TECHNICAL NOTE

# DNA quantification using the VICTOR Nivo and micro-volume plates.


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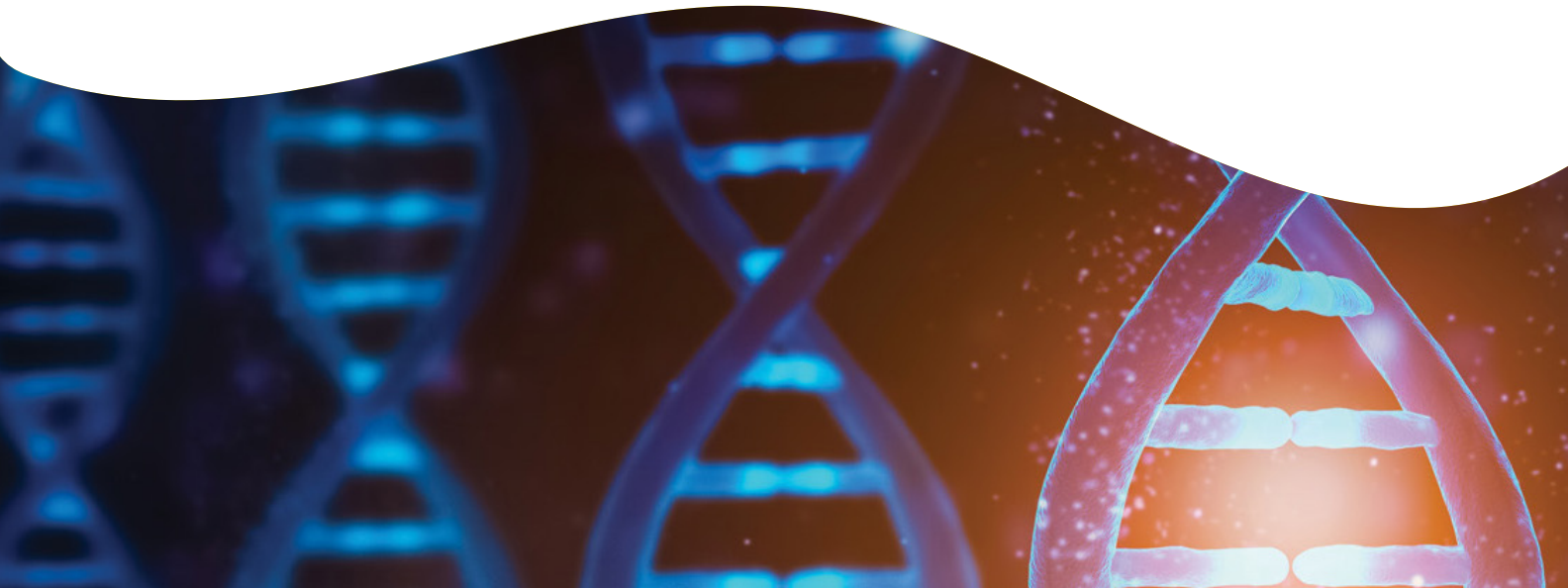
## Introduction

All life science related fields from basic research in academia to commercial diagnostics laboratories rely on quick and simple, yet highly accurate quantification of nucleic acids. Commonly, photometers are employed to determine quality, purity, and concentration by measuring the absorption of the probe using light in the ultra-violet (UV) range with wavelengths of 230, 260, 280, and 320 nm. While the absorption of 230 nm light indicates contamination with organic compounds, salt, sugar, or phenol, 320 nm serves as background correction. Nucleic acids and proteins show absorption maxima at 260 and 280 nm, respectively.

Photometers show high sensitivity but require extra space and maintenance while offering only limited functionality. Additionally, some devices perform measurements in cuvettes which require comparatively large sample volumes.

The VICTOR® Nivo™ multimode microplate reader combines a small footprint with a multitude of applications including absorbance-measurements to sensitively quantify nucleic acids[1]. Also, microplates improve cost- and time-effectiveness by enabling the measurement of many samples at the same time while using small volumes. VICTOR Nivo's internal software is easy to use and does not require special training.

In combination with micro-volume plates like the µDrop™ plate (Thermo Scientific™), which complies with the SBS- standard, even small volumes of 2-5 µL can be analyzed[2].



