Sensitive fluorometric quantification of DNA in microplate format

Goal

This technical note demonstrates the feasibility of using the Thermo Scientific™ Fluoroskan™ or Fluoroskan FL instruments with the Invitrogen™ Quant-iT™ dsDNA high-sensitivity assays. Sensitive and specific quantification of double-stranded DNA is carried out in microplate format. This is part of a series of technical notes aiming to help customers choose the best reagents and instruments for their sample type, throughput, and expected concentration range.

Introduction

Fluorescent detection of nucleic acids is much more sensitive than the most commonly used absorbance measurement at 260 nm (A_{260}). The Quant-iT dsDNA assays are well-adapted to high-throughput use, and can be performed either in 96-well or 384-well plates. Samples processed using the Quant-iT dsDNA Assay Kits are read with a fluorometric microplate reader—here using a Fluoroskan or Fluoroskan FL instrument. Data can theoretically be plotted with any graphics software package of the user's choice. However, Thermo Scientific™ SkanIt™ Software provides ease of use and a range of other benefits.

In combination with Fluoroskan or Fluoroskan FL microplate fluorometers, the Quant-iT reagents provide rapid, specific, and accurate determination of nucleic acid concentrations over a wide range.

Materials	Cat. No.
Instrument	
Fluoroskan Microplate Fluorometer	5200110
Fluoroskan FL Microplate Fluorometer and Luminometer	5200220
Reagents	
Quant-iT dsDNA Assay Kit, High Sensitivity	Q33120
Microplates	
Nunc F96 MicroWell Black Polystyrene Plates	237108
Software	
SkanIt Software 5.0	5187139

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Quant-iT assays

The Quant-iT assays were performed according to the kit instructions. Briefly, the Quant-iT™ dsDNA reagent was diluted 1:200 (working solution). 200 µL of working solution and 10 µL of DNA (Quant-iT λ DNA standards) were loaded into each well of a 96-well microplate. The samples were thoroughly mixed with a multichannel pipette (Thermo Scientific™ Finnpipette™ F1, Cat. No. 4661030N). Fluorescence was measured using 485 nm excitation and 538 nm emission wavelengths on Fluoroskan and Fluoroskan FL instruments. Calibration curves and basic statistics were calculated using the SkanIt 5.0 software, using blank-subtracted values. Preconfigured SkanIt protocols can be downloaded from the SkanIt Protocol Library, accessible through SkanIt software 5.0.

Quant-iT dsDNA Assay Kit, High Sensitivity

Results

The Quant-iT High-Sensitivity dsDNA Assay Kit with Fluoroskan gives a linear standard curve between 5 and 100 ng of DNA (Figure 1). The average coefficient of variation (CV) of the standards was typically less than 2%.

To assess the reliability of the assay in the low range, we further diluted the standards in HS assay buffer. The standard curve was also linear in this lower range. We were able to experimentally detect 0.2 ng of λ DNA (Figure 2), which is in good agreement with the kit specifications. The calculated limit of detection (LOD), as determined by the IUPAC standard 3 SD method, was 0.04 ng in this particular experiment.

Standard curves were in close agreement between two Fluoroskan instruments (Figure 3). We studied the repeatability of the standard curve from assay to assay. Figure 4 shows the results of two repeated experiments that were run 4 months apart from each other on the same instrument with the same reagent lot.

Fluoroskan and Fluoroskan FL instruments give equally good, highly consistent standard curves (Figure 5).

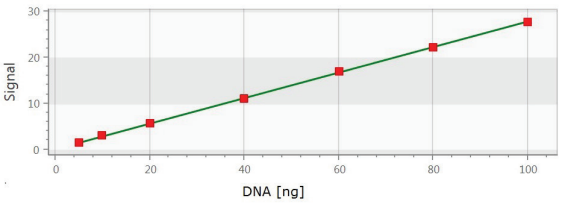

Figure 1. Standard curve generated using the Quant-iT High-Sensitivity dsDNA Assay Kit on a Fluoroskan instrument. DNA sample volume was 10 µL. Data points are the averages of 8 replicates.

