

## Assay RNA quality with the updated Qubit benchtop fluorometer

### Introducing the Qubit 4 Fluorometer and Qubit RNA IQ Assay.

The Invitrogen™ Qubit™ 4 Fluorometer is the newest generation of the popular benchtop fluorometer designed to quantify DNA, RNA, and protein using the highly sensitive Invitrogen™ Qubit™ assays. This latest version of the Qubit fluorometer has been reengineered to run the Invitrogen™ Qubit™ RNA IQ Assay for assessing RNA integrity and quality. The Qubit 4 Fluorometer and Qubit RNA IQ Assay work together to accurately distinguish viable from degraded RNA in just a few simple steps: mix reagent, add sample, and read.

#### Qubit 4 Fluorometer

As with its predecessors, the Qubit 4 Fluorometer (Figure 1) provides sensitive and accurate detection and quantitation of DNA, RNA, and protein using 1 to 20  $\mu\text{L}$  of sample. For over a decade, life science researchers have relied on the Qubit fluorometer when handling valuable or complex samples (e.g., blood or formalin-fixed, paraffin-embedded (FFPE) tissue), and when preparing samples for critical downstream applications such as next-generation sequencing (NGS)—particularly in cases when UV absorbance is unable to accurately detect the concentration or to discern between RNA and DNA (Figure 2). With easy-to-use touchscreen menus, state-of-the-art algorithms, and Wi-Fi connectivity, the Qubit 4 Fluorometer quickly and accurately quantifies DNA, RNA, and protein, with a read time of less than 3 seconds per sample, and can store up to 1,000 sample results.

#### Qubit RNA IQ Assay

The updated Qubit 4 Fluorometer is designed to enable RNA qualification with the Qubit RNA IQ (Integrity & Quality) Assay, which distinguishes between intact and degraded RNA. In contrast with classic gel or capillary electrophoresis methods, the Qubit RNA IQ Assay utilizes two unique fluorescent dyes to assess whether the RNA sample is viable for use or degraded. One dye binds to large intact RNA with or without secondary or tertiary structure; the second dye selectively binds to small, degraded RNA. To perform the RNA IQ assay, simply prepare the RNA IQ working solution, add samples, and then measure the signal using the Qubit 4 Fluorometer. No special handling, tedious sample preparation, or waiting for results are required. With as little as 1  $\mu\text{L}$  of your sample (containing 0.5–1.5  $\mu\text{g}$  RNA), you can determine the quality and integrity of the sample RNA in just a few seconds.

The Qubit 4 Fluorometer reports the results from the RNA IQ assay as an RNA IQ value, which is based on a scale of 1 to 10 and is similar to RNA quality scores used in other methods. A high IQ value indicates that the majority of the sample consists of large or structured RNA; a low IQ value indicates that the sample contains small RNA with limited secondary and tertiary structure (Figure 3). Additionally, both of the fluorescent dyes used in the RNA IQ assay emit signal only when bound to the target molecule, minimizing the impact of contaminants on the readout accuracy.

#### Better DNA and RNA quantitation

In addition, we have developed improved versions of the classic Qubit DNA and RNA quantitation assays. The updated Invitrogen™ Qubit™ 1X dsDNA HS (high sensitivity) Assay provides the reagents in a ready-to-use formulation that is stable for up to 1 year at 2–8°C. This new Qubit 1X dsDNA assay provides the same dynamic range (10  $\text{pg}/\mu\text{L}$  to 100  $\mu\text{g}/\mu\text{L}$ ), limit of detection (0.2 ng), and accurate results at very low DNA concentrations as the original Qubit dsDNA HS Assay (Figure 4).



Figure 1. Qubit 4 Fluorometer.

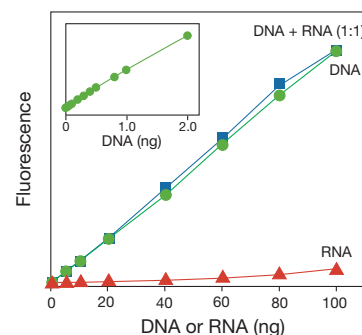
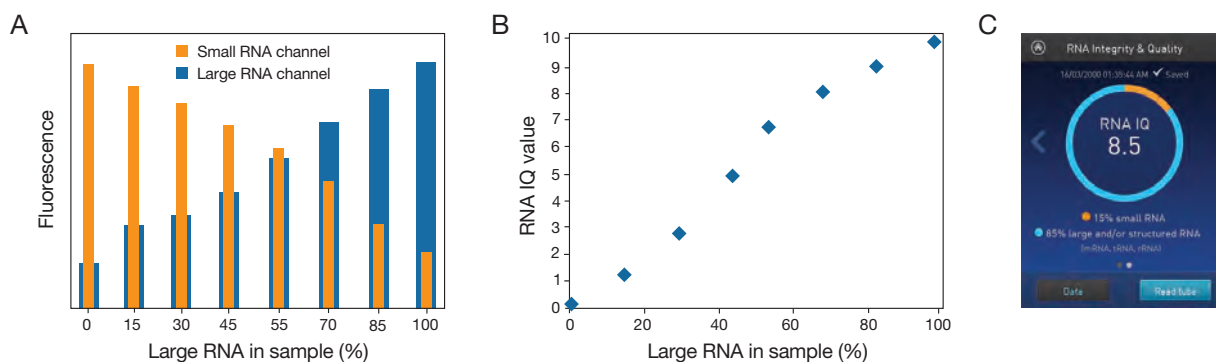


Figure 2. Performance of the Qubit dsDNA HS Assay. The Invitrogen™ Qubit™ dsDNA HS Assay has a linear detection range of 0.2–100 ng and is selective for dsDNA, even in the presence of an equal mass of RNA.



