

# Absorbance or Fluorescence: Which Is the Best Way to Quantify Nucleic Acids?

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## Executive Summary

When performing molecular experiments on nucleic acids, it is a basic requirement to determine the concentration as well as the quality of the sample. The standard method which serves this purpose is UV-Vis spectrophotometry – but is it always the right choice? In the present White Paper, the advantages and disadvantages of this technique will be described and it will be compared with nucleic acid quantification via fluorescence. In this context, it will be discussed which situations warrant the use of which one of the two methods. The decision will generally depend on the condition of the sample as well as on the requirements of downstream applications. Since the advantages of both methods complement each other well, it is most practical to have the ability to perform both methods on a single instrument in a flexible manner.

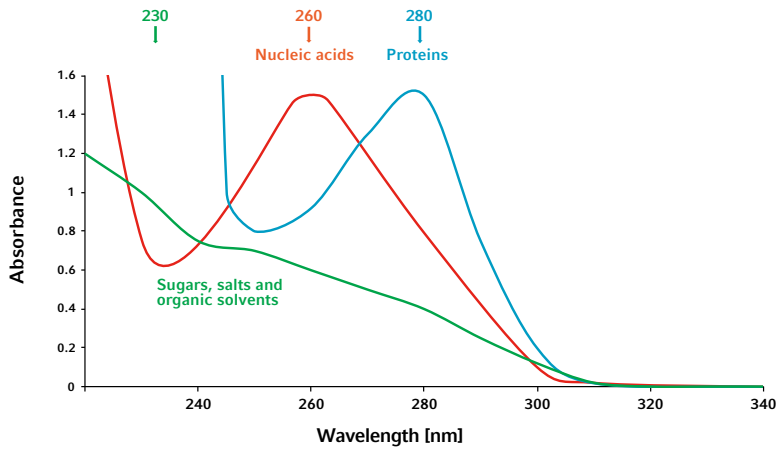


## Introduction

Experiments involving nucleic acids are a mainstay in any molecular laboratory. DNA and RNA are isolated from microorganisms and from cells of higher order organisms in order to be employed in a broad variety of processing steps and analyses. It is crucial for any type of downstream application that a defined amount of nucleic acid is used and that the sample is free from contaminations that may impact the experiments. To this end, subsequent to the isolation of the nucleic acid, its concentration and, if required, its purity are determined on a routine basis.

One tried and true method is the quantification of DNA/RNA solutions by measuring absorbance at a wavelength of 260 nm in a UV-Vis (spectro-)photometer. Sample purity may be

evaluated by measuring the sample at additional wavelengths (230 nm, 280 nm) and calculating the purity ratios, i.e. the ratios of the values obtained at 260/230 nm and at 260/280 nm, respectively. In this way, it will be evident whether cellular debris or remainders of reagent used during purification such as proteins, sugar molecules, certain salts or phenols, are present in the solution, as these will generate a different absorbance spectrum from nucleic acids (figure 1). In addition, turbidity measurements, frequently conducted at 320 nm, are capable of detecting particles within the sample [1]. A scan which covers the entire UV spectrum will provide even more information on impurities, and particularly on possible errors which may have occurred during the course of the measurement [2].



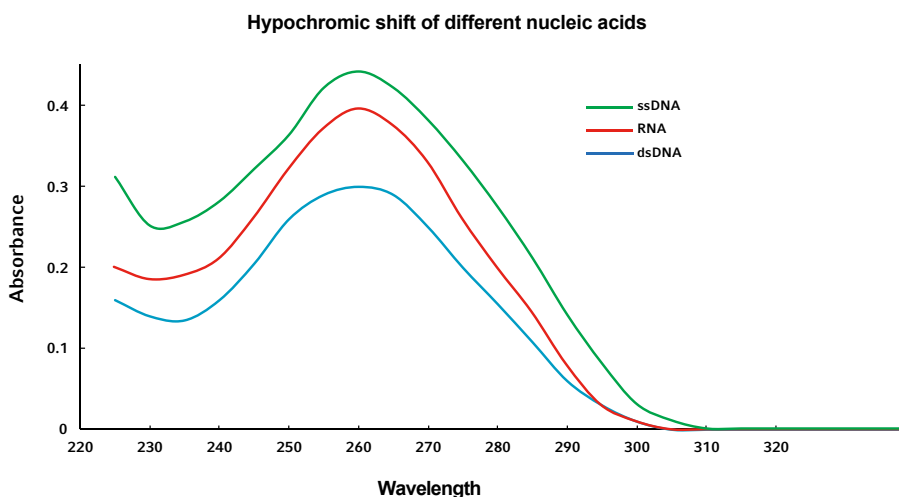
**Figure 1:** Spectra obtained from nucleic acids and possible contaminants

