# 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 variation 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 Invitrogen™ Qubit™ Fluorometer and the Thermo Scientific™ NanoDrop™ ND-1000 Spectrophotometer. We conclude that Qubit fluorometric quantitation is a more selective, sensitive, and accurate method for quantitating nucleic acids than UV absorbance measurements obtained with the NanoDrop ND-1000 Spectrophotometer. However, the Qubit Fluorometer and NanoDrop Spectrophotometer may be used together to determine RNA or DNA concentration—the Qubit Fluorometer for accurate quantitation, and the NanoDrop Spectrophotometer to show the presence of contaminants.

### 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 Invitrogen™ Qubit™ assay kits for routine DNA, RNA, and protein quantitation (Table 1). All settings and calculations are selected and 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.

**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–1,000 ng/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–1,000 ng/µL |
| Qubit RNA XR assay | 1 ng/µL–8 µg/µL |
| Qubit protein assay | 12.5 µg/mL–5 mg/µL |

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.

### Comparison of selectivity for DNA or RNA

The most significant difference between Qubit fluorometric quantitation and UV absorbance for measuring nucleic acid concentrations is the selectivity of the Qubit assays, which are very specific for the molecule of interest. With UV analysis, results for samples containing both DNA and RNA 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 1). In this experiment, the DNA concentration of a sample containing equal parts of 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 the NanoDrop Spectrophotometer, potentially increasing the likelihood of error in subsequent applications.

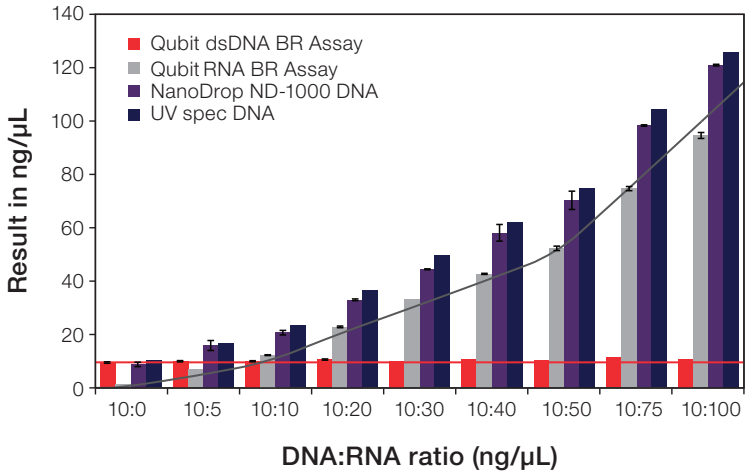


**Figure 1. Selectivity of the Qubit assays compared to UV spectrophotometry.** Triplicate samples containing lambda DNA (10 ng/µL) and varying amounts of *E. coli* ribosomal RNA (0–100 ng/µL) were assayed using Qubit dsDNA BR and Qubit RNA BR assays on the Qubit Fluorometer according to kit protocols. The same samples were subsequently measured in triplicate using a NanoDrop ND-1000 Spectrophotometer, and single measurements were made using a PerkinElmer™ Lambda 35 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 gray trendlines indicate the actual concentrations of DNA and RNA, respectively, in the starting samples. The actual concentration of nucleic acid was designated by diluting pure, concentrated solutions of DNA and RNA (separately) to an optical density of 1.0 at 260 nm using a PerkinElmer Lambda 35 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 cannot distinguish one from the other.

## 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. Using the Qubit dsDNA HS Assay Kit, the Qubit Fluorometer quantifies DNA in samples with starting concentrations as low as 10 pg/µL, to within 12% of the actual concentration (Figure 2A). In contrast, the concentrations of these same samples were overestimated 46-fold when measured with the NanoDrop Spectrophotometer. Samples containing 10 ng/µL of DNA (the reported low end of quantitation for the NanoDrop Spectrophotometer is 2 ng/µL) are accurately read to within 1% of the actual concentration when using the Qubit Fluorometer and 5% when using the NanoDrop Spectrophotometer. In addition, the coefficient of variation (CV) of replicates for all samples containing at least 0.5 ng/µL of DNA was ≤1% using the Qubit Fluorometer (Figure 2B). Only samples containing 4 ng/µL of DNA or higher resulted in CV values lower than 9% using the NanoDrop Spectrophotometer.

