 1 cm cuvette whereby OD₉₀₀ should be used as a blank (3).

$$c_{DNA} = OD_{260} * sc * d/l \quad (1)$$

sc: standard coefficient at 1 cm pathlength
(50 µg/mL for dsDNA)

OD_{260} : optical density at 260 nm

l: pathlength

d: dilution

$$V = a * b * l \quad (2)$$

V: volume

a, b: width and depth of the well

l: pathlength

$$l = 1 \text{ cm} * (OD_{977 \text{ micro}} - OD_{900 \text{ micro}}) / (OD_{977 \text{ cuvette}} - OD_{900 \text{ cuvette}}) \quad (3)$$

l: pathlength

OD_{micro} : optical density measured in a microplate

OD_{cuvette} : optical density measured in a 1 cm cuvette

$$(OD_{977 \text{ cuvette}} - OD_{900 \text{ cuvette}} = 0.18)$$

Using the well dimensions for the UV compatible microplates and the detected OD_{977} values for dH_2O , pathlengths of 0.92 (2) and 0.94 (3) cm were determined for 100 µL sample volume in a 384 well microplate (UV-Star® Microplates Prod. no. 781801, Greiner Bio-One) (Figure 1). In parallel with the calculation of the DNA concentration using the standard coefficient and the pathlength correction, a linear regression line was measured using a commercially available DNA standard (Deoxyribonucleic acid sodium salt from calf thymus, Prod. no. D1501-100 MG, Sigma Aldrich). The detected absorbance showed a good linear correlation in the range of 0.8 - 100 µg/mL DNA. Calculated concentrations were in line with the DNA standard concentrations used. (Figure 2). Similar results were obtained for the 96-well plate (Figure 2).

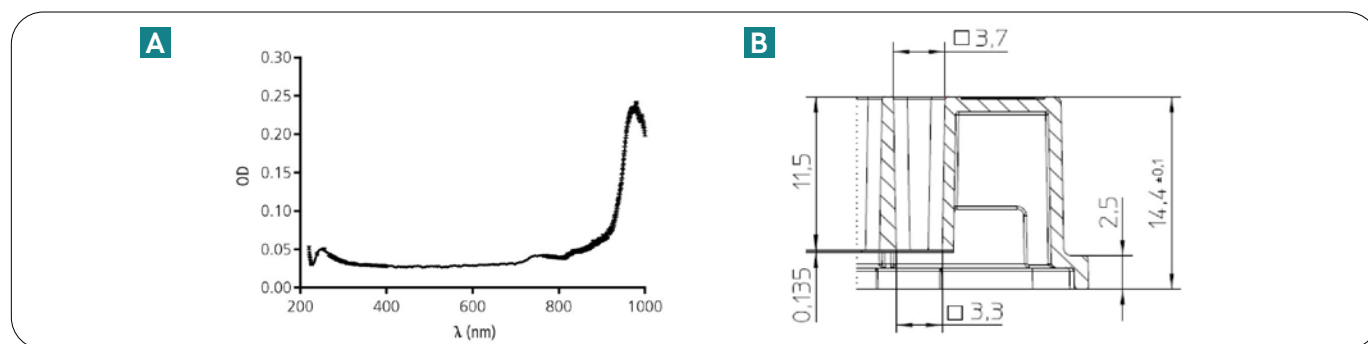


Figure 1. Correction of the pathlength for OD measurements in a microplate. A: Measured absorbance spectrum of water. Values represent single points. B: Well dimensions of the UV-Star® Microplates (#781801, Greiner Bio-One).

