

Quantification of dsDNA Using Qubit dsDNA Broad Range (BR) Assay Kit

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Abstract

Nucleic acid quantitation remains a central component of myriad workflows across a wide spectrum of biological sciences. Many of these workflows require accurate determination of sample concentration from small sample volumes. The use of enhancing reagents provides a means to perform such analyses while conserving precious sample.

Introduction

Quantitative analysis of double-stranded deoxyribonucleic acid (dsDNA) can be performed by many methods. The method selected will often depend on the nature and concentration of the sample being analyzed. Native measurements taking advantage of absorption of light by dsDNA in the ultraviolet (UV) wavelength range will often be the first choice due to simplicity. However, limited sample volume or samples with contaminating species may require a more selective and sensitive assay method. Often fluorescence binding dyes that chelate with target analyte are used resulting in a signal proportional to analyte concentration with high specificity. This allows the analyte of interest to be quantified in a complex mixture such as those typically found during the initial steps of a purification protocol or molecular genetics process. In such cases, UV absorbance determinants may result in overestimation of sample concentration due to absorption by small amounts of residual contaminant. This becomes increasingly critical when the efficiency of downstream processes such as qPCR and mammalian cell transformation depends on a reliable known starting concentration of nucleic acid.

Materials and methods

Microplate-based assay

Herring sperm DNA, part number D6898, was purchased from Sigma-Aldrich (St. Louis, MO). Qubit dsDNA BR Assay Kit, part number Q32850, was purchased from Thermo Fisher Scientific (Waltham, MA). Solid 96-well black microplates (part number 3915), were from Corning (Corning, NY). Herring sperm DNA was diluted to 20 $\mu\text{g}/\text{mL}$ with TE (10 mM Tris, 1 mM EDTA, pH 7.5) as the diluent. The final concentration was confirmed using 260 nm absorbance. A series of dilutions ranging from 0.0 to 100 $\text{ng}/\mu\text{L}$ of purified herring sperm was made using TE and 10 μL aliquots pipetted into microplate wells. Working Qubit reagent was prepared by diluting the concentrated Qubit stock solution 1:200 with Qubit dsDNA BR buffer according to the manufacturers' recommendations. Qubit working quantitation reagent (190 μL) was added to each well with mixing and incubated for two minutes at room temperature, protected from light.

Instrumentation

Fluorescence was determined using an Agilent BioTek Synergy LX multimode reader. The onboard software and touch screen interface were used to select the appropriate filter cube, define shaking parameters, add delays, and select well locations and protocol parameters. A GFP filter cube (EX 485/20 nm, EM 528/25 nm, and 510 nm cutoff dichroic mirror) was used for all measurements. The gain value was set to 100 for microplate measurements, with Extended Dynamic Range selected. Alternatively, the instrument can be controlled and analysis performed in the Agilent BioTek Gen5 microplate reader and imager software.

Results and discussion

Many workflows involving dsDNA will require conservation of sample during routine procedures such as quantification for downstream processes. Reagents, such as fluorescent intercalating dyes, have been developed to enhance signal from a low volume of nonrecoverable sample. Fluorescence enhancing reagents can provide quantification of nucleic acids down to subpicogram per microliter concentrations. The Qubit dsDNA BR Assay kit contains a highly selective enhancing reagent specific for quantifying dsDNA using volumes as low as 1 μL in a standard 96-well microplate format. The assay is a homogeneous, mix-and-read format where the fluorescence is proportional to analyte present. The use of a serial dilution of dsDNA standards while performing the assay provides a means to extrapolate sample concentrations from the standard curve. Excellent linearity is seen across a wide range of concentrations (Figure 1). Sample volumes can vary from 1 to 20 μL by adjusting the reagent volume to a final assay volume of 200 μL .

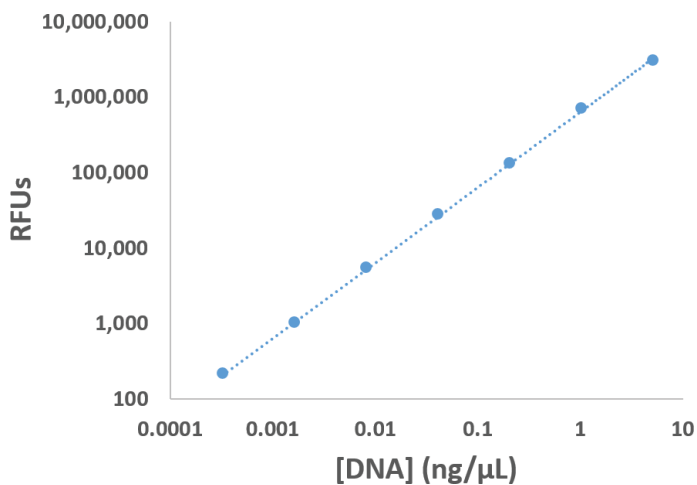


Figure 1. Microplate-based assay. Double-stranded DNA standards were analyzed in triplicate at a volume of 200 μL in a solid black microplate. DNA concentrations are those in the final assay volume.

Conclusion

The use of enhancing reagents for analysis of biomolecules increases the dynamic range of concentrations that can be accurately quantified. Furthermore, many of these reagents have increased specificity for the desired target molecule allowing accurate quantification if small amounts of impurities remain from upstream processes. When used in a microplate-based assay format increased throughput can be realized, with small sample volumes, for quantification down to the low nanogram per microliter range, typical of many midstream yields in various experimental workflows.