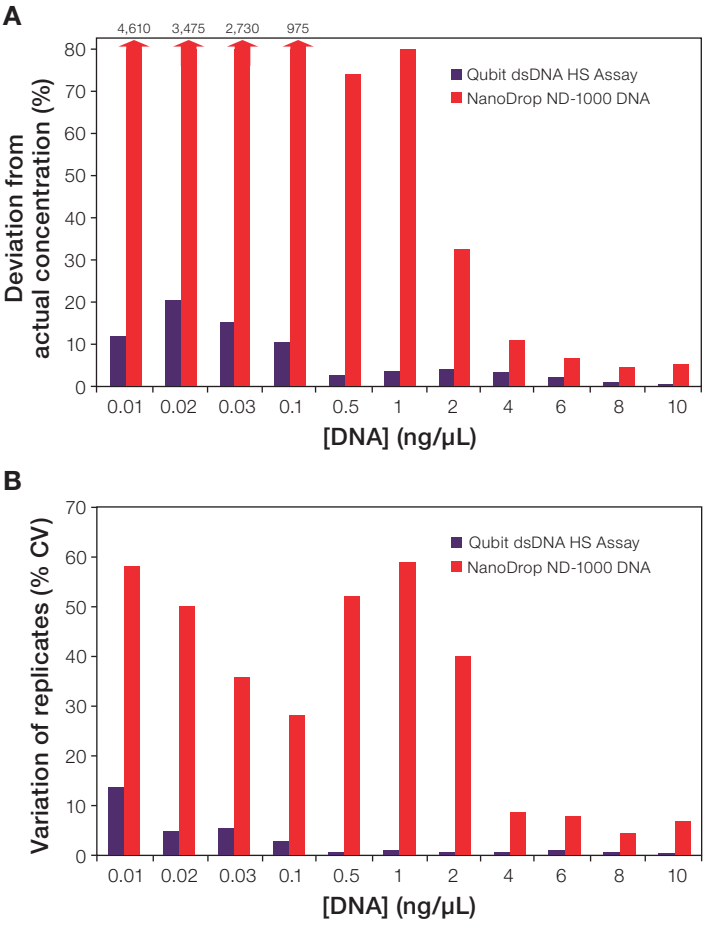


**Figure 2. Accuracy and precision of Qubit fluorometric quantitation.** Ten replicates of lambda DNA at concentrations from 0.01 to 10 ng/µL were assayed using the Qubit dsDNA HS Assay on the Qubit Fluorometer according to the standard kit protocol. The same concentrations of DNA were measured in 10 replicates using a NanoDrop ND-1000 Spectrophotometer, and results were compared for both **(A)** accuracy and **(B)** precision. 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 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 3). Together, the Qubit dsDNA HS and BR assays cover a starting sample concentration range of 10 pg/µL to 1,000 ng/µL (1 µg/µL) DNA. Similarly, the Qubit RNA and RNA BR assays cover a starting sample concentration range of 250 pg/µL to 1,000 ng/µL (1 µg/µL). The NanoDrop Spectrophotometer covers a starting sample concentration range of 2 ng/µL to 15 µg/µL.

## The NanoDrop Spectrophotometer is able to detect the presence of contaminants

Full-spectrum absorption readings on the NanoDrop Spectrophotometer can give peaks revealing the presence of contaminants. This may be useful information for downstream applications in which the contaminants might be detrimental.

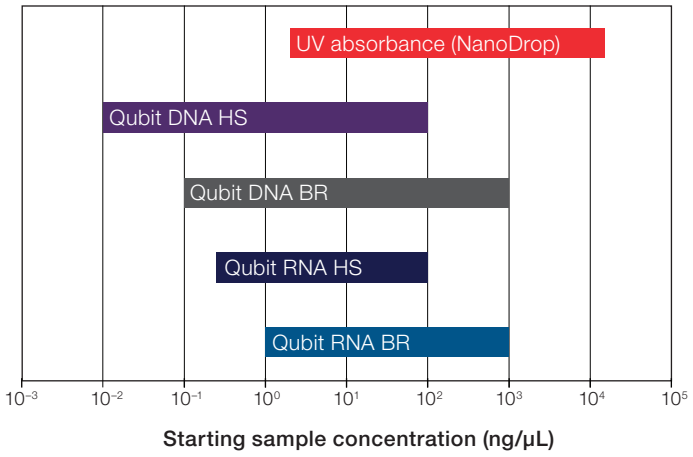


**Figure 3. Comparison of sample concentration ranges for the Qubit assays and UV absorbance measurements using the NanoDrop Spectrophotometer.**

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References

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## Ordering information

| Product | Initial sample concentration | Quantitation range | Quantity | Cat. No. |
|---|---|---|---|---|
| **DNA kits** | | | | |
| Qubit ssDNA Assay Kit | 50 pg/µL to 200 ng/µL | 1–200 ng | 100 assays | Q10212 |
| Qubit dsDNA BR Assay Kit | 100 pg/µL to 1,000 ng/µL | 2–1,000 ng | 100 assays | Q32850 |
| | | | 500 assays | Q32853 |
| Qubit dsDNA HS Assay Kit | 10 pg/µL to 100 ng/µL | 0.2–100 ng | 100 assays | Q32851 |
| | | | 500 assays | Q32854 |
| Qubit 1X dsDNA HS Assay Kit | 10 pg/µL to 100 ng/µL | 0.2–100 ng | 100 assays | Q33230 |
| | | | 500 assays | Q33231 |
| **RNA kits** | | | | |
| Qubit RNA BR Assay Kit | 1 ng/µL to 1 µg/µL | 20–1,000 ng | 100 assays | Q10210 |
| | | | 500 assays | Q10211 |
| Qubit RNA HS Assay Kit | 250 pg/µL to 100 ng/µL | 5–100 ng | 100 assays | Q32852 |
| | | | 500 assays | Q32855 |
| Qubit RNA XR Assay Kit | 1 ng/µL to 8 µg/µL | 20 ng–8 µg | 100 assays | Q33223 |
| | | | 500 assays | Q33224 |
| Qubit microRNA Assay Kit | 50 ng/mL to 100 µg/mL | 1–100 ng | 100 assays | Q322880 |
| | | | 500 assays | Q322881 |
| **Instrument and accessories** | | | | |
| Qubit 4 Fluorometer | | | 1 | Q33226 |
| Qubit 4 Quantitation Starter Kit | | | 1 kit | Q33227 |
| Qubit 4 NGS Starter Kit | | | 1 kit | Q33228 |
| Qubit Assay Tubes | | | 500 tubes | Q32856 |

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