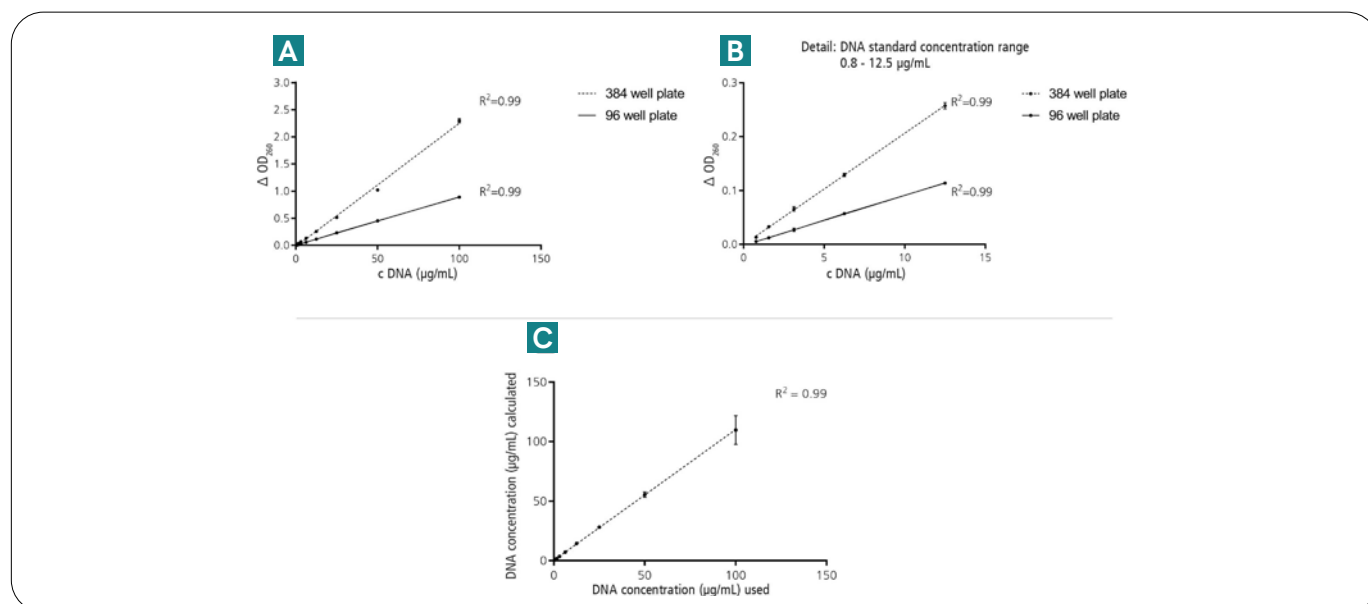


Figure 2. OD_{260} detection of DNA sodium salt standard from calf thymus. A: Linear fit of the DNA standard dilution series in 384-well and 96-well formats. B: Graph represents a magnified region of detected signal for low DNA concentrations. C: Correlation of the used DNA concentration and calculated DNA concentration values using the pathlength correction. Values represent mean of four replicates ± SD.

In order to test the reproducibility of the assay, the inter-plate variability was determined. Four plates were used to measure the OD_{260} of the DNA standard dilution series using DNA concentrations up to 400 $\mu\text{g/mL}$. The results showed good linear correlation of DNA concentration and absorbance in the range of 0.8 - 100 μg DNA (Figure 3).

Higher DNA concentrations had to be pre-diluted for reliable concentration determination. Low variability in OD values and thus in calculated DNA concentrations was observed, whereas calculated concentrations were slightly increased compared to experimentally used concentrations (Table 1).

