Introducing the new Qubit Flex Fluorometer: DNA, RNA, and protein quantitation with flexible throughput

Accurate and precise quantitation of up to 8 samples simultaneously.

DNA, RNA, and proteins are typically quantified using fluorescence or UV absorbance. Previous studies have shown that measurements using selective fluorescent stains are far more accurate and sensitive than those that rely on UV absorbance, which detects any molecule that absorbs at 260 nm, including DNA, RNA, protein, free nucleotides, and excess salts. Moreover, UV spectrophotometry is often not sensitive enough to accurately measure low concentrations of DNA and RNA. The Invitrogen[™] Qubit[™] platform combines benchtop fluorometers with Invitrogen[™] Qubit[™] assays to provide an optimized, easy-to-use, dedicated system for the accurate and reliable fluorescence-based quantitation of nucleic acid and protein.

Current fluorometers read one sample at a time, which works well for low-throughput needs. When processing multiple samples, however, this low-throughput workflow will significantly increase the time required to obtain results. Additionally, sequential processing of samples can contribute to a decrease in the accuracy and precision of the quantitation results. A benchtop fluorometer that can read a group of samples simultaneously provides significant time savings and reduced variability.

To address this need, we have developed the Invitrogen™ Qubit™ Flex Fluorometer, the newest member of the Qubit™ family of fluorescence-based quantitation instruments. Similar to the Invitrogen™ Qubit[™] 4 Fluorometer, the Qubit Flex Fluorometer is a benchtop device with an intuitive interface and large 8-inch, state-of-the-art color touchscreen, designed explicitly for the highly accurate quantitation of DNA, RNA, and protein. The Qubit Flex Fluorometer increases quantitation throughput with the ability to measure up to 8 samples at once (Figure 1). The time it takes to generate quantitation measurements using the Qubit Flex Fluorometer is significantly less than that required with single-sample readers, due in part to a 50% reduction in hands-on time. In addition to time savings, the Qubit Flex Fluorometer's ability to read 8 samples simultaneously helps to reduce assay variability and yield reproducible data because each sample is exposed to identical quantitation conditions. The Qubit Flex Fluorometer is compatible with the same optimized Qubit assay reagents as the Qubit 4 Fluorometer, each of which has been formulated to cover a broad range of target



Figure 1. Flex your throughput—introducing the Qubit Flex Fluorometer. The Invitrogen™ Qubit™ Flex Fluorometer (Cat. No. Q33327) provides the same great performance you expect from the Invitrogen™ Qubit™ Fluorometers but now has built-in flexibility to quantify DNA, RNA, or protein in 1 to 8 samples simultaneously.

molecule concentrations and provide sensitive quantitation with as little as 1 μL of sample.

Speedy quantitation

To measure the time needed to quantify an increased number of samples, we used the Invitrogen[™] Qubit[™] 1X dsDNA HS Assay Kit (Cat. No. Q33231) with the Qubit Flex Fluorometer, the Qubit 4 Fluorometer, and a single-sample fluorometer from another supplier. The time required to prepare and quantify 1, 8, 24, 48, and 96 samples of 4 different concentrations of lambda DNA (0.1, 1, 5, and 10 ng/µL) on the each of the three fluorometers was recorded. This study demonstrates that time savings are realized with the Qubit Flex Fluorometer using batches of as few as 8 samples, and efficiency multiplies as the number of samples increases (Figure 2). When compared with a single-sample fluorometer, the Qubit Flex Fluorometer provides a reduction of up to 50% in total time-to-data (from preparation to measurement of the samples) for higher numbers of samples. In addition, the Qubit Flex Fluorometer can save data for up to 10,000 samples, and you can transfer data to the Connect cloud-based platform using Wi-Fi, a USB drive, or ethernet cable.