Here, we highlight the VICTOR Nivo's robust capability to quantify DNA concentration using micro-volume plates in three experiments:

- DNA purity verification
- Comparison of DNA concentration series between the µDrop™ plate and samples in a UV-Star® 96-well microplate
- Comparison of DNA concentration series between spectrometer and filter based configurations of VICTOR Nivo

## Materials and methods

### Sample preparation

For the concentration series, a 3x-dilution series of Salmon Sperm DNA (10 mg/mL, Agilent Technologies) ranging from 10,000 to 1.5 µg/mL was prepared in $dH_2O$. Additionally, a 100 mg/mL bovine serum albumin (BSA) solution in phosphate-buffered saline (PBS) (Sigma, Cat. No. A9647) was diluted in $dH_2O$ and mixed with DNA to simulate protein contamination. For each of the five measurements, 3 µL of the samples were pipetted into the wells of a µDrop™ plate (Thermo Scientific™, Cat. No. N12391). The low-volume measurement area of the µDrop™ plate contains 16 sample positions and has a path length of 0.5 mm. This setup allows for measurements of volumes of 2-5 µL and higher concentrations of the sample when compared to standard cuvettes with a path length of 10 mm. Subsequently, we prepared the same samples in a 96-well plate (UV-Star® microplate, 96-well, COC, F-Bottom, chimney well, REF655801, Greiner Bio-One) with five replicates. Each well was filled with 170 µL DNA sample. For the diluted samples, 3 µL of DNA was mixed with 167 µL of $dH_2O$. The path length of 4.9 mm was determined empirically by using the specific absorbance peak of water near infrared at 977 nm ($OD_{977}$) and comparing it to the $OD_{900}$, where no absorbance is expected. The path length is calculated with the following formula:

$$l = 10\ mm * \frac{OD_{977\ microplate} - OD_{900\ microplate}}{0.18^*} = 10\ mm * \left(\frac{0.089}{0.18}\right) = 4.9\ mm$$

l: path length
0.18*: $OD_{977\ cuvette} - OD_{900\ cuvette}$ as for a standard cuvette with 10 mm path length

The path length in the microplate as it was used in this preparation is about 10 times larger than in the µDrop™ plate.

### Measurements

Measurements were performed immediately after sample preparation to avoid evaporation. A full spectrum from 220 to 1000 nm (step size 1 nm) of all samples, as well as single wavelength measurements at 230, 260 and 280 nm, were acquired on the VICTOR Nivo S and VICTOR Nivo F reader using the parameters shown in Table 1.

Table 1: Settings used for data acquisition

| MMD reader | VICTOR Nivo S | VICTOR Nivo F |
|---|---|---|
| Plate Type | General 96 | General 96 |
| Plate Format | 96-wells (8x12) | 96-wells (8x12) |
| Plate Map | Wells A2-H3 | Wells A2-H3 |
| Operation | ABS (S) - Spectrum<br>ABS (S) - Scan | ABS (F) -<br>End Point |
| Measurement Unit | OD | OD |
| Measurement Mode | Band or whole spectrum | / |
| Bandwidth | 5 nm | / |
| Wavelengths | 230/260/280 nm<br>or 220 – 1000 nm<br>(1nm steps) | 235/10*<br>260/10<br>280/10 |
| Measurement Time | 250 ms | 500 ms |
| Measurement Order | Bi-directional by rows | Bi-directional by rows |

*non-standard absorbance filter. Order # HH35000992-001-A

### DNA quantification and determination of DNA purity

The concentration of DNA in a sample can be calculated from the $OD_{260}$ value and the path length of light using the following formula:

$$C_{DNA} = OD_{260} * sc * \frac{d}{l}$$

sc: standard coefficient at 10 mm path length (50 µg/mL for dsDNA)
d: dilution
l: path length

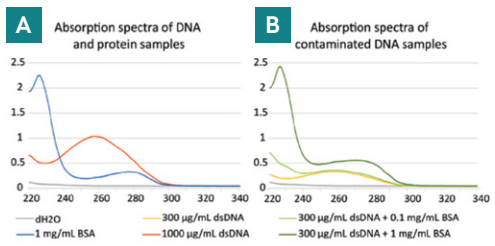
We also used a well-specific correction factor for the µDrop™ plate which was determined by measuring a DNA sample with a known OD of 1.1 in all 16 wells. Averages calculated from 5 measurements resulted in correction factors between 0.99 and 1.05. The purity of the DNA can be determined by calculating the ratio between $OD_{260}$ and $OD_{280}$ (contamination by proteins) and between $OD_{260}$ and $OD_{230}$ (contamination by salts, phenol etc.). A strong deviation from the ideal values ($OD_{260}/OD_{280}$ ~ 1.8 and $OD_{260}/OD_{230}$~2) is an indication for contamination.

To determine the dynamic range for the measurement of DNA concentrations, a serial dilution of dsDNA from 1.5 to 10,000 µg/mL was measured with a µDrop™ plate and compared to measurements using a 96-well UV-Star® microplate with 170 µL sample per well resulting in a path length of 4.9 mm.