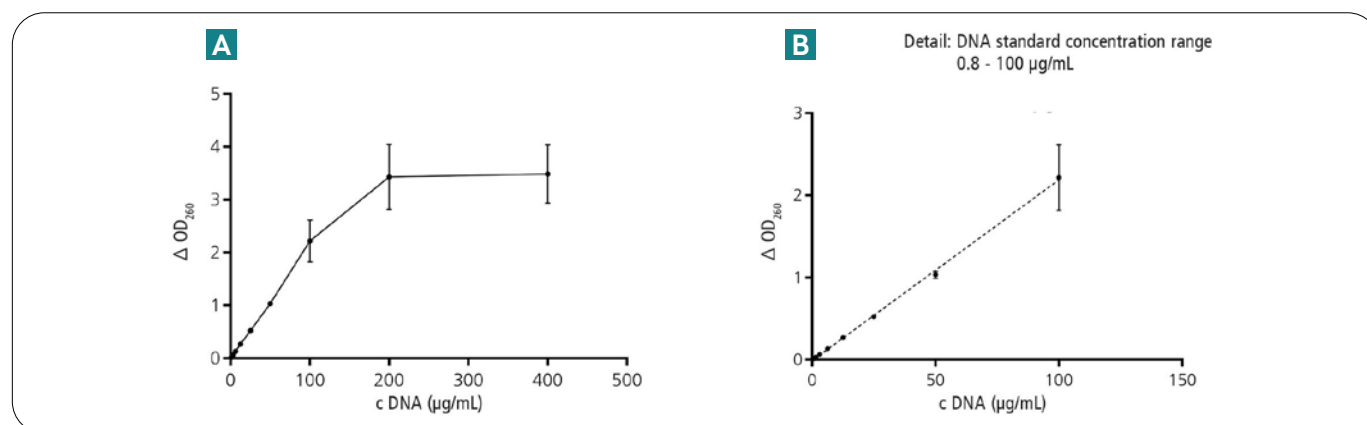


Figure 3. Inter-plate variation of OD_{260} measured using the DNA sodium salt standard from calf thymus. Data points represent mean of four plate replicates \pm SD. B represents a magnified region of detected signal for low DNA concentrations. Data show good linear correlation with low SD. Saturation of the OD_{260} was observed for ≥ 100 $\mu\text{g/mL}$ DNA.

Table 1. Linear correlation and inter-plate variation of OD_{260} measured for DNA sodium salt standard from calf thymus of four 384-well plates. Values for 50 $\mu\text{g}/\mu\text{L}$ DNA standard were taken as representative examples for the dilution series.

	Plate 1	Plate 2	Plate 3	Plate 4	AV	STD
R^2	0.998	0.988	0.999	0.997	0.996	0.004
OD_{260} (50 $\mu\text{g/mL}$)	1.02	0.99	1.09	1.04	1.03	0.04
Concentration DNA _{calculated} ($\mu\text{g/mL}$)	54.95	52.96	58.54	55.93	55.60	2.01

DNA quantification using Quant-iT™ PicoGreen®

The Quant-iT™ PicoGreen® assay was tested to overcome limitations of the UV absorbance assay at very low DNA concentrations. According to the obtained results, the assay presents a linear correlation in a wide range of 0.5 - 1,000 ng/mL DNA with a high dynamic range (Fig.4). Similar results were obtained for the 96-well plate, whereby signal saturation was observed for > 1 $\mu\text{g/mL}$ DNA standard. The sensitivity of the assay enables its use for very low concentration probes or high dilutions of DNA stocks, therefore saving material and cost.

In order to evaluate assay stability and inter-plate signal variation, two separate serial dilutions of the DNA standard were prepared according to the Quant-iT™ PicoGreen® protocol. Both dilution series showed good linearity in the range 0.5 and 1,000 ng/mL DNA. Only minor signal variation was observed between two plates demonstrating good assay and detection reproducibility (Fig.4).

Protein quantification using bradford reagent

The Bradford assay uses the shift in the absorbance maximum of the dye from 465 to 595 nm owing to the formation of a complex with a protein, to quantify the protein concentration. Detected absorption is proportional to the amount of protein present in the sample. According to the manufacturer's manual, the assay can be easily adapted to a microplate format. Serial dilutions of a BSA protein standard were used to determine assay sensitivity and dynamic range. Linear correlation was observed in the range $\sim 1 - 55$ $\mu\text{g/mL}$ (Figure 5). Similar results were obtained for the 96-well plate. Good assay reproducibility was confirmed by determination of the absorbance OD_{595} in four independent microplates (Figure 5). Signal saturation was observed for > 50 $\mu\text{g/mL}$ BSA, indicating the need for pre-dilution of higher concentration samples for a reliable determination of concentration using linear regression.

