

# Protein and DNA/RNA quantification assays with EnSpire: Monochromator-based absorbance and fluorescence technologies



EnSpire Multilabel Plate Reader

## Introduction

Protein and nucleic acid (DNA/RNA) quantitation or estimation assays are one of the most widely used methods in life science research. Estimation of macromolecule concentration is necessary in protein purification, electrophoresis, biochemistry, cell biology, molecular biology, and other research applications. Quantitation methods include spectrophotometry, fluorometry, and hybridization assays.

The most commonly used technique for determining nucleic acid concentration is based on measuring the absorbance at 260 nm with a spectrophotometer. Purity

(i.e., protein contamination) is checked by determining the ratio of absorbance measurements at 260 nm and 280 nm. This can be further combined with a 320 nm background correction.

Traditional methods for protein concentration measurement include colorimetric Bradford, Lowry and BCA assays. These assays measure visible absorbance and are performed on standard clear microplates. UV-compatible plates and readers are not required. Determination of protein absorbance at 280 nm represents a convenient label-free method for quantifying protein concentration.

## Authors

Ville-Veikko Oksa, Miika Talvitie  
 and Petri Kivelä  
 PerkinElmer, Inc.  
 Turku, Finland

Several quantification methodologies are frequently needed in research environments since the advantages and limitations of these assays are weighed based on the application in mind. Fluorescence technology, utilizing fluorescent dyes, is used when more sensitivity is needed and evaluation of sample purity is not required.

The EnSpire™ Multilabel Plate Reader supports the measurement of a variety of quantification applications. A flexible, easy-to-use instrument, EnSpire includes intuitive user software for running any absorbance or prompt fluorescence-based quantification assay. The software is controlled by using the touch screen.

Setting up measurement protocols with the EnSpire reader has been made simple and easy. The flexible monochromator-based system makes it possible to do scanning assays in addition to single wavelength measurements. New or existing measurement protocols are easily started without any prior instrument knowledge or training, fitting well in multi-user environments.

This application note describes the use of the EnSpire Multilabel Plate Reader to determine nucleic acid and protein concentration using UV-absorbance at 260 nm, the Quant-iT™ PicoGreen® assay and the Bradford assay.

## Materials and Methods

### DNA quantification with UV-absorbance (260 nm)

A DNA stock solution of 2 mg /mL was prepared by dissolving purified and lyophilized DNA (Salmon testes DNA; #D1626, Sigma) in water. A dilution series from 0.1 to 100 µg /mL was prepared in triplicate and transferred into a UV-absorbance compatible microplate (96-well UV-Star® plate; #655801, Greiner). DNA quantification was performed by reading the absorbance at 260 nm.

### Quant-iT™ PicoGreen® dsDNA

#### Quantification Assay

The Quant-iT™ PicoGreen® dsDNA Assay Kit (# P11496, Molecular Probes) contained both the purified Lambda DNA standard and TE buffer stock used in the assay. A working solution of 1xTE was made by diluting the provided stock buffer with sterile water (#KKF7113, Baxter). A working solution of the Quant-iT™ PicoGreen® reagent was similarly prepared according to the kit information sheet by making a 1:200 dilution with the 1xTE buffer. A dilution series of the Lambda DNA standard was prepared in 1xTE buffer to cover a range of 0.16–1,000 ng /mL. Samples with the added PicoGreen® reagent were pipetted into a black opaque 96-well microplate (black OptiPlate™; #6005279, PerkinElmer) in triplicate and the fluorescence signal read. The wavelengths used for excitation and emission (496/525 nm) were chosen based on the excitation and emission spectra of PicoGreen dye (Figure 3).

#### Bradford Protein Quantification Assay

Albumin from bovine serum (BSA; #05479, BioChemika) was dissolved in water to 2 mg/mL and a dilution series was prepared

following Bio-Rad's microassay procedure for microtiter plates (0–80 µg /mL, 4 replicates). The assay was performed in a clear bottom 96-well SpectraPlate MB (#6005279, PerkinElmer). The Protein Assay Dye Reagent concentrate (#500-0006, Bio-Rad) was mixed with the protein dilution samples and added to the wells. The plate was shaken and incubated at room temperature prior to reading the absorbance at 595 nm. All data was analyzed with GraphPad Prism®.

## Results and Discussion

### UV-absorbance (260 nm) DNA Quantification Assay

The absorbance assay at 260 nm shows a good linearity over the full range of concentrations used (Figure 1). The true linear concentration range of this absorbance assay is confined to two decades (1–100 µg /mL) and necessitates the use of multiple sample dilutions for accurate quantitation of samples with DNA concentration above the standard curve's linear range.

### Quant-iT PicoGreen dsDNA Assay

The fluorescence-based dsDNA quantification assay presents a linear range covering

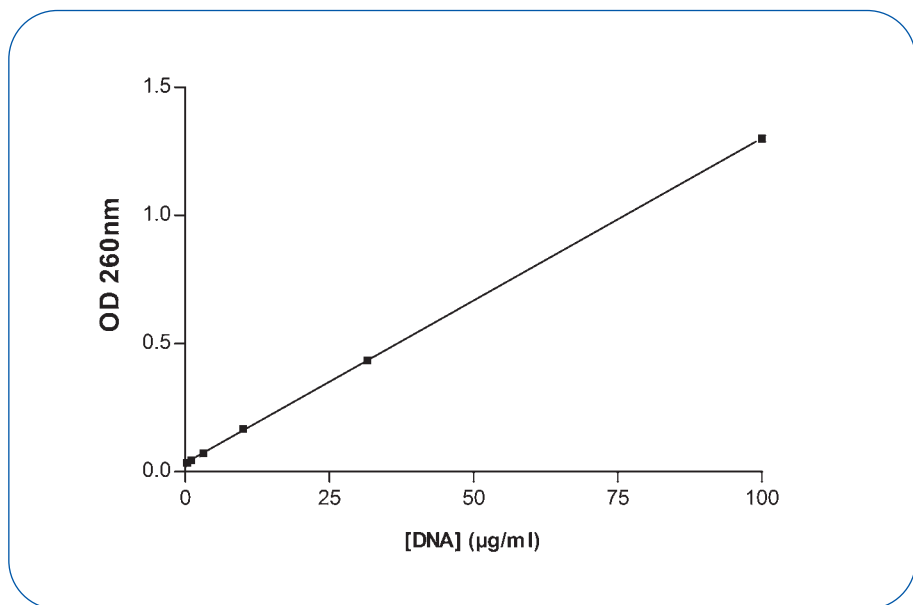


Figure 1. A DNA dilution series resulted in a linear fit, demonstrating that this is a robust method for nucleic acid quantification, although it suffers from relatively low dynamic and linear range.

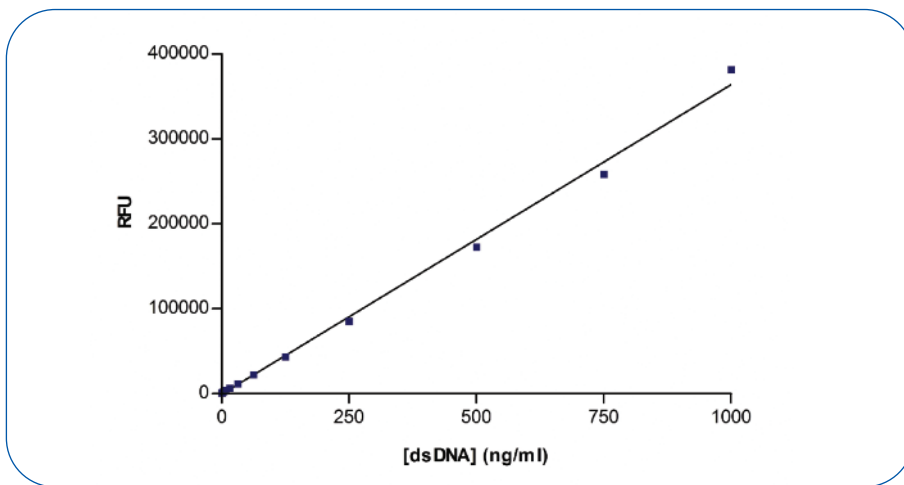


Figure 2. Quant-iT™ PicoGreen® dsDNA reagent responds to the increasing double stranded DNA concentration linearly and over a large range of concentrations. The highest sample concentration was used to determine the instrument settings (primarily the detector gain) used for the whole assay. Illustration made with GraphPad Prism® software.

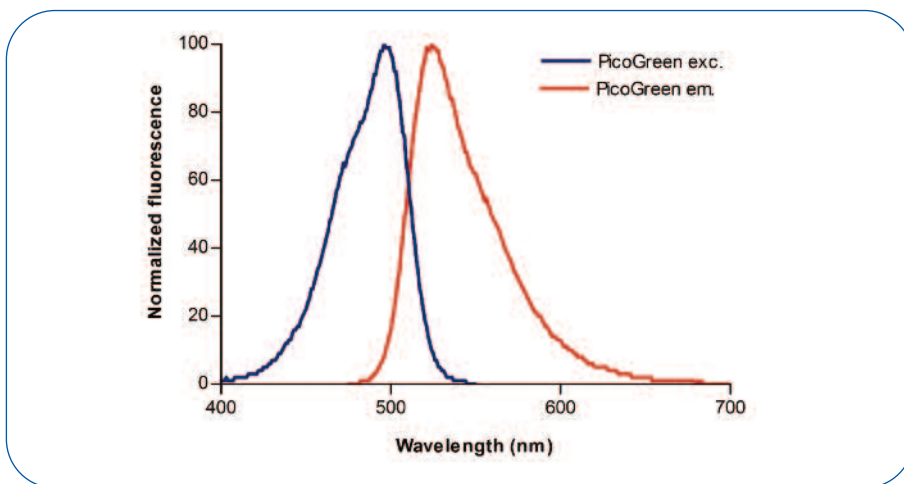


Figure 3. Spectra of Quant-iT™ PicoGreen® dye illustrates its excitation and emission properties and was used to define the best wavelength settings for the instrument. Fixed wavelength at 450 nm and 575 nm were used in scanning the spectra for emission and excitation, respectively.

