Quantification of dsDNA using Qubit® dsDNA Broad Range (BR) Assay Kit

Peter J. Brescia, Applications Scientist, BioTek Instruments, Inc., Winooski, VT USA

Key Words:

Qubit
dsDNA Quantification
Fluorescence

BioTek Instruments, Inc.
P.O. Box 998, Highland Park,
Winooski, Vermont 05404-0998 USA
Phone: 888-451-5171
Outside the USA: 802-655-4740
Email: **customercare@biotek.com**
www.biotek.com
Copyright © 2018

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 very 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 a variety of 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 ultra-violet (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 over-estimation of sample concentration due to absorption by small amounts of residual contaminant. This become increasing 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, cat # D6898 was purchased from Sigma-Aldrich. Qubit® dsDNA BR Assay Kit, cat #Q32850 was purchased from Thermo-Fisher. Solid 96-well black microplates, (cat # 3915), were from Corning. Herring sperm DNA was diluted to 20 µg/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 ng/µL 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 2 minutes at room temperature, protected from light.

Instrumentation

Fluorescence was determined using a Synergy™ LX Multi-Mode Reader. The onboard software and touchscreen 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 Gen5™ software.

Results and Discussion

Many workflows involving dsDNA will require conservation of sample during routine procedures such as quantification for downstream processes. Reagent such as fluorescent intercolating dyes have been developed to enhance signal from a very low volume of non-recoverable sample. Fluorescence enhancing reagents can provide quantification of nucleic acids down to sub-picogram 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 – 20 µL by adjusting the reagent volume to a final assay volume of 200 µL.

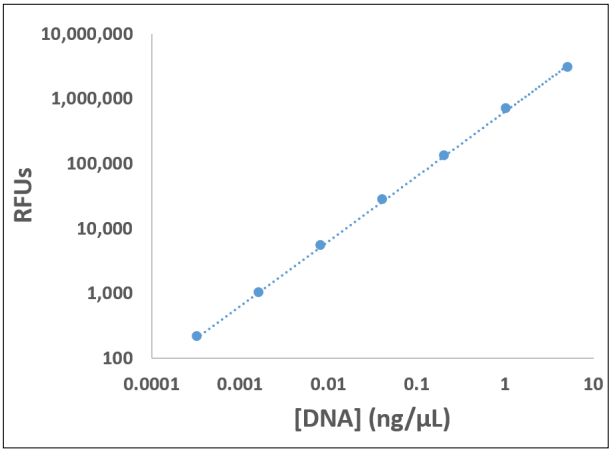

Figure 1. **Microplate based assay.** Double-stranded DNA standards were analyzed in triplicate at a volume of 200 µL in a solid black microplate. DNA concentrations are those in the final assay volume.

Conclusions

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, in combination with small sample volumes, for quantification down to the low nanogram per microliter range, typical of many mid-stream yields in various experimental workflows.