## Results

### Quick and robust acquisition of DNA sample spectra and selected wavelengths

The VICTOR Nivo S is provided with a spectrometer which allows for simultaneous acquisition of the whole absorption spectrum from 220 to 1000 nm in less than 1 second per well. In Figure 1a, the absorption spectra of a DNA sample (1 mg/mL, red), a protein sample (1 mg/mL BSA, grey) in PBS and distilled water (blue) are plotted. The DNA sample shows a maximum at ~260 nm while the protein/PBS sample shows maxima at ~230 nm and ~280 nm. Since the spectra of DNA and proteins overlap, protein contamination can seriously affect the accurate calculation of the DNA concentration determined only from the $OD_{260}$ as illustrated in Figure 1b (light and dark green). The ratios $OD_{260}/OD_{280}$ and $OD_{260}/OD_{230}$ are reliable indicators of sample quality and help to find and avoid contaminated samples, see Figure 1c.




C Quality parameters of contaminated DNA samples

| Protein added | dsDNA* (µg/mL) | 260/280 | 260/230 |
|---|---|---|---|
| none | 290 | 2.0 | 2.5 |
| BSA (0.1 mg/mL) | 313 | 1.8 | 0.9 |
| BSA (1 mg/mL) | 492 | 1.1 | 0.3 |

* calculated from absorption at 260 nm

Figure 1: Absorption spectra from 220-340 nm. a) Absorption spectra of water (grey), dsDNA (1000 µg/mL, red) and Bovine Serum Albumin (BSA, 1 mg/mL, blue) were determined with a VICTOR Nivo S and a µDrop™ plate. b) Absorption spectra of a DNA sample (300 µg/mL) with different degrees of protein contamination (0.1 and 1 mg/mL BSA). c) DNA concentration as determined by calculation using the $OD_{260}$ value of the three different samples shown in b and its quality according to $OD_{260}$/ $OD_{280}$ and $OD_{260}/OD_{230}$.

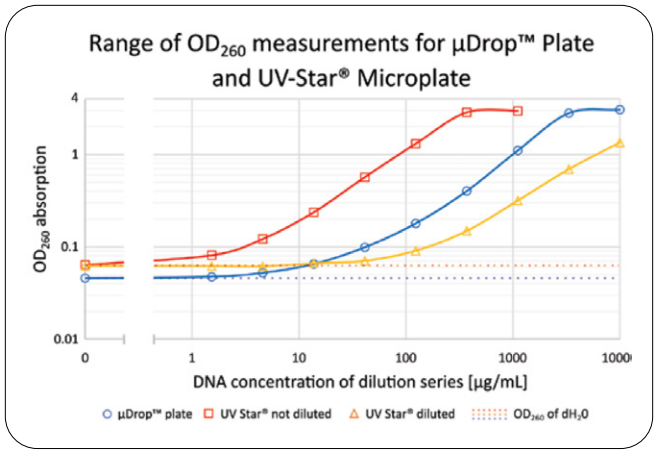



Figure 2: Range of $OD_{260}$ measurements with a VICTOR Nivo S. A dilution series of dsDNA was measured using a µDrop™ plate (blue) and a 96-well UV-Star® microplate (red and orange). For the measurement in the UV-Star® microplate, both 170 µL of the DNA solution (red) and diluted samples (3 µL in 167 µL $dH_2O$, orange) were measured. The $OD_{260}$ values for $dH_2O$ are shown as dotted lines (red/ orange for UV-Star® microplate measurements and blue for the µDrop™ plate). All circles in the plot represent the mean of 5 independent measurements.

### Combining the VICTOR Nivo and micro-volume plates extends the detection of sample concentrations above 3000 µg/mL

One parameter affecting the dynamic range of an absorption measurement is the optical path length. While in the wells of a 96-well UV-Star® microplate (filled with 170 µL) the path length of approximately 5 mm allows detection of slightly lower DNA concentrations without additional dilution step, the much shorter path length of a µDrop™ plate of only 0.5 mm allows also for the detection of higher concentrated DNA samples. Concentrations between 10 and 300 µg/mL were accurately measured with both methods (Figure 2 for $OD_{260}$ values and Figure 3 for the calculated DNA concentrations). While the samples containing <3 µg/mL were determined with a higher precision with the non-diluted samples in the 96-well plate, the samples with DNA concentrations >300 µg/mL were still determined accurately with the µDrop™ plate (see Figure 3). This highlights the optimal working range of >3 µg/ mL for micro-volume plates and <300 µg/ mL for microtiter plates. To extend the working range of microtiter plates to concentration >300µg/mL DNA samples can be diluted, see Figure 2 and Figure 3 (UV-Star® diluted, 3 µL in 167 µL $dH_2O$, orange circles). Samples with high DNA concentrations dilution in microtiter plates represents a convenient alternative to micro-volume plates. However, for lower DNA concentrations the micro-volume plates are better suited (see Figure 2 and 3), particularly if the available amount of DNA sample is restricted as in this test.