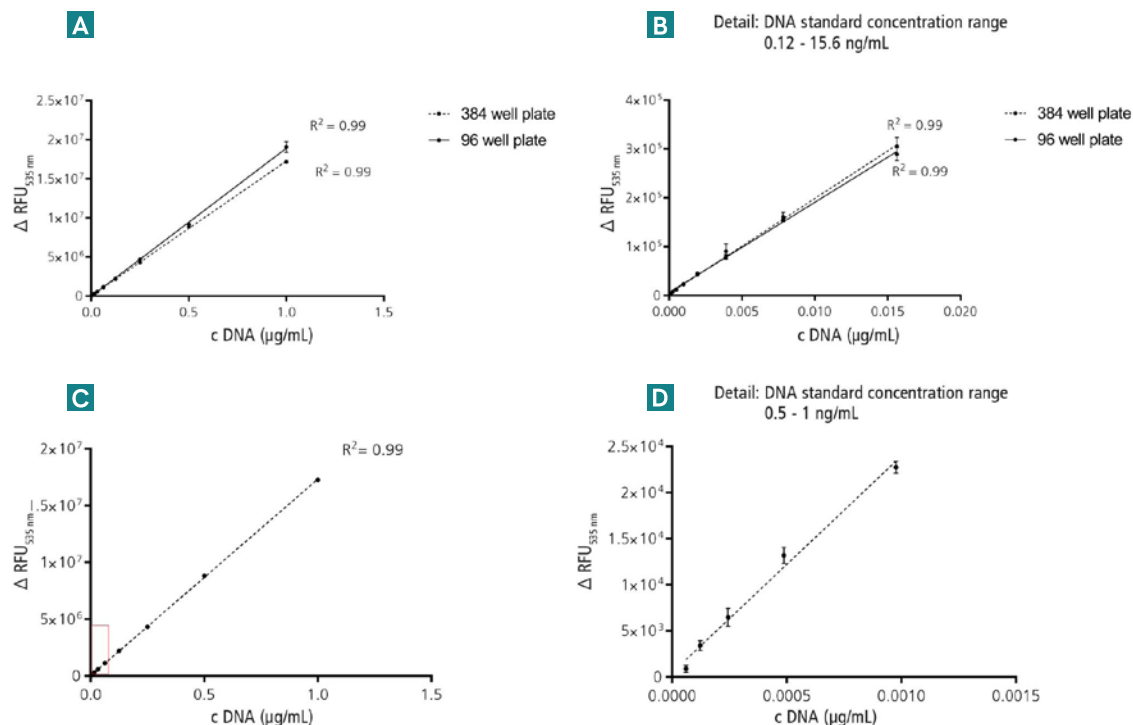


Figure 4. Fluorescence signal measured for the DNA sodium salt standard from calf thymus using the Quant-iT[™] PicoGreen[®] assay. A: Linear fit of the DNA standard dilution series in 384-well and 96-well formats. B: Graph represents magnified region of detected signal for low DNA concentrations. Data points represent mean of four replicates \pm SD. C: Inter-plate variation of the fluorescence signal detected for the DNA sodium salt standard from calf thymus using the Quant-iT[™] PicoGreen[®] assay. Data points represent mean of two plate replicates \pm SD. D: Graph represents a magnified region of detected signal for low DNA concentrations. Data show good linear correlation with low SD in the tested range 0.5 - 1,000 ng/mL DNA.

