Application Note

# QUANTIFYING DNA WITH THE QUANT-IT™ PICOGREEN® dsDNA KIT AND BERTHOLD TRISTAR MULTIMODE READERS

## FAST, SIMPLE, AND ACCURATE NUCLEIC ACID QUANTIFICATION


## Abstract

DNA quantification is mostly done by absorbance at 260 nm, but suffers from low throughput, sensitivity, and specificity. The Quant-iT™ PicoGreen® dsDNA reagent is specific for dsDNA and is suitable for microplate readers. In combination with the Tristar 3 and Tristar 5 Multimode Microplate Readers, it allows the specific quantification of dsDNA in 96-well plates achieving a limit of detection below 0.1 pg/µL.


## Introduction

DNA quantification is an important pre-analytical method, which is of great importance for many molecular biological analysis methods and can even determine their success. It is also a routine technique in procedures for translational research such as Next-Generation Sequencing (NGS), Polymerase Chain Reaction (PCR) or Real-Time PCR (quantitative PCR; qPCR), cloning or transfection, which initiates the subsequent workflow.


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The most popular DNA quantification methods are based on UV-Vis- or fluorescence spectroscopy. Both methods have advantages and disadvantages.

**Quantification using absorbance**

Absorbance at 260 nm has been the method of choice for routine quantification of DNA and RNA since decades. It is simple and convenient to use as no further sample treatment (other than DNA extraction) is required. However, it is not very specific (it measures all nucleic acids as a whole) and it is sensitive to contaminants, so it demands very pure DNA to be accurate. Many of those contaminants can be estimated by measuring the absorbance of the sample at wavelengths other than 260 nm (usually at 230, 280 and 340 nm).

Absorbance of DNA samples at 260 nm is currently most often measured using a microvolume spectrophotometer, but it is also possible to use a microplate reader. Microplate readers can measure many samples in a short time (typical plate formats are 96- and 384-well), but they require larger sample volumes for the measurement than a microvolume spectrophotometer (up to 50 µL in standard 96-well plates, less in other microplate formats). However, microvolume microplates are available which use small sample volumes (usually 2 µL). While they don't

have as many sample positions as standard microplates (normally 16 instead of 96), they offer a good compromise between sample volume and throughput.

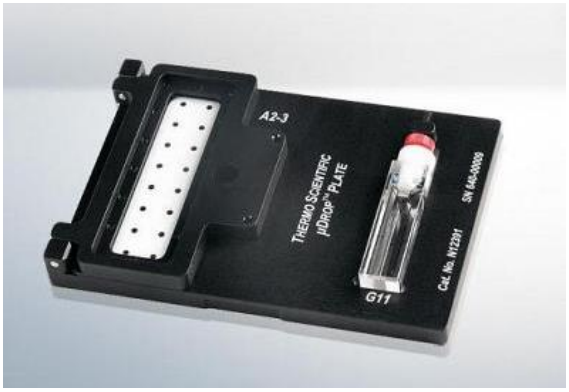


**Figure 1**: The µDrop plate can be used for DNA quantification using absorbance in a microplate reader using only 2 µL of sample and also features a cuvette port for increased flexibility.

**Quantification using fluorescence**

The use of fluorescent dyes permits the quantification of DNA with much higher sensitivity than measuring absorbance of DNA itself (typically 10-1000 times higher, depending on the specific methods compared). In addition, specific dyes can be used to stain only specific types of nucleic acid, such as dsDNA or RNA, thereby increasing the specificity of the quantification and reducing the effect of contaminants. However, fluorescence-based methods are more expensive than measuring absorbance at 260 nm, often require a standard curve to be prepared, and do not provide a direct estimate of the presence of contaminants (which may be important, for example, to evaluate the possible effects on downstream methods). Fluorescence measurement is performed using a microplate reader or a single tube fluorometer.

Quant-iT™ PicoGreen® dsDNA reagent is an ultrasensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution. Detecting and quantitating small amounts of DNA is extremely important in a wide variety of biological applications. The PicoGreen dsDNA quantitation reagent and kits are ideal for PCR-based assays, microarray samples, DNA damage assays, enzyme activity assays, genomic DNA quantitation, measuring dsDNA in complex mixtures, and viral DNA quantitation.

In this Application Note we report the suitability of the Tristar Multimode Microplate Readers to quantify dsDNA using the Quant-iT™ PicoGreen® dsDNA reagent and the recommended settings for this method.

# Tristar Series

## Application flexibility you can count on


Developed for high flexibility and equipped with the proprietary ONE-4-ALL optical system, the Tristar Serie combines the user friendliness of a multimodal optical system with the sensitivity and performance of a dedicated optical device. You can choose between the affordable Tristar 3 and the more advanced and flexible Tristar 5. The Tristar series provides you with flexibility for today, tomorrow, and beyond.

- Monochromator Technology
- High-sensitivity Luminescence
- BRET
- UV/VIS Absorbance
- UV/VIS Fluorescence
- UV/VIS FRET
- Time-Resolved Fluorescence (TRF)
- Time-Resolved FRET (TR-FRET / HTRF®)
- Fluorescence Polarization
- AlphaScreen®
- Top and Bottom Reading
- Incubation

## Materials

- Tristar 3 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69173-30).
- Tristar 5 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69185-15).
- Quant-iT™ PicoGreen® dsDNA Assay Kit from Invitrogen™ (Cat. # P7589).
- Black 96-well microplates from Berthold Technologies (Id. Nr. 23302).
- Tubes of various volumes.
- Pipettes and pipette tips (various volumes).

## Instrument settings

- Reading mode: Fluorescence Endpoint
- Excitation filter: 485/14
- Emission filter: 535/25
- Counting time: 0.1 s
- All other settings with default values

## Methods

Reagents were prepared following the manufacturer's instructions. Using the DNA standard included with the kit, two different standard curves were prepared: a high range standard curve, which could be used to quantify high concentration samples, and a low range standard curve, which could be used to quantify low concentration samples.

Equal volumes of standard and Quant-iT™ PicoGreen® reagent were mixed for each standard point. The final concentrations of each curve were the following ones:

- High range: 1, 10, 100, 250, 500, 750 and 1000 pg/µL dsDNA.
- Low range: 0.025, 0.25, 2.5, 5, 10, 20 and 25 pg/µL dsDNA

The mix was incubated for 5 minutes at room temperature in the dark. 200 µL of each mix were pipetted in triplicate in the wells of a black 96-well TE buffer was used as blank.

The plate was inserted in the multimode reader and measured with the settings detailed above. Blank values were subtracted from the values of the standard. Data were exported from the ICE software to xls format, and standard curves were drawn in Excel.

## Results

The High Range and Low range standard curves of the Tristar 3 and Tristar 5 Multimode Microplate Readers are displayed in Figure 2. All curves exhibit excellent linearity.

The calculated Limit of Detection in 96-well microplates was 0.069 pg/µL dsDNA (13.8 pg/well) for the Tristar 3 and 0.083 pg/µL dsDNA (16.6 pg/well) for the Tristar 5. When the measurement was performed in 384-well plates, the calculated Limit of Detection was 0.272 pg/µL dsDNA (19.0 pg/well) for the Tristar 3 and 0.110 pg/µL dsDNA (7.6 pg/well) for the Tristar 5.

With the settings used, a full 96-well plate can be measured in 33 seconds.