The major advantage of this direct method is the fact that it is very simple and quick to perform. With the right equipment (micro-volume instrument or micro-volume cuvette), it is possible to measure very small volumes (in the range of 1  $\mu$ L) and thus save sample material. In addition, as elaborated above, this method is capable of determining sample purity.

This method, however, comes with certain drawbacks: on the one hand, with a detection limit in the range of approximately 1  $\mu$ g/mL dsDNA (based on the common path length of 10 mm), it is not very sensitive. Therefore, in order to guarantee accurate quantification, samples may need to be concentrated prior to measurement, which, in turn, entails additional effort and which also frequently leads to considerable sample loss. In extreme cases, the amount of sample will not be

sufficient to even fill a cuvette. Microvolume systems, which require sample volumes of only 1 – 2  $\mu$ L, may offer a solution. It is, however, important to keep in mind that, due to the shorter path lengths of these systems (compared to a path length of 10 mm in common cuvettes), their detection limits are correspondingly higher. For this reason, considerably higher sample concentrations are required in order to obtain accurate and reproducible measurement values.

Furthermore, all nucleic acids generate the same absorbance spectrum (figure 2), thus potentially leading to an overestimation of the concentration of the DNA at hand in cases where RNA is present alongside dsDNA [3]. Contaminations are also frequently capable of absorbing light at 260 nm and thus contribute to elevated readings (figure 1).

