Determine microRNA concentration in solution

Fluorescence-based small RNA quantitation for both conventional and high-throughput assays.

Small noncoding RNA molecules such as microRNA (miRNA) and small interfering RNA (siRNA) play a critical role in posttranscriptional gene regulation, though their significance and conservation among species were not fully recognized until the early 2000s. These small single- or double-stranded RNA molecules (~20 nucleotides or base pairs) are difficult to quantify accurately because of both their small size and their typically low concentrations in total RNA samples.

Conventional spectrophotometric A_{260} readings require relatively large sample volumes (typically 500 µL or more), lack sensitivity, and cannot differentiate between intact RNA, degraded RNA, and contaminants that absorb at 260 nm. The NanoDrop® 2000 UV-Vis Spectrophotometer requires only 0.5 µL of sample; however, it has a detection limit of 1.5 µg/mL for RNA and provides absorption-based measurements that cannot discriminate between large and small RNAs and free nucleotides. Traditional fluorescence-based nucleic acid assays are more sensitive and can better distinguish intact vs. degraded RNA, but they often inadequately detect small RNAs.

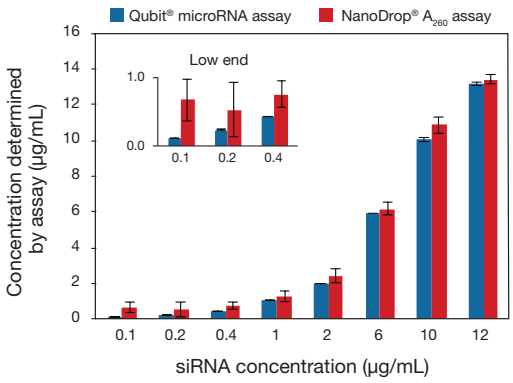

Figure 1. Accuracy and precision of the Qubit® and Quant-iT™ microRNA assays. Six experiments were run to test the accuracy and precision of the Qubit® and Quant-iT™ microRNA assays with pure siRNA; results of a typical experiment are shown here. In this experiment, eight replicates of GAPDH siRNA in concentrations from 0.1 µg/mL to 12 µg/mL were measured using the Qubit® MicroRNA Assay Kit with the Qubit® 2.0 Fluorometer and using A_{260} measurements on the NanoDrop® ND-1000 Spectrophotometer. The detection limit for the NanoDrop® instrument is reported to be 1.5 µg/mL; lower siRNA concentration results are shown for comparison. Accuracy for the Qubit® microRNA assay was within 15% of expected. CVs (1 standard deviation/average of 8 data points) were 0.63% to 2.9%. For all six experiments, the Qubit® microRNA assay accuracy was within 15% of expected, and CVs were <5%.

Introducing a fluorescence-based microRNA assay

Unlike traditional A_{260} or fluorescence-based measurements, the newly developed Qubit® and Quant-iT™ microRNA assays efficiently detect small RNAs (all types) in an RNA sample but detect little of the large RNAs (>1,000 nt). These microRNA assays detect small RNAs such as miRNA and siRNA (17–25 nucleotides or base pairs) with a detection limit of 50 ng/mL in the sample, using a sample volume of only 1–20 µL. Furthermore, these assays can detect between 1 and 100 ng of RNA in the assay tube, allowing initial sample concentrations from 50 ng/mL to 100 µg/mL—an extremely broad usable range.

Both the Qubit® and Quant-iT™ microRNA assays are based on the use of a proprietary fluorogenic dye that exhibits >200-fold enhancement upon binding to small RNAs, and this enhancement is maintained in the presence of common contaