

Qubit 1X dsDNA assays: simplified workflow and improved performance

Introduction

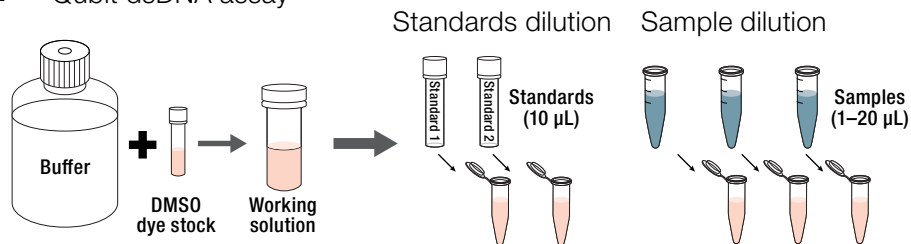
Nucleic acid quantification is a critical component of genomic research techniques, including next-generation sequencing (NGS), gene expression analysis, and polymerase chain reaction (PCR). Often, fluorometry is used to obtain accurate and precise measurements of DNA concentration prior to these methods. Invitrogen™ Qubit™ instruments and assays enable rapid and reliable fluorescence-based quantification of nucleic acids with as little as 1 µL of sample.

The Invitrogen™ Qubit™ 4 and Flex Fluorometers both use highly specific Invitrogen™ Qubit™ assays, which contain selective dyes that fluoresce only when bound to the target molecule. Qubit assay kits are available for double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), RNA, miRNA, and protein.

To further simplify the nucleic acid quantification workflow, Invitrogen™ Qubit™ 1X assays were developed. These assays feature ready-to-use working solutions, each with a specially formulated fluorogenic dye and assay buffer (Figure 1). The 1X formulations enable users to simply combine their DNA samples with the provided 1X working solution without the need for assay preparation. The 1X working solution can be used reliably for 6 months after receipt, while working solutions prepared from standard Qubit dsDNA assay kits are intended to be used within 3 hours of preparation.

Both standard Qubit assay kits and the latest 1X assay kits are highly selective for dsDNA over RNA, ssDNA, and free nucleotides. Additionally, these assays tolerate many contaminants common to nucleic acid isolation and purification workflows. Here we demonstrate the performance of the standard Invitrogen™ Qubit™ dsDNA high sensitivity (HS) and dsDNA broad range (BR) assays and the latest Qubit 1X dsDNA HS and BR assays.

A Qubit dsDNA assay



B Qubit 1X dsDNA assay

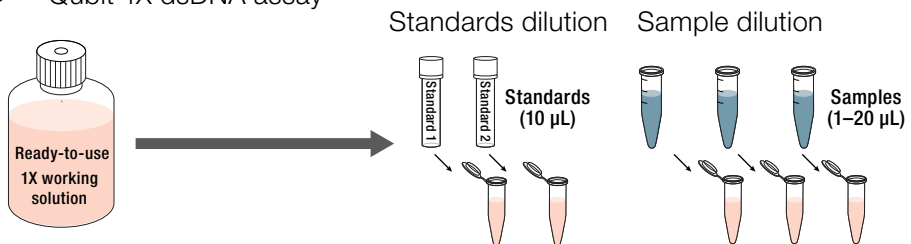


Figure 1. Workflow comparison for the (A) Qubit dsDNA and (B) Qubit 1X dsDNA assays.

Standard Invitrogen™ Qubit™ dsDNA High Sensitivity (HS) and Qubit™ dsDNA Broad Range (BR) Assay Kits include a fluorogenic dye, buffer, and dsDNA standards. Prior to each assay, a fresh aqueous working solution needs to be prepared by diluting the dye stock in the provided buffer in a 1:200 ratio. Qubit 1X dsDNA assay kits eliminate this step by providing a ready-to-use working solution.

Methods

The standard Qubit assay formulations and the latest Qubit 1X assay formulations were compared for performance across the core quantification ranges (Table 1). For each assay, 10 μL of Thermo Scientific™ lambda dsDNA was combined with 190 μL of working solution to yield a final dsDNA amount that is within the core quantification range, with 8 replicates per concentration. Samples were vortexed to ensure homogeneity and incubated for 2 min. The Qubit Flex Fluorometer was used to quantify the samples, streamlining the sample read process.

Results

Accurate quantification

For the 1X formulations of the HS and BR kits, the quantitation results were within 5% of those of the standard assay formulations (Figures 2 and 3). Additionally, the Qubit 1X dsDNA BR assay provided a higher quantification range, enabling accurate quantification of up to 4,000 ng of DNA, compared to 2,000 ng with the standard Qubit dsDNA BR assay (Table 1). With both kit configurations, the error relative to expected values was <10% with a coefficient of variation of <5%.

Table 1. Qubit dsDNA assay quantification ranges. Samples with concentrations within the core range of an assay will provide high-confidence results with <15% relative error. Samples within the extended range of an assay will provide moderate-confidence results. These Qubit assays are intended to be used with 1–20 μL of sample.