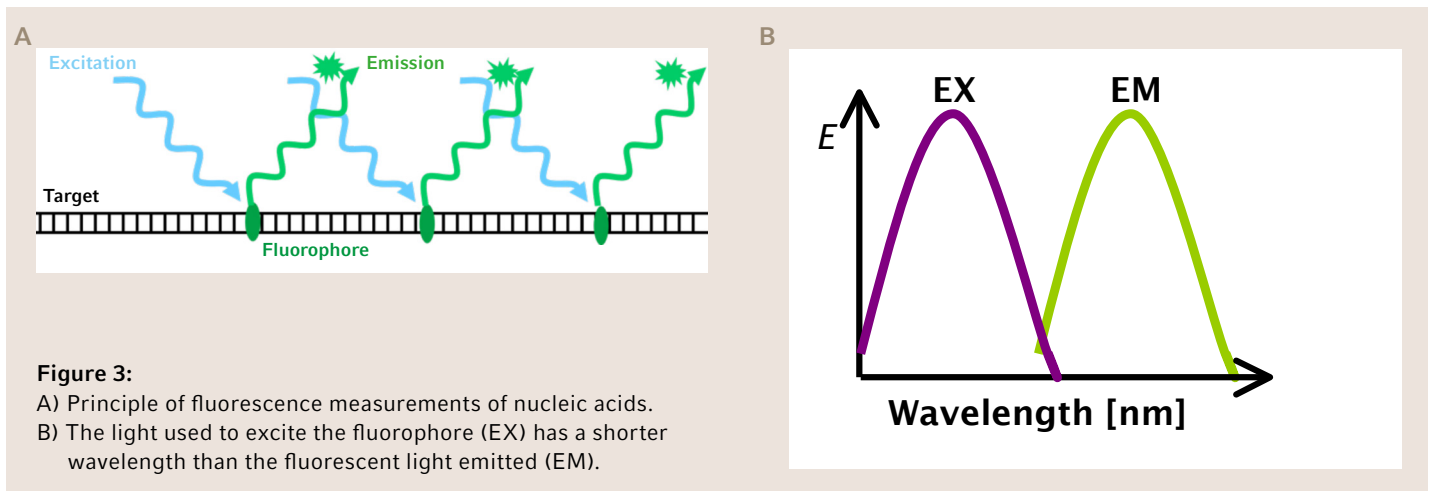


**Figure 2:** Absorbance spectra of different types of nucleic acids.

## Fluorescence measurements – the alternative?

Common alternatives employed in the quantification of nucleic acids include measurements of fluorescence, qPCR and capillary electrophoresis systems that are chip-based. The two latter processes are mainly used for specific tasks (e.g. qPCR: exact quantification of specific target sequences; capillary electrophoresis: exact determination of fragment size and integrity) as both methods are relatively costly as well as time-consuming.

Quantification of nucleic acids via fluorescence is based on the use of fluorescent dyes which bind to the molecule of interest. Only the complex consisting of nucleic acid and dye is excited by light of a specific wavelength (dependent on the dye) and will subsequently emit light of a slightly longer wavelength (figure 3). The intensity of the fluorescent signal is dependent on the amount of nucleic acid. For excitation and detection, a plate reader with fluorescence function, a fluorimeter or a photometer with a fluorescence module is required.



One major advantage of this method is its high sensitivity, which is 1000-fold higher for the fluorescent dye PicoGreen® as compared to absorbance measurements. In this way, even samples of very low concentrations can be quantified accurately [4]. A further positive aspect is the fact that this detection method requires only very small amounts of sample. Fluorescent dyes bind in a highly specific manner – possible contaminations such as salt or protein will therefore not lead to artificially elevated readings, which equals higher accuracy. Certain variants are available which bind specifically to one type of nucleic acid. This is the case for dyes which detect dsDNA in particular, thus practically eliminating the impact of possible ssDNA or RNA on the result.

Detection of nucleic acids via fluorescence is, however, an indirect method that necessitates the generation of a standard curve prior to the actual measurement. Since samples and standards must be prepared with the fluorescent dye, this method is slightly more cumbersome than UV-Vis spectrophotometry (figure 4). Furthermore, this method does not allow one to draw any conclusions regarding the purity of the sample.

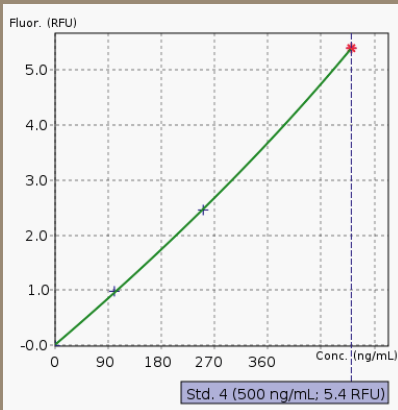
Workflow fluorescence measurements

Sample preparation

- > Add fluorescence reagent to blank, standards and samples
- > Incubate

A Generate standard curve

- > Measure blank and standards
- > Choose regression mode



B Determine sample concentration

- > Measure samples
- > Calculate concentration

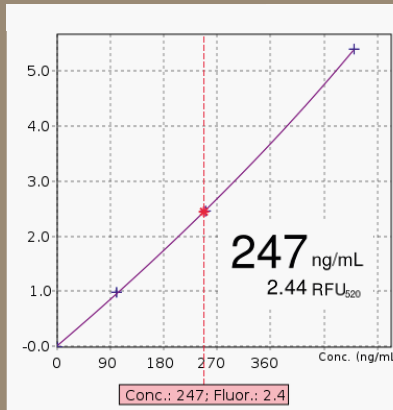


Figure 4: Workflow for the quantification of nucleic acids via fluorescence measurements.

The decision which of the two methods is to be used for nucleic acid quantification depends on a number of factors including, on the one hand, the amount and quality of the sample, and on the other hand, the requirements of the downstream application. It is crucial to weigh the importance of accurate determination of the sample concentration against the potential impact of impurities on subsequent applications. In general, absorbance measurement is the method of choice for samples of medium to high concentration, as well as high purity. Alternatively, this method is

ideally suited to the verification of sample purity. Fluorescence measurements are beneficial in cases where the exact quantification of samples of low concentration, or of samples containing impurities, is of the essence.