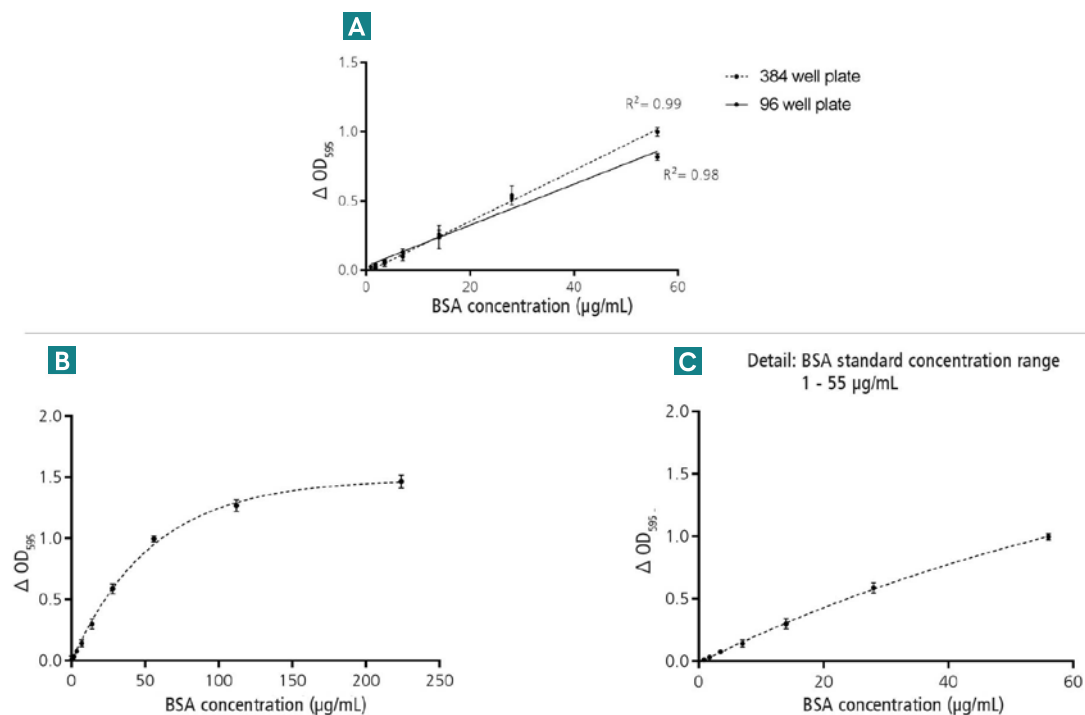


Figure 5. Detected OD_{595} of the BSA standard. A: Linear fit of the BSA standard dilution series in 384-well and 96-well formats. Values represent the mean of four replicates \pm SD. B: Inter-plate variation of OD_{595} measured using the BSA standard. Data points represent mean of four plate replicates \pm SD. C: Graph represents a magnified view of detected signal for low BSA concentrations. Linear correlation was observed in the tested range 1 - 55 $\mu\text{g/mL}$ protein.

Protein Quantification Using Qubit® Protein Assay

The Qubit® Protein Assay is a fluorescent stain-based method for determination of protein concentration in a sample. A serial dilution of 0.3 – 80 µg/mL of a BSA protein standard was used to determine the assay sensitivity and dynamic range. The assay was tested in both 384- and 96-well formats. Signal linearity was observed in a narrow range between 0.3 and 10 µg/mL protein standard. Similar results were obtained for both the 96- and 384-well plates (Figure 6). For concentrations > 10 µg/mL, saturation of the fluorescence signal was observed. Testing of the plate to plate variability revealed good reproducibility of the measurement and assay with only one outlier at 5 µg/mL.

Conclusions

This study shows the suitability of the VICTOR Nivo multimode plate reader for a variety of commonly-used DNA and protein quantification assays in both 96- and 384-well microplate formats. The pre-configured user-friendly protocols of the reader were easily adapted to the specific requirements of each assay. The measured dynamic range and assay linearity were in line with the assay manuals. The high data quality, shown by the low standard deviation and inter-plate variation, reveals good reproducibility and suitability for tests of various DNA and protein samples, therefore saving material and cost. The results demonstrate that fluorescent stains show good linearity in low µg/mL DNA concentration range whereas absorption based measurements are better suited for higher concentration samples. Therefore, the method of quantitation should be selected according to the expected concentration in the sample.