	Qubit dsDNA HS assay	Qubit 1X dsDNA HS assay	Qubit dsDNA BR assay	Qubit 1X dsDNA BR assay
Extended lower range (ng of DNA)	0.1–0.2	0.1–0.2	–	–
Core dynamic range (ng of DNA)	0.2–100	0.2–100	4–1,000	4–2,000
Extended upper range (ng of DNA)	100–120	100–120	1,000–2,000	2,000–4,000

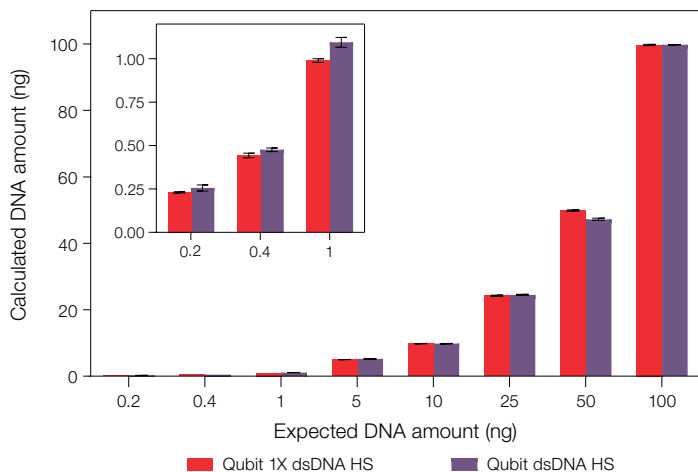


Figure 2. Comparison of DNA quantification over the core range for the 1X and standard Qubit dsDNA HS Assay Kits. The core quantification range for both assay formats is 0.2–100 ng of DNA.

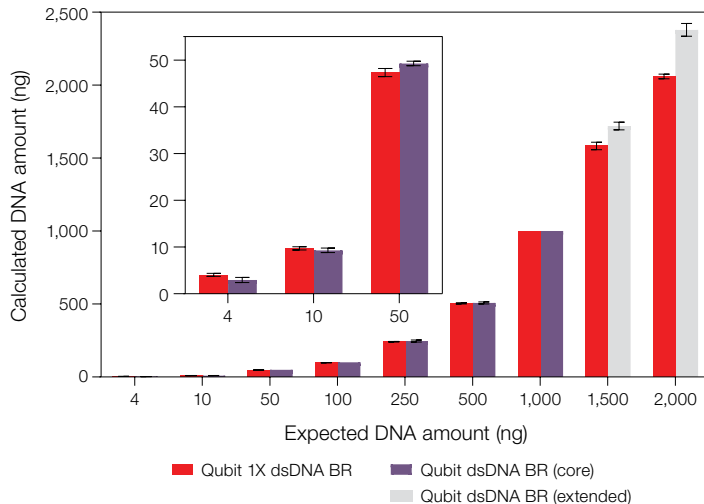


Figure 3. Comparison of DNA quantification over the core range for the 1X and standard Qubit dsDNA BR Assay Kits. Note that the core ranges for the two assay kits differ. For the Qubit 1X dsDNA BR assay, the core range is up to 2,000 ng of DNA. The gray bars on the graph indicate that this is outside of the core quantification range of the standard Qubit dsDNA BR assay.

Assay selectivity

The Qubit 1X dsDNA HS and BR assays maintain the selectivity that users rely on for accurate quantification of complex samples. The assays are highly selective for dsDNA, and accurately quantitate dsDNA in the presence of RNA, ssDNA, and free nucleotides. Figure 4 shows that the measured amount of dsDNA deviates by less than 10% from the expected value even in the presence of RNA. The assays also tolerate common contaminants of processed biological samples, such as salts, solvents, and detergents. These results are outlined in the product information sheet of each assay.

Conclusion

Overall, the new Qubit 1X assays simplify the nucleic acid quantification workflow while providing accurate and precise results.

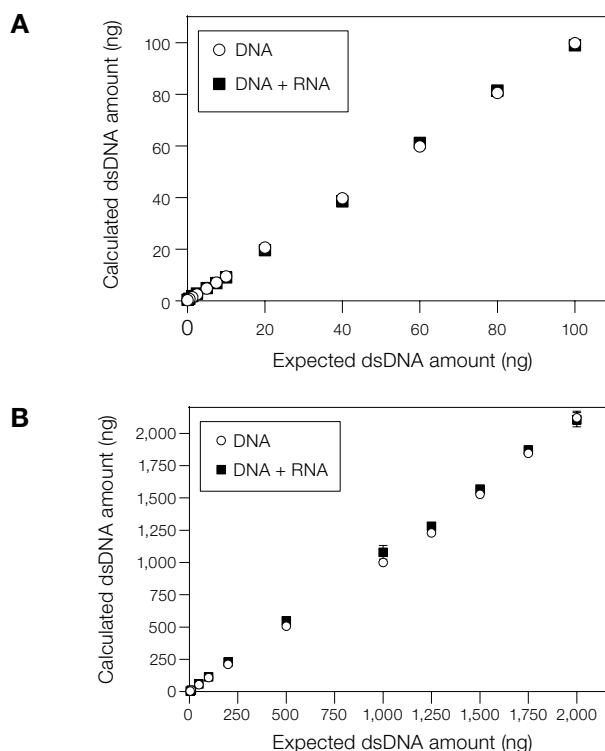


Figure 4. Selectivity results for the (A) Qubit 1X dsDNA HS assay and (B) Qubit 1X dsDNA BR assay. Both plots show the quantification results of two different sample types vs. expected values. Circles represent samples with 10 µL of DNA plus 190 µL of working solution, at varying concentrations. Squares represent samples with 10 µL of RNA and 10 µL of DNA plus 180 µL of working solution, at varying concentrations.

Ordering information

Product	Cat. No.
Qubit 1X dsDNA HS Assay Kit, 100 assays	Q33230
Qubit 1X dsDNA HS Assay Kit, 500 assays	Q33231
Qubit 1X dsDNA HS Assay Lambda Standard	Q33233
Qubit 1X dsDNA BR Assay Kit, 100 assays	Q33265
Qubit 1X dsDNA BR Assay Kit, 500 assays	Q33266
Qubit 1X dsDNA BR Assay Lambda Standards	Q33263
Qubit 4 Fluorometer with WiFi	Q33238
Qubit Flex Fluorometer	Q33327

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