It is frequently a good idea to combine both methods, thus allowing the respective benefits to complement each other. The advantages of both detection methods are summarized in table 1.

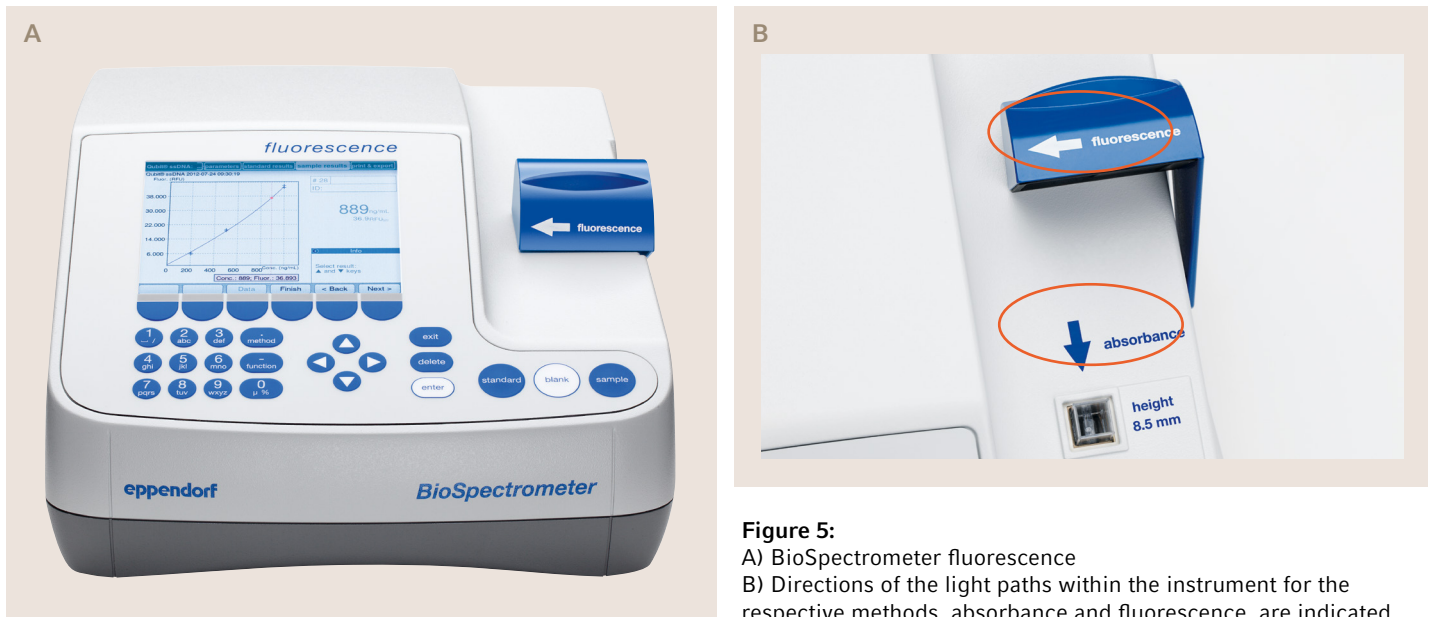
Table 1: Advantages of absorbance measurements and fluorescence measurements, respectively

Absorbance	Quick and easy as this is a direct method of measurement
	Non-hazardous and very stable as no chemicals are required
	Provides information on sample purity
Fluorescence	High sensitivity; therefore ideally suited to the analysis of samples of low concentration and saving of valuable sample material
	High accuracy due to high specificity for the target molecule