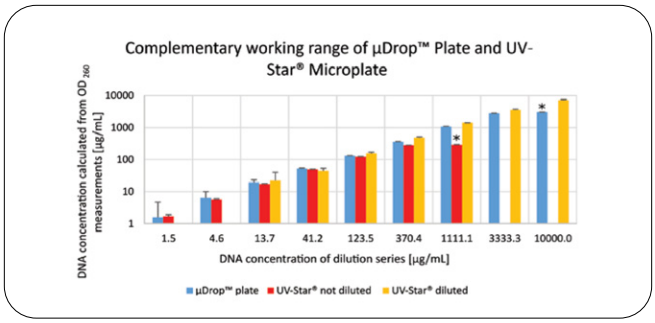



Figure 3: Showcase of the complementary working range of µDrop™ plates (blue) and a 96-well UV-Star® microplate (red and orange) for the quantification of DNA concentration using the VICTOR Nivo. For the measurement in the UV-Star® microplate, both 170 µL of the DNA solution (red) and diluted samples (3 µL in 167 µL $dH_2O$, orange) were measured. All bars represent the mean of 5 independent measurements. The error bars show the standard deviation. Measurements which already exceeded the working range are indicated by an asterisk.

The measurements show high reproducibility as indicated by the low standard deviation (Figure 3). Within the dynamic range of the respective plate type, the $OD_{260}/OD_{280}$ ratio of 1.8 confirmed the expected purity of the samples. Regardless of device configuration, the VICTOR Nivo provides high quality data. The sensitive detection results in convincing reproducibility in measurements comparing the filter based (VICTOR Nivo F) and spectrometer based (VICTOR Nivo S) multimode plate readers which show a correlation of $R^2$=0.999, see Figure 4.

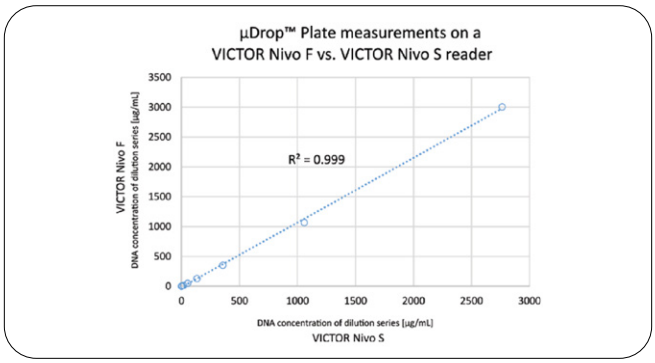



Figure 4: Measurement of a DNA dilution series with a µDrop™ Plate on a VICTOR Nivo S with a spectrometer in comparison to a measurement on a VICTOR Nivo F with a 260/10 nm filter. The measurements show a high coefficient of correlation $R^2$. For calculation of the $OD_{260}/OD_{280}$ and $OD_{260}/OD_{230}$ ratio, the respective filters must be purchased for the VICTOR Nivo F.

## Conclusion

Reliable quantification of nucleic acids is essential for all downstream processes. The VICTOR Nivo system integrates high quality assay readouts with broad functionality. VICTOR Nivo's software offers assay protocols that are ready-to- use and can be easily adapted to special experimental conditions. At the same time, the VICTOR Nivo system offers a small footprint and allows for the use of all SBS format- complying sample carriers. Compared to traditional cuvette measurements, this improves cost and time-effectiveness. In combination with micro-volume plates, the low volume reduces the consumption of precious samples and the fixed path length allows for a direct calculation of the nucleic acid concentration.

Here, we showed the effective use of the VICTOR Nivo in combination with micro-volume plates for DNA quantification. The compatibility and high-quality data are demonstrated by a small standard deviation as well as a convincing correlation of data acquired on micro-volume plates and microtiter plates. Contaminations like proteins were sensitively detected.

The VICTOR Nivo S allows for additional readouts such as $OD_{900}$ and $OD_{977}$ for the determination of the path length by rapidly acquiring the whole absorption spectrum.

The µDrop™ plate can be used as easily as any standard microplate on the VICTOR Nivo. Furthermore, we could show the complementary function of micro-volume plates to microplates. Micro-volume plates are effective in order to increase the detection range of samples with higher DNA concentrations using either a spectrometer or filter based VICTOR Nivo microplate reader.

## References

1. Rapid and Simple Quantification of DNA and Protein Using the VICTOR Nivo Multimode Plate Reader. Revvity Application Note 2017

2. Quantification of Proteins by UV-Vis Absorbance Using VICTOR Nivo with Microvolume Plates. Revvity Application Note 2020

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