Comparison of fluorescence-based quantitation with UV absorbance measurements

Qubit® fluorometric quantitation vs. spectrophotometer measurements

Detection and quantitation of nucleic acids are vital to many biological studies. Historically, DNA and RNA have been quantified using spectrophotometry to measure absorbance at 260 nm. Although this method is commonly used, it can be unreliable and inaccurate [1–4]. UV absorbance measurements are not selective and cannot distinguish DNA, RNA, or protein. Values are easily affected by other contaminants (e.g., free nucleotides, salts, and organic compounds) and variations in base composition. In addition, the sensitivity of spectrophotometry is often inadequate, prohibiting quantitation of DNA and RNA at low concentrations.

In light of these drawbacks, the use of fluorescent dyes to quantitate nucleic acids has become a common alternative [5–8]. Fluorescence-based quantitation is more sensitive and is often specific for the nucleic acid of interest. We compared the two quantitation methods using the Qubit® Fluorometer and a microvolume UV spectrophotometer. We conclude that Qubit® fluorometric quantitation provides a more selective, sensitive, and accurate method for quantitating nucleic acids than UV absorbance measurements. However, the Qubit® Fluorometer and microvolume UV spectrophotometer may be used together to determine RNA or DNA concentration—the Qubit® Fluorometer for accurate concentration determination, and the microvolume UV spectrophotometer to show the presence of contaminants.

Table 1. Qubit® assay kits for use with the Qubit® Fluorometer.

Kit	Sample starting concentration range
Qubit® dsDNA HS assay	10 pg/µL–100 ng/µL
Qubit® dsDNA BR assay	100 pg/µL–1 µg/µL
Qubit® ssDNA assay	50 pg/µL–200 ng/µL
Qubit® RNA assay	250 pg/µL–100 ng/µL
Qubit® RNA BR assay	1 ng/µL–1 µg/µL
Qubit® protein assay	12.5 µg/mL–5 mg/mL

Overview of Qubit® fluorometric quantitation

Qubit® fluorometric quantitation combines a user-friendly fluorometer with highly sensitive fluorescence-based quantitation assays. The Qubit® Fluorometer is a small, economical instrument designed to work seamlessly with Qubit® assay kits for routine DNA, RNA, and protein quantitation (Table 1). All settings and calculations are performed for you. The system is simple, fast, and easy to use, yet enables you to consistently obtain accurate results so that you can be confident moving forward with subsequent applications. Each Qubit® assay kit is highly specific for a single analyte, and all are more sensitive than absorbance-based measurements. Only small sample volumes of 1–20 µL are required, which means less sample is used for quantitation and more sample is available for analysis.

life technologies

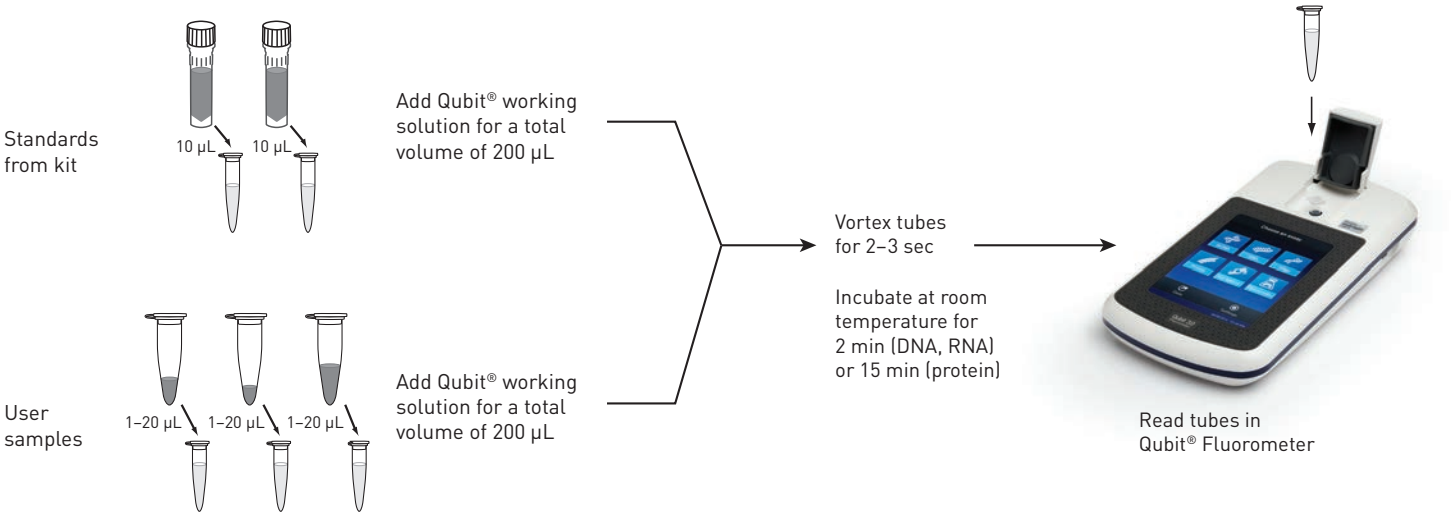

Figure 1. Workflow for the Qubit® assays using the Qubit® 2.0 Fluorometer.

The Qubit® assays, designed for use with the Qubit® Fluorometer, are all performed using the same general protocol. A simple mix-and-read format is used, with an incubation time of only 2 minutes required for DNA and RNA assays (Figure 1).

Comparison of selectivity for DNA or RNA

The most significant difference between using Qubit® fluorometric quantitation and UV absorbance to measure nucleic acid concentrations is the selectivity of the Qubit® assays, which are very specific for the molecule of interest and provide much more accurate information than UV absorbance. With UV analysis, results for samples containing both DNA and RNA are nondiscriminatory—you cannot distinguish one from the other. In contrast, Qubit® fluorometric quantitation is able to accurately measure both DNA and RNA in the same sample (Figure 2). In this experiment, the DNA concentration of a sample containing equal parts DNA and RNA was measured within 2% of the actual concentration using the Qubit® dsDNA BR Assay Kit. Furthermore, in a sample containing a 10-fold excess of RNA over DNA, the concentration determined in the DNA assay was only 7% higher than the actual concentration. DNA and RNA in samples such as these could not be accurately measured by UV absorbance on a microvolume UV spectrophotometer, potentially increasing the likelihood of error in subsequent applications.

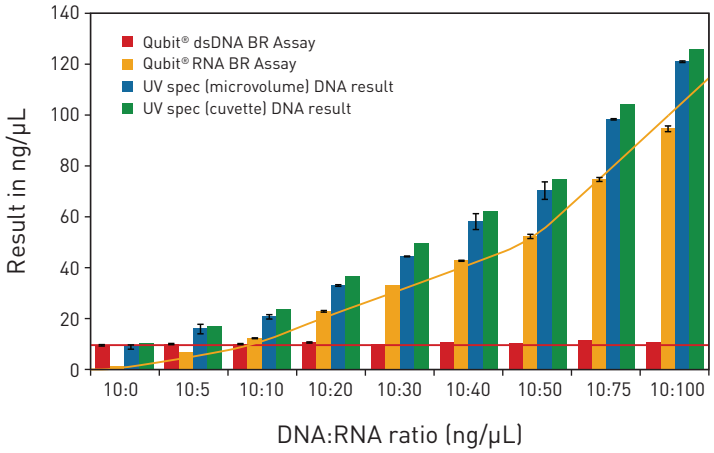

Figure 2. Selectivity of the Qubit® assays compared to UV spectrophotometry. Triplicate samples containing lambda DNA (10 ng/µL) and varying amounts of *E. coli* ribosomal RNA (0–100 ng/µL) were assayed using Qubit® dsDNA BR and Qubit® RNA BR assays on the Qubit® Fluorometer according to kit protocols. The same samples were subsequently measured in triplicate using a microvolume UV spectrophotometer, and single measurements were made using a cuvette-based UV spectrophotometer. The concentrations indicated are the concentrations of DNA and RNA in the starting samples, before dilution in the Qubit® assay tubes. The red and orange trendlines indicate the actual concentrations of DNA and RNA, respectively, in the starting samples. The actual concentration of nucleic acid was set by diluting pure, concentrated solutions of DNA and RNA (separately) to an optical density of 1.0 at 260 nm using a cuvette-based UV spectrophotometer. The concentrations of the stock solutions were then calculated and used for all subsequent dilutions. With UV analysis, results for samples containing both DNA and RNA are nondiscriminatory—you cannot distinguish one from the other.

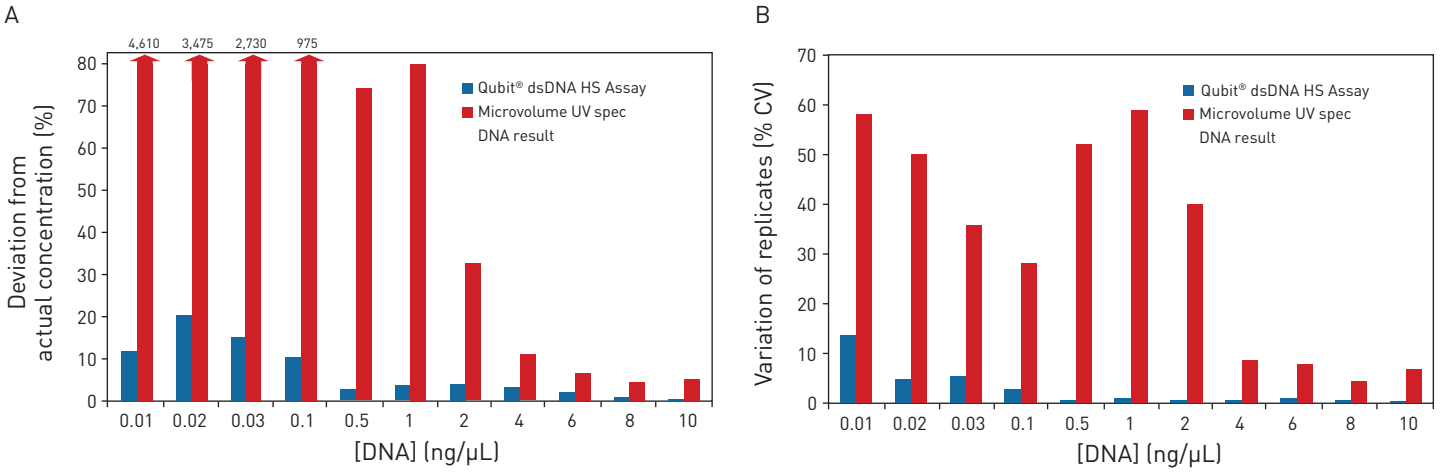

Figure 3. Accuracy and precision of Qubit® fluorometric quantitation. Ten replicates of lambda DNA at concentrations from 0.01 to 10 ng/µL were assayed using the Qubit® dsDNA HS Assay on the Qubit® Fluorometer according to the standard kit protocol. The same concentrations of DNA were measured in 10 replicates using a microvolume UV spectrophotometer, and results were compared for both accuracy (A) and precision (B). Accuracy was defined as the average deviation from the known concentration. The concentrations indicated are the concentrations of DNA in the starting samples, before dilution in the Qubit® assay tubes.