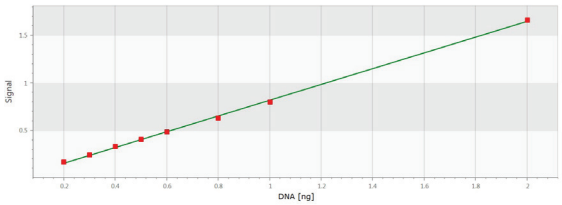

Figure 2. Standard curve in the low DNA concentration range.

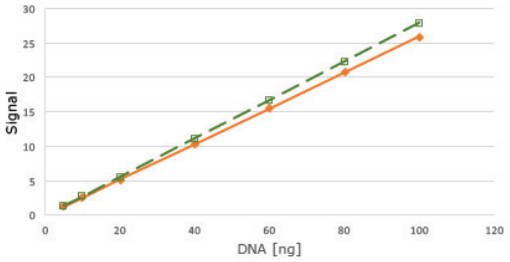

Figure 3. The standard curves are repeatable between instruments using the Quant-iT High-Sensitivity dsDNA Assay Kit in a 96-well plate format on a Fluoroskan instrument.

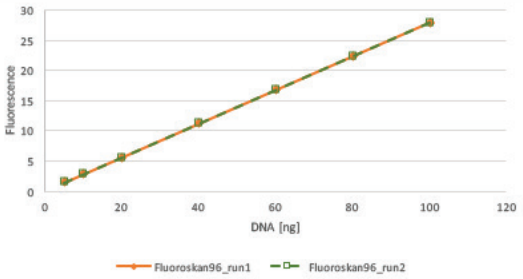

Figure 4. The standard curves are highly repeatable from assay to assay when using the same reagent lot.

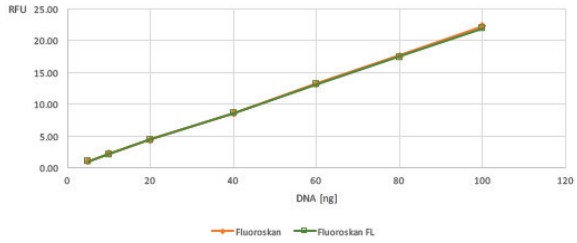

Figure 5. Quant-iT High-Sensitivity dsDNA Assay Kit standards measured on Fluoroskan and Fluoroskan FL instruments.

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Procedural notes

- Black microplates are recommended for fluorescence measurements, with minimum back-scattered light and background fluorescence.
- Thorough mixing of samples is very important to ensure accuracy of quantification.
- The Qubit dsDNA assay kits provide the same sensitivity, and they are convenient for fewer samples, since the samples are read one at a time in the Qubit™ fluorometer. The Quant-iT assays are available as a high-sensitivity (HS) assay kit and a broad-range (BR) assay kit. For both assays, sample processing is easy and the kits contain prediluted DNA standards. The samples can be read in a microplate reader in 96-well or 384-well plate format. Therefore, these assays are ideal for automated, high-throughput measurements. Separate kits are available for quantifying DNA, RNA, or protein, with minimal interference from common contaminants.
- We can calculate the LOD as an average of signal of blanks + 3 SD of blank. If one wants to express the LOD as a theoretically detectable amount of DNA, a standard curve is needed. Ideal experimental circumstances should be determined by the user. The results, including the calculated LOD, might diverge in different laboratories or over time. Ideal experimental conditions include the use of calibrated pipettes, fresh reagents, nondegraded DNA, appropriate numbers of repeats of each standard, and performance of the reader.

Conclusions

The Quant-iT fluorescence assays are several orders of magnitude more sensitive than UV absorbance measurements. Unlike measurements of UV absorbance, these assays are not affected by the presence of proteins, free nucleotides, or very short oligonucleotides, making the quantification of intact oligonucleotides and nucleic acids much more accurate in complex mixtures such as serum or whole blood.

The Fluoroskan and Fluoroskan FL readers with the SkanIt software provide a range of benefits for Quant-iT assay measurements, including:

- Simple protocol setup aided by the intuitive interface of the SkanIt software
- Effortless data analysis using SkanIt software's built-in calculations
- Ready-to-run protocols, including data analysis steps, available from the SkanIt Protocol Library, accessible through SkanIt software 5.0

Find out more at **thermofisher.com/platereaders**

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