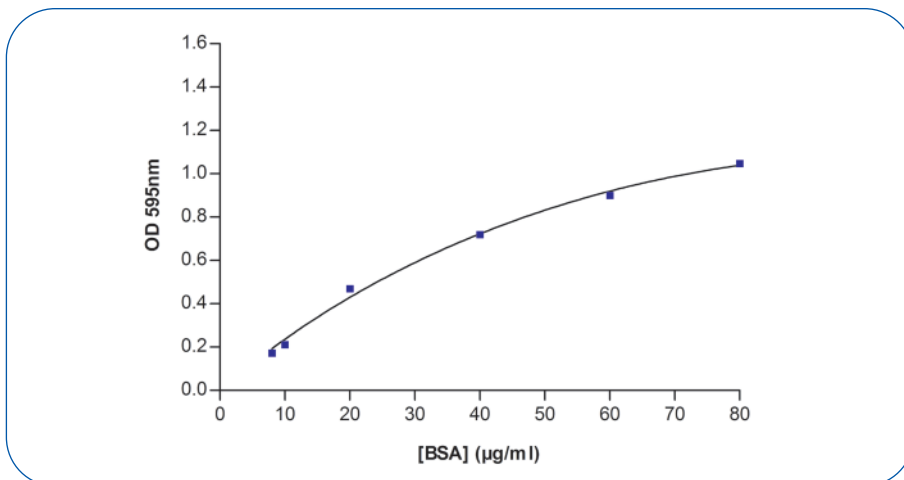


Figure 4. Bradford assay with background subtracted absorbance values. The assay presents a considerably narrow linear concentration response. The linear range limited to one order of magnitude often requires extra sample dilutions.

over four decades of DNA concentrations (approx. 0.1–1,000 ng /mL) highlighting the benefits of using a fluorescence-based approach over UV absorbance methods for DNA quantitation applications (Figure 2). With a larger dynamic range, the necessity of sample dilutions is minimized, smaller concentrations can be detected, and more of the samples may be saved for further studies.

### Bradford Protein Quantification Assay

Protein quantification using absorbance at 595 nm is based on the ability of the Bradford reagent to undergo a shift in absorbance as a protein binds to a Coomassie® Brilliant Blue G-250 dye. The absorbance is monitored at 595 nm as it exhibits the greatest shift in the dye’s absorbance when the protein concentration is changed. The microassay procedure used produced results consistent with the manufacturer’s reported linear range for this method (8–80 µg /mL for BSA). Despite the narrow linear response range, the overall robustness of the assay makes it an easy and popular method for protein quantification (Figure 4).

### Summary

The EnSpire Multilabel Plate Reader combines an easy-to-use and high performance instrument in one package. As demonstrated by these three common quantification assays, this reader’s interactive, touch screen-based software interface and built-in data analysis capabilities make it an excellent choice for many types of laboratories. In this study, the EnSpire produced linear and precise assay data, which was well in line with the kit inserts.

**PerkinElmer, Inc.**  
940 Winter Street  
Waltham, MA 02451 USA  
Phone: (800) 762-4000 or  
(+1) 203-925-4602  
[www.perkinelmer.com](http://www.perkinelmer.com)



---

For a complete listing of our global offices, visit [www.perkinelmer.com/ContactUs](http://www.perkinelmer.com/ContactUs)

©2009 PerkinElmer, Inc. All rights reserved. The PerkinElmer logo and design are registered trademarks of PerkinElmer, Inc. GraphPad Prism is a registered trademark of GraphPad Software Inc. EnSpire and OptiPlate are trademarks of PerkinElmer, Inc. or its subsidiaries, in the United States and other countries. UV-Star is a registered trademark of Grenier Bio-One. Quant-iT is a trademark and PicoGreen is a registered trademark of Molecular Probes, Incorporated. Coomassie is a registered trademark of Imperial Chemicals Industries, Ltd. All other trademarks not owned by PerkinElmer, Inc. or its subsidiaries that are depicted herein are the property of their respective owners. PerkinElmer reserves the right to change this document at any time without notice and disclaims liability for editorial, pictorial or typographical errors.