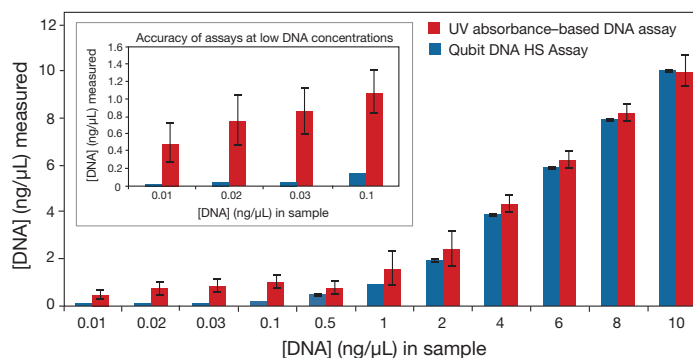
**Figure 3.** Qubit RNA IQ fluorescence response and IQ value for solutions containing various proportions of large and small RNA. Triplicate samples containing a total of 100 ng/mL RNA, comprising small RNA (Invitrogen™ Silencer™ Select GAPDH Positive Control siRNA, Cat. No. 4390849) and large RNA (*E. coli* rRNA), were assayed with the Invitrogen™ Qubit™ RNA IQ Assay (Cat. No. Q33221, Q33222) on the Invitrogen™ Qubit™ 4 Fluorometer. (A) Relative fluorescence and (B) RNA IQ values were plotted for all samples. (C) A Qubit 4 Fluorometer readout shows the RNA IQ assay results for one sample.

The Invitrogen™ Qubit™ RNA XR (extended range) Assay can detect up to 8 µg of RNA, whereas the Invitrogen™ Qubit™ RNA BR (broad range) Assay is only able to detect a maximum of 1 µg. Both Qubit RNA assays provide the same lower limit of detection (20 ng).

The Qubit 1X dsDNA HS and Qubit RNA XR Assays are preprogrammed on the Qubit 4 Fluorometer. If you already have a Qubit 2 or Qubit 3 Fluorometer, programs for these assays can be downloaded at [thermofisher.com/qubit](http://thermofisher.com/qubit). The improved dsDNA and RNA assays are also available as Invitrogen™ Quant-iT™ Assay Kits, which are optimized for use with fluorescence microplate readers (but can also be used on the Qubit fluorometer) and include 8 standards.

### Get started with a Qubit Starter Kit

The Qubit Starter Kits provide a convenient and efficient way to get started with Qubit DNA, RNA, or protein quantitation. They include a Qubit 4 Fluorometer, Qubit quantitation kit(s), and Qubit assay tubes. Visit [thermofisher.com/qubitbp77](http://thermofisher.com/qubitbp77) for more information about the Qubit 4 Fluorometer, the Qubit RNA IQ Assay, and the family of Qubit and Quant-iT quantitation assays. ■



**Figure 4.** Accuracy and precision of the Qubit Quantitation Platform. Ten replicates of lambda DNA at concentrations from 0.01 to 10 ng/µL were assayed using the Invitrogen™ Qubit™ dsDNA HS Assay on the Invitrogen™ Qubit™ Fluorometer according to the standard kit protocol. The same concentrations of DNA were assayed in 10 replicates using UV absorbance measurements and a microvolume spectrophotometer, and results were compared for both accuracy and precision. Each bar represents the average of 10 replicates. Error bars represent the standard deviations of the 10 replicates. The concentrations indicated are the concentrations of DNA in the starting samples, before dilution in the Invitrogen™ Qubit™ Assay Tubes (Cat. No. Q32856).

Product	Quantity	Cat. No.
<b>Qubit™ 4 Fluorometer and starter kits</b>		
Qubit™ 4 Fluorometer	1 fluorometer	Q33226
Qubit™ 4 Quantitation Starter Kit	1 fluorometer, 6 kits	Q33227
Qubit™ 4 NGS Starter Kit, for next-generation sequencing	1 fluorometer, 1 kit	Q33228
Qubit™ 4 RNA IQ Starter Kit, for RNA integrity & quality	1 fluorometer, 1 kit	Q33229
<b>Qubit™ RNA and DNA assays</b>		
Qubit™ RNA IQ Assay	1 kit, 75 assays	Q33221
	1 kit, 275 assays	Q33222
Qubit™ RNA XR Assay Kit	1 kit, 100 assays	Q33223
	1 kit, 500 assays	Q33224
Quant-iT™ RNA XR Assay Kit*	1 kit, 1,000 assays	Q33225
Qubit™ 1X dsDNA HS Assay Kit	1 kit, 100 assays	Q33230
	1 kit, 500 assays	Q33231
Quant-iT™ 1X dsDNA Assay Kit*	1 kit, 1,000 assays	Q33232

\*To use a Quant-iT assay on a Qubit Fluorometer, simply replace standards 1 and 2 with standards 1 and 8 from the Quant-iT assay.