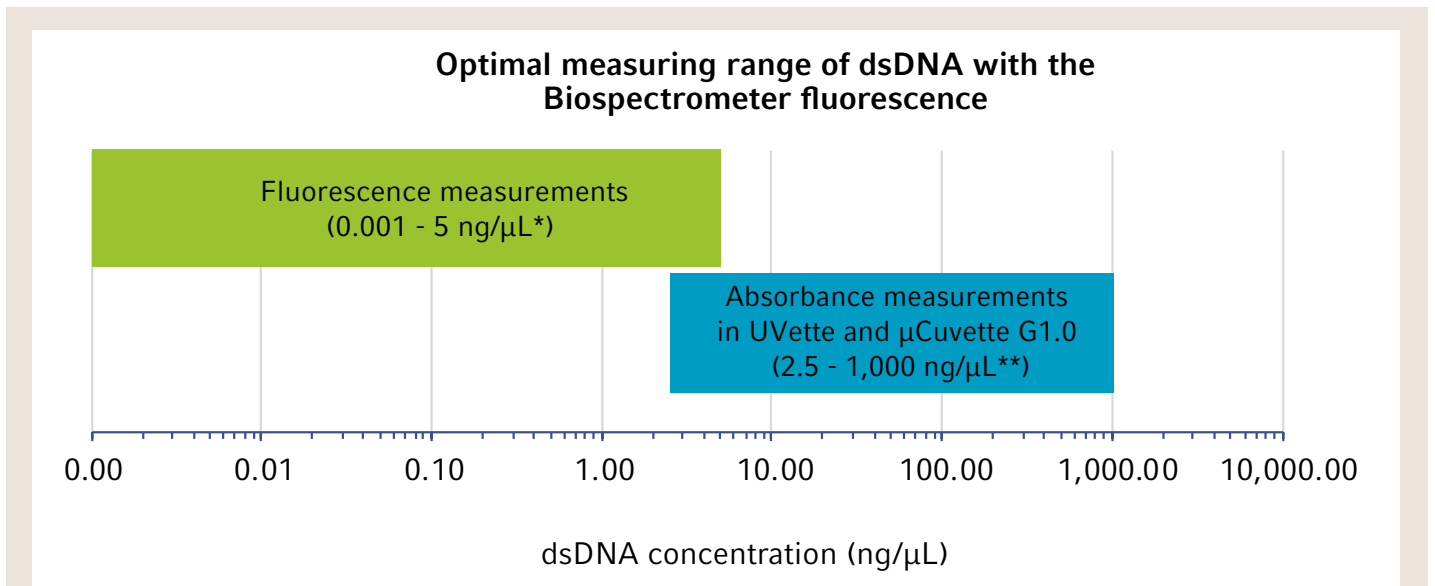
## Solutions by Eppendorf

The Eppendorf BioSpectrometer® fluorescence is an instrument that enables both absorbance measurements as well as fluorescence measurements (figure 5). This combination allows accurate quantification of nucleic acid solutions

across a broad measurement range (figure 6). In addition, sample purity is easily determined via absorbance measurements, whereas the fluorescence method is highly specific.



**Figure 5:**  
 A) BioSpectrometer fluorescence  
 B) Directions of the light paths within the instrument for the respective methods, absorbance and fluorescence, are indicated.



**Figure 6:** Depiction of the dsDNA concentrations that can be quantified accurately and reproducibly via fluorescence and absorbance measurements, respectively, in the BioSpectrometer fluorescence.

\* With respect to a combination of the following assay kits: Quant-iT™ PicoGreen dsDNA, Qubit™ dsDNA HS, Qubit dsDNA BR as measured in cuvettes with path lengths of 10 mm.

