

# DNA CONCENTRATION MEASUREMENT AT 260 nm USING PHOTOPETTE® BIO

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- Photopette Bio makes measurement of DNA concentration efficient.
- Sample purity ( $A_{260}/A_{280}$ ) could be measured concurrently.

## OBJECTIVE

The objective of this application note is to demonstrate how Photopette Bio can be used for direct DNA concentration measurement and DNA purity for unknown samples. Furthermore, it acts as a handy guide to get you started with Photopette, and outlines application-specific parameters for reference.

## INTRODUCTION

DNA (deoxyribonucleic acid) concentration measurement is a commonly performed procedure in life science and biomedical research laboratories. A spectrophotometer is able to determine DNA concentration as well as its purity [1]. It is based on the principles that nucleic acids absorb ultraviolet (UV) light at a specific wavelength. For pure DNA samples, the maximum absorbance occurs over a broad peak at around 260 nm; at 280 nm it only absorbs about half as much UV light compared to 260 nm [2]. DNA absorbs UV light due to heterocyclic rings of the nucleotides, its sugar-phosphate backbone does not contribute to this absorption [3]. Factors such as pH and ionic strength can further affect the absorbance spectrum. Typical spectrums of UV absorbance for different concentrations of purified DNA are presented in Figure 1 below.

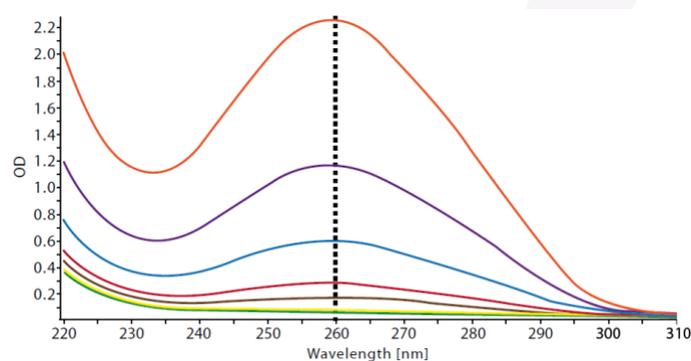


Fig 1: UV absorbance spectrums for different DNA concentrations

The DNA concentration of an unknown sample can be determined at a wavelength of 260 nm using Beer-Lambert

Law. The method does not require any other additional reagents or preparations, or the generation of a standard curve in advance. The ratio of the absorbance at 260 nm and at 280 nm ( $A_{260}/A_{280}$ ) is used to assess purity of the DNA sample.

This approach is only useful for pure DNA samples. Impurities such as protein, RNA and insoluble cell lysate factors also absorb in similar UV range and therefore, could interfere.  $A_{260}/A_{280}$  for a pure DNA sample is usually about 1.8 [2]. Since pure RNA has an  $A_{260}/A_{280}$  ratio of 2.0, a DNA sample with  $A_{260}/A_{280}$  ratio greater than 1.8 suggests RNA contamination [4].

## MATERIALS AND APPARATUS

### Instruments:

- Photopette® Bio.

### Reagents:

- Human DNA Quantitation Standard (NIST [SRM372](#))
- Tris-EDTA buffer (Sigma Aldrich #[93283](#))

## METHOD

Highlighted in this section are the steps followed to perform measurement for DNA standards. It is advised to perform an application specific risk-assessment analysis before performing an experiment. Please refer to the Photopette User Manual for operating and safety precautions [5].

## EXPERIMENTAL PROCEDURE

Turn-on the Photopette device and connect to the Photopette iOS/Android app. Select 'ssDNA' or 'dsDNA' as the measurement type, depending upon the sample type. Select dataset and set additional settings (if needed) before selecting 'Start Measurement'. Please follow the video-tutorials available online at [www.tipbiosystems.com](http://www.tipbiosystems.com) to get familiar with the measurement process.