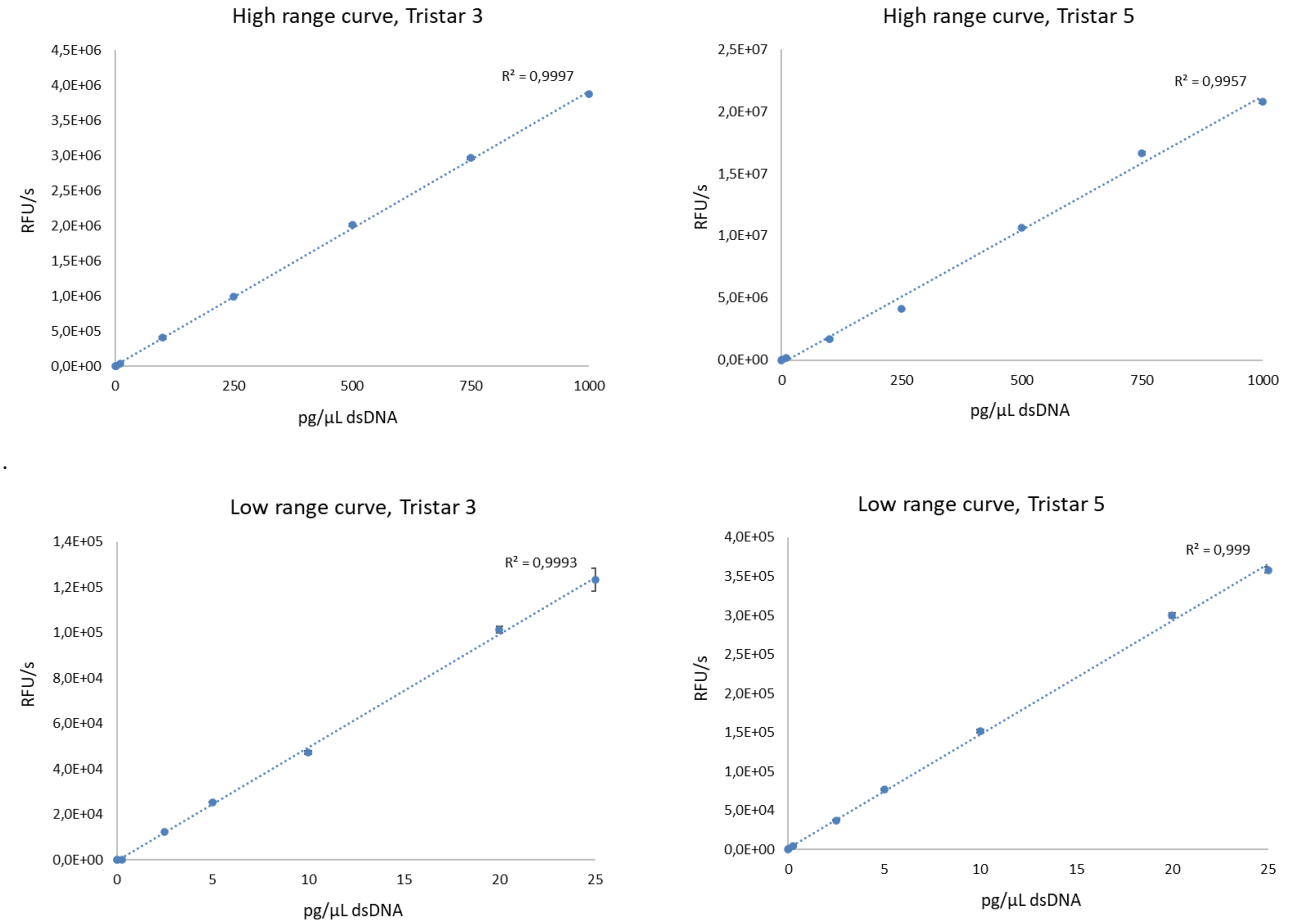


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**Figure 2:** dsDNA standard curves measured with a Tristar 3 (left) and Tristar 5 (right) Multimode Reader with standard fluorescein filters. All measurements in triplicate. Data are average ± SEM.

## Discussion and conclusions

Quantification of dsDNA is very often performed using a microvolume spectrophotometer. However, this method suffers from low throughput, as samples have to be measured one by one, and of low sensitivity, as the limit of detection is typically 2 ng/µL dsDNA (2000 pg/µL). While using the Quant-iT™ PicoGreen® dsDNA reagent involves some increase in costs and preparation time (as a standard curve must be prepared and measured), when used to measure dsDNA concentrations in the Tristar Multimode Microplate Readers, it allows to measure 96 wells under a minute while achieving a limit of detection below 0.100 pg/µL. This represents an improvement of sensitivity of 20,000-fold over the detection limit of a microvolume spectrophotometer. The limit of detection obtained with Tristar instruments is clearly better than the typical result for microplate readers which, according to the kit insert, is of 0.25 pg/µL.

The results demonstrate the high performance of the Tristar Multimode Microplate Readers for the quantification of dsDNA using the Quant-iT™ PicoGreen® dsDNA reagent. The system not only enables dsDNA concentration measurements over a wide dynamic range but can also help increase the throughput of this qualification step in important applications such as PCR assays, microarray samples, DNA damage assays, enzyme activity tests, or the quantification of viral DNA.

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