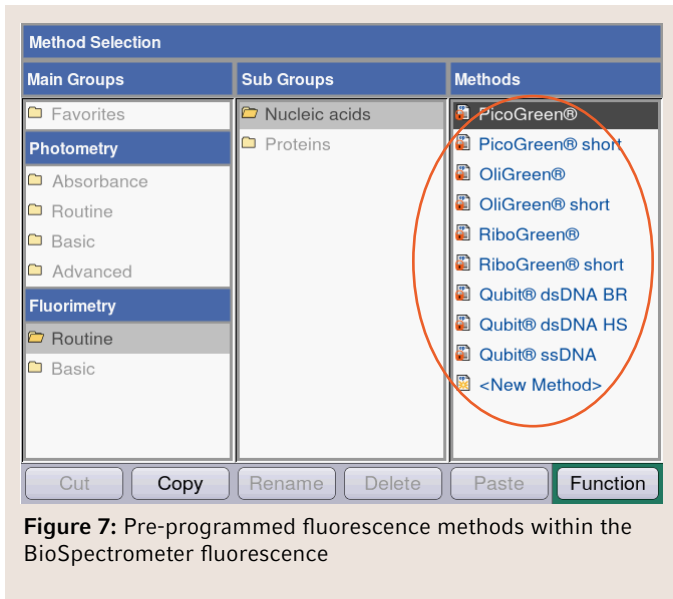
\*\* With respect to a combination of the path lengths 10 mm and 1 mm.

The fluorescence module of the BioSpectrometer fluorescence generates an excitation wavelength of 470 nm, whereas emitted light can be detected at 520 nm and 560 nm. It is compatible with standard nucleic acid quantification kits by different manufacturers [4, 5, 6, 7, 8, 9] and thus constitutes an open system. The instrument

features pre-programmed methods (figure 7) which simplify operation while simultaneously offering the option of adapting the protocols as needed. Many applications allow 2-point calibrations which, in the form of 'short methods', provide good alternatives that are quick and save reagent at the same time.

In addition to the instrument, Eppendorf offers a range of different cuvettes (table 2). For absorbance measurements at 260 nm, the UVette®, made from a UV-transparent plastic, is a good choice. With two optical path lengths to choose from (10 mm and 2 mm), it enables measurements across a broader range of concentrations than standard cuvettes, requiring a minimum sample volume of only 50 µL. Solutions of higher concentrations may be quantified using the Eppendorf µCuvette® G1.0, which features a path length of 1 mm. When using this micro-volume quartz cuvette, the sample volume may be reduced to 1.5 µL.

Fluorescence measurements may be performed with simple Vis cuvettes, such as the Eppendorf semi-micro or macro Vis cuvettes. The UVette and the µCuvette G1.0 are also well suited to this application, with the added advantage that smaller sample volumes are required, thus saving sample as well as reagents [5, 6, 7, 8, 9].



**Figure 7:** Pre-programmed fluorescence methods within the BioSpectrometer fluorescence

**Table 2: Overview of Eppendorf cuvettes**

Eppendorf cuvettes	Eppendorf Semi-micro + macro Vis cuvettes	Eppendorf UVette	Eppendorf µCuvette G1.0
Wavelength range	300 – 900 nm	220 – 1600 nm	180 – 2000 nm
Single-use item	yes	yes	no
Minimum volume Absorbance	400 µL/1000 µL	50 µL	1.5 µL
Minimum volume Fluorescence	400 µL/1000 µL	60 µL	5 µL

## Conclusion

Common methods for the quantification of nucleic acids include direct absorbance measurements as well as indirect measurements involving fluorescence. Both techniques exhibit specific advantages and disadvantages, and the choice is therefore dependent on the concentration and the purity of the sample, as well as on the demands of the downstream application. The BioSpectrometer fluorescence combines both functions in one compact instrument and

thus allows the benefits of both methods to complement each other perfectly. To enhance flexibility even further, a selection of cuvettes, comprising the Eppendorf Vis cuvettes, the UVette and the  $\mu$ Cuvette G1.0, is available to suit each need and application. The combination of both technologies covers a very broad measurement range (figure 6), while at the same time allowing determination of sample purity.

## Literature

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## About Eppendorf

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide. Its product range includes pipettes and automated pipetting systems, dispensers, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, CO<sub>2</sub> incubators, shakers, and cell manipulation systems. Associated consumables like pipette tips, test tubes, microtiter plates, and disposable bioreactors complement the instruments for highest quality workflow solutions.

Eppendorf was founded in Hamburg, Germany in 1945 and has about 3,000 employees worldwide. The company has subsidiaries in 25 countries and is represented in all other markets by distributors.

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