Tris-EDTA buffer (TE buffer) can be used as blank sample. A CuveTip™ was mounted on the Photopette and it was dipped in the blank sample to perform auto-zero measurement. Please ensure that there is no air-bubble trapped in the CuveTip Cavity. Note: Presence of air-bubbles may disrupt the optical path and will lead to inaccurate measurements.

Subsequently, DNA solutions with different concentrations were prepared by serial dilutions using TE buffer. DNA concentration was calculated based on the information provided in NIST SRM certificate of analysis [6]. For each concentration, five replicates were taken. Measurement results for 260 nm are tabulated below in Table 1.

**Table 1:** Absorbance values for prepared DNA standards

DNA Conc. (ng/μl)	Average Absorbance at 260 nm (AU)	Standard Deviation
1	0.05	0.000
2	0.10	0.002
5	0.12	0.001
10	0.19	0.001
15	0.24	0.000
20	0.32	0.001
25	0.38	0.001
30	0.46	0.001
40	0.52	0.002
50	0.67	0.001

#### DETERMING CONCENTRATION OF AN UNKNOWN SAMPLE

Following a similar approach, DNA concentration for an unknown sample can be conveniently measured using Photopette Bio device by substituting the absorbance value in the equation of the standard curve.

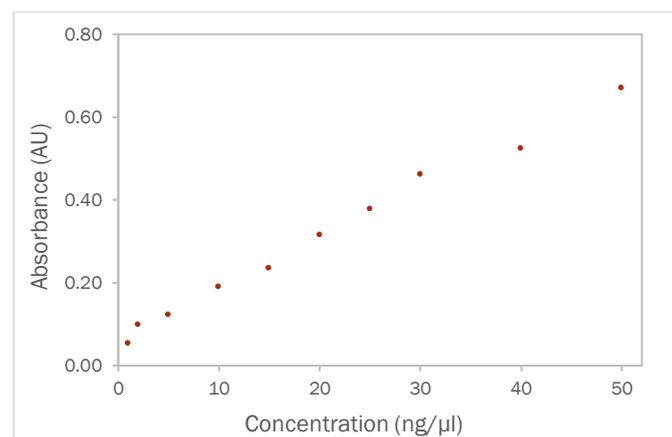
#### DISCUSSIONS

The data was used for generation of a standard curve, and to determine experimental parameters such as limit of detection, linear range and dynamic range. The absorbance values in Table 1 were plotted in the Figure 2.

#### EXPERIMENTAL PARAMETERS

##### *Upper measurement-limit and linear range*

Based on dynamic range From Figure 2, we can deduce that the upper measurement-limit for DNA measurements is beyond 50 ng/μl. Regression analysis indicates the linear range to be beyond 50 ng/μl as well.



**Fig 2:** Absorbance value plot for the prepared turbidity standards

#### Sample purity and $A_{260}/A_{280}$ ratio

The absorbance values for 260 nm and 280 nm are tabulated below in Table 2.  $A_{260}/A_{280}$  ratio (i.e. ratio for sample purity) was thus calculated and highlighted in adjacent column.

**Table 2:** Absorbance values for prepared DNA standards and their purity ratio.

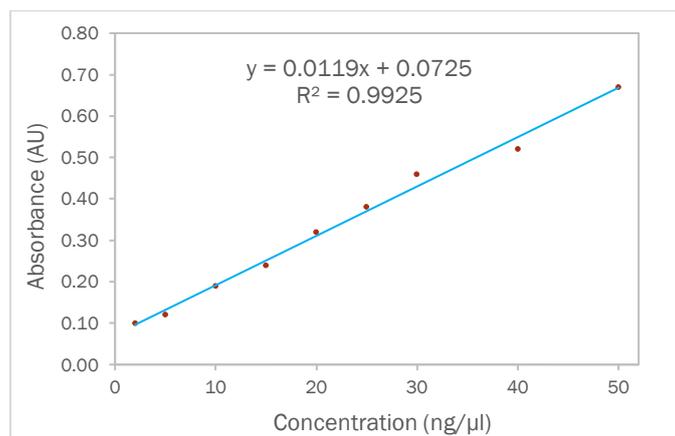
DNA Conc. (ng/μl)	Avg. Absorbance at 260 nm (AU)	Avg. Absorbance at 280 nm (AU)	$A_{260} / A_{280}$
1	0.05	-0.05	1.05
2	0.10	0.01	10.33
5	0.12	0.02	8.27
10	0.19	0.02	11.33
15	0.24	0.08	2.88
20	0.32	0.12	2.59
25	0.38	0.14	2.74
30	0.46	0.17	2.71
40	0.52	0.25	2.12
50	0.67	0.24	2.83