Figure 2. The Qubit Flex Fluorometer reduces time-to-data by up to 50%. A time study comparing the Invitrogen[™] Qubit[™] Flex Fluorometer with both the Invitrogen[™] Qubit[™] 4 Fluorometer and another supplier's single-sample fluorometer showed time-to-data reduced by up to 50% using the Invitrogen[™] Qubit[™] 1X dsDNA HS Assay Kit (Cat. No. Q33230) with up to 96 samples.



Figure 3. Qubit platforms deliver better accuracy and precision than other manufacturers. Invitrogen[™] Qubit[™] 1X dsDNA HS and Qubit[™] dsDNA BR Assay Kits (Cat. No. Q33230, Q32850) were run with 4 DNA sample concentrations (BR assay: 2, 20, 50, and 100 ng/µL; HS assay: 0.1, 1, 5, and 10 ng/µL) in 8 replicates on the Invitrogen[™] Qubit[™] Flex Fluorometer, Invitrogen[™] Qubit[™] 4 Fluorometer, and another supplier's single-sample fluorometer. (A) The percent relative error (lower is more accurate) and (B) percent CV (lower is more precise) were measured for each data point and averaged across all concentrations for each instrument.

Accurate, precise measurements

To determine the accuracy and precision of quantitation measurements, we used the Invitrogen[™] Qubit[™] 1X dsDNA HS and Qubit[™] dsDNA BR Assay Kits with the Qubit Flex Fluorometer, the Qubit 4 Fluorometer, and another supplier's single-sample fluorometer. The samples used for these assays consisted of 0.1, 1, 5, and 10 ng/µL lambda DNA for the Qubit 1X dsDNA HS Assay Kit; and 2, 20, 50, and 100 ng/µL lambda DNA for the Qubit dsDNA BR Assay Kit. Samples were run in replicates of eight in individual Invitrogen™ Qubit[™] Assay Tubes (Cat. No. Q32856) on all three fluorometers. The samples were run according to the assay protocols, and the percent error and percent coefficient of variation (CV) were calculated for each sample measurement and averaged across all concentrations for each instrument.

The accuracy of quantitation (represented as % error) was assessed using the Qubit 1X dsDNA HS Assay Kit with the Qubit 4 Fluorometer, Qubit Flex Fluorometer, and another supplier's single-sample fluorometer (Figure 3A). The Qubit Flex Fluorometer was the most accurate, with an error of only 3.5%, followed by the Qubit 4 Fluorometer with a 4.5% error. The other supplier's fluorometer had lower accuracy, with a 7.9% error. Similarly, the precision of quantitation was evaluated using the Qubit dsDNA BR Assay Kit with the Qubit 4 Fluorometer, Qubit Flex Fluorometer, and another supplier's single-sample fluorometer. The CV for all samples was measured for each data point and averaged across all concentrations for each instrument. The Qubit Flex Fluorometer measurements had the lowest percent CV, indicating higher precision than the other two fluorometers tested (Figure 3B).

Improved user interface

In addition to its higher throughput, the Qubit Flex Fluorometer includes several improvements in the user interface. The instrument's user interface can be personalized to display in the language of your choice, including English, French, Spanish, Italian, German, Japanese, and simplified Chinese. Additionally, to help improve accuracy and reliability and reduce time-to-data, the Qubit Flex Fluorometer includes calculators for reagent, assay range, molarity, and normalization.



Figure 4. The Qubit Flex Fluorometer includes new calculators. (A) The Reagent Calculator helps you determine how much working solution to prepare for the number of samples. (B) The Assay Range Calculator displays the core sample concentration range for which the selected assay is most accurate, along with the extended low and high ranges, based on the sample volume. (C) The Molarity Calculator allows you to calculate the molarity of a sample based on nucleic acid length and the measured concentration. (D) The Normalization Calculator replaces the need to transfer the data to a spreadsheet to normalize samples during library preparation for sequencing.