Comparison of accuracy and precision at low concentrations

Qubit® fluorometric quantitation is designed to generate more accurate and precise results across a lower concentration range than those obtained by UV absorbance measurements such as on a microvolume UV spectrophotometer. Using the Qubit® dsDNA HS Assay Kit, the Qubit® Fluorometer quantifies DNA in samples with concentrations as low as 10 pg/µL to within 12% of the actual concentration (Figure 3A). In contrast, a microvolume spectrophotometer cannot measure samples at this low concentration. Samples containing 10 ng/µL of DNA (the reported low end of quantitation for a microvolume UV spectrophotometer is 2 ng/µL) are accurately read to within 1% of the actual concentration when using the Qubit® Fluorometer and 5% when using a microvolume UV spectrophotometer. In addition, the variation of replicates (% CV) for all samples containing at least 0.5 ng/µL of DNA was ⩽1% using the Qubit® Fluorometer (Figure 3B). Only samples above 10 ng/uL yielded similar results for the microvolume UV.

Comparison of sensitivity and range

Qubit® assays used on the Qubit® Fluorometer are more sensitive than UV absorbance measurements, and because the assays can tolerate 1–20 µL of sample, the effective range of the assays can be increased (Figure 4). Together, the Qubit® dsDNA HS and BR assays cover a sample concentration range of 10 pg/µL to 1 µg/µL DNA. Similarly, the Qubit® RNA and RNA BR assays cover a sample concentration range of 250 pg/µL to 1 µg/µL. The microvolume UV spectrophotometer covers a sample concentration range of 2 ng/µL to 15 µg/µL, as reported by the manufacturer.

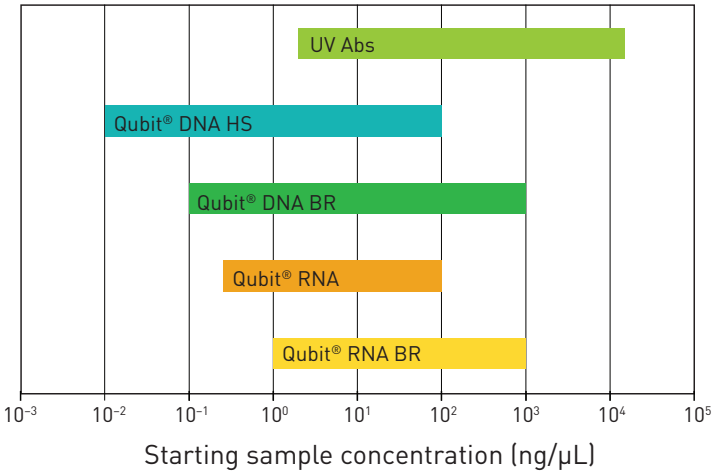

Figure 4. Comparison of sample concentration ranges for the Qubit® assays and UV absorbance measurements using a microvolume UV spectrophotometer.

Ability to detect the presence of contaminants

Microvolume UV spectrophotometer full-spectrum absorption readings can give peaks revealing the presence of contaminants. This may be useful information for downstream applications in which the contaminants might be detrimental.

References

1. Glasel JA (1995) *Biotechniques* 18:62–63.
2. Huberman JA (1995) *Biotechniques* 18:636.
3. Manchester KL (1995) *Biotechniques* 19:208–210.
4. Manchester KL (1996) *Biotechniques* 20:968–970.
5. Singer VL, Jones LJ, Yue ST et al. (1997) *Anal Biochem* 249:228–238.
6. Jones LJ, Yue ST, Cheung CY et al. (1998) *Anal Biochem* 265:368–374.
7. LePecq JB (1966) *Anal Biochem* 17:100–107.
8. Kapuscinski J (1995) *Biotech Histochem* 70:220–233.

Find out more at **lifetechnologies.com/qubit**

life technologies

A Thermo Fisher Scientific Brand

For Research Use Only. Not for use in diagnostic procedures. © 2014 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. PerkinElmer is a trademark of PerkinElmer, Inc. NanoDrop is a trademark of NanoDrop Technologies LLC. **CO29244 1214**