The recommended absorbance range starts at 0.1 AU, thus measurement readings below 0.1 AU were ignored and are highlighted in red in the Table 2. The  $A_{260}/A_{280}$  ratio is highly sensitive to the peak wavelength and bandwidth for 260 nm and 280 nm [7]. Since Photopette relies on LED technology, the peak wavelength tolerance and bandwidth is higher. Therefore, the ratio of about 2.5 should be used as the purity.

#### STANDARD CURVE

A standard curve was plotted in Figure 3 for data within 2 ng/μl to 50 ng/μl. A linear regression was performed on the data using Microsoft's Excel® software, and a linear fit was performed. The equation of standard curve along with its R-

squared value was obtained. The slope of the linear component of the standard curve is 0.012 AU  $\mu$ l/ng.



**Fig 3:** Standard Curve for DNA measurement using Photopette

Photopette users may download a pre-configured worksheet for the DNA measurement from our '[online resource section](#)'. The worksheet is compatible with Microsoft Excel® and similar worksheet software, and will aid users in performing the calculations and generating the standard curve.

#### Limit of Detection

The Limit of Detection (LOD) for this assay using Photopette is determined by factoring in the standard-deviation for blank measurements as well as experimental data using the equation given below:

$$\text{LOD} = 3 \times \text{SD}_{\text{blank}} / \text{Slope}_{\text{(Standard Curve)}}$$

Standard Deviation for blank measurements with 50 repeats using same CuveTip was found to be 0.005 AU. Thus, the limit of detection for the DNA measurement with Photopette using the equation above was 1.25 ng/ $\mu$ l.

$$\begin{aligned} \text{LOD} &= 3 \times (0.005 \text{ AU}) / (0.012 \text{ AU } \mu\text{l/ng}) \\ &= 1.25 \text{ ng}/\mu\text{l} \end{aligned}$$

#### LIMITATIONS

It is important that the DNA sample to be measured using this approach should be pure in order to avoid other interfering biological entities and proteins.

#### SUMMARY

The application note guides the user for determining DNA concentration in a given sample. It highlights experimental parameters such as limit of detection, upper measurement-limit and linear range for such measurements.

#### REFERENCES

- [1] "Understanding and measuring variations in DNA sample quality." [Online]. Available: [https://www.ogt.com/resources/literature/483\\_understanding\\_and\\_measuring\\_variations\\_in\\_dna\\_sample\\_quality](https://www.ogt.com/resources/literature/483_understanding_and_measuring_variations_in_dna_sample_quality). [Dated: 30-Mar-2017].
- [2] Rothman R., "Determination of DNA Concentration and Purity by Ultraviolet Spectrophotometry."
- [3] H. F. Lodish, *Molecular cell biology*. W.H. Freeman, 1999.
- [4] "Thermo Scientific NanoDrop Spectrophotometers Protein A280 Protein A280 Protein A280," 2010.
- [5] Tip Biosystems, F. Omar, "Photopette User Manual V1.0.0," Singapore, 2016.
- [6] "Certificate of Analysis." NIST Humanized DNA Standard.
- [7] "T042 - Assessment of Nucleic Acid Purity."