The Reagent Calculator (Figure 4A) helps to determine how much working solution to prepare for the number of samples in your experiment. To help choose the best assay for the samples, the Assay Range Calculator (Figure 4B) displays the core sample concentration range for which the selected assay is most accurate, along with the extended low and high ranges, based on sample volume. To aid in various downstream applications, the Molarity Calculator (Figure 4C) allows you to calculate the molarity of a sample based on nucleic acid length and measured concentration, and the Normalization Calculator (Figure 4D) replaces the need to transfer the data to a spreadsheet to normalize samples during library preparation for sequencing. With the Normalization Calculator, the results are easily normalized to a desired mass, concentration, or molarity.

Exceptional selectivity and sensitivity provided by the Qubit Assay Kits

The Qubit Flex Fluorometer in combination with the Qubit assays yields analyte-specific quantitation of DNA, RNA (including miRNA), and protein over a broad concentration range and with unprecedented accuracy, sensitivity, and simplicity, for both routine analyses and rare or hard-to-obtain samples (Figure 5). The basis of each Qubit assay is a target-selective fluorescent dye that emits fluorescence only when bound to the specific target molecule, even when present at low concentrations.

The most significant advantage of the Qubit DNA and RNA assays is their selectivity. UV absorbance–based analysis cannot be used to separately quantify DNA or RNA in samples containing a mixture of both. In contrast, the Qubit DNA and RNA assays are able to accurately measure DNA and RNA, respectively, in the same sample. We found that the DNA concentration of a sample containing equal parts DNA and RNA can be measured within 2% of the actual concentration using the Qubit dsDNA BR Assay Kit. In a sample containing a 10-fold excess of RNA over DNA, the DNA concentration was measured to be only 7% higher than the actual concentration.

Each Qubit Assay Kit provides concentrated assay reagent, dilution buffer, and prediluted standards, and all assay reagents are stored at room temperature. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 and 20 µL), and read the concentration using a Qubit Fluorometer after a 2-minute incubation (allow 15 minutes for the protein assay). The Qubit assay signal is stable for 3 hours. With the Qubit Flex Fluorometer, all assay settings and calculations are performed automatically.



Figure 5. Comparison of sample concentration ranges for Qubit assays and UV absorbance measurements. UV absorbance readings are not selective for RNA vs. DNA. The Qubit Flex Fluorometer and Qubit assays are optimized for use together.

Bring the Qubit Flex Fluorometer to your benchtop

When laboratories need to quantify nucleic acid or protein in an increased number of samples due to changes in applications or scope of a project, a single-sample quantitation reader is not only tedious but a bottleneck in the workflow. The Qubit Flex Fluorometer is designed to address the growing need for higher-throughput DNA and RNA quantitation while maintaining all the benefits of accuracy and reproducibility that have long been associated with Qubit fluorometers and assays. The advanced optics and data analysis algorithms built into the Qubit Flex Fluorometer are optimized to work together with the Qubit assays, resulting in a seamless solution that generates highly reliable, sensitive, and specific results. To learn more or request an in-lab demonstration, visit **thermofisher.com/qubit**.

Product	Quantity	Cat. No.
Qubit™ Flex Fluorometer	1 fluorometer	Q33327
Qubit™ Flex NGS Starter Kit	1 kit	Q45893
Qubit™ Flex Quantitation Starter Kit	1 kit	Q45894
Qubit™ Flex Assay Tube Strips	125 tube strips	Q33252
Qubit™ Flex Assay Reservoirs	100 reservoirs	Q33253
Qubit™ 1X dsDNA HS Assay Kit	100 assays 500 assays	Q33230 Q33231
Qubit™ dsDNA BR Assay Kit	100 assays 500 assays	Q32850 Q32853
Qubit™ ssDNA Assay Kit	100 assays	Q10212
Qubit™ microRNA Assay Kit	100 assays 500 assays	Q32880 Q32881
Qubit [™] RNA HS Assay Kit	100 assays 500 assays	Q32852 Q32855
Qubit™ Protein Assay Kit	100 assays 500 assays	Q33211 Q33212