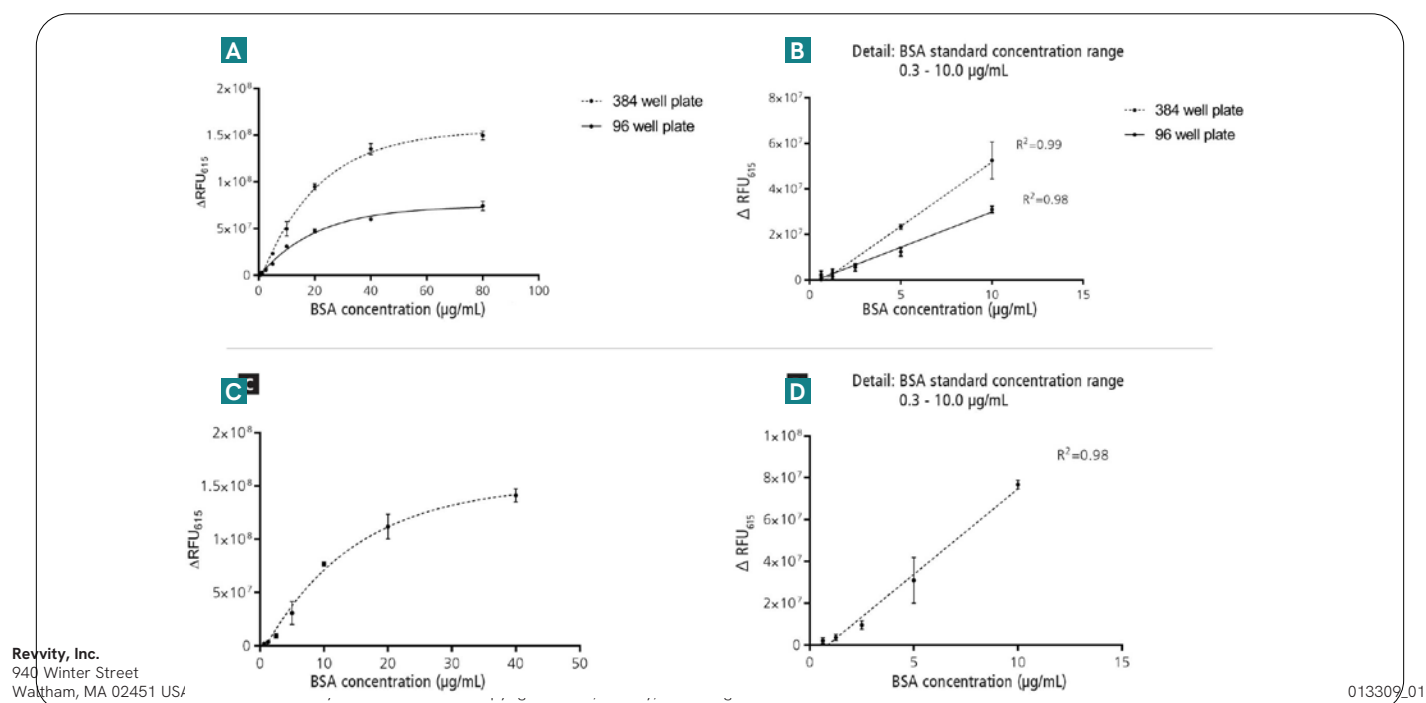


Figure 6. Fluorescence signal measured for the BSA standard from calf thymus using the Qubit® Protein Assay Kit. A: Fit of the BSA standard dilution series in 384-well and 96-well formats. Values represent the mean of four replicates \pm SD. B: Graph represents a magnified region of detected signal for low DNA concentrations. Values represent the mean of four replicates \pm SD. C: Inter-plate variation of OD595 measured using the BSA standard. Data points represent the mean of four plate replicates \pm SD. D: Graph represents a magnified region of detected signal for low BSA concentrations. Linear correlation was observed in the tested range 0.3 – 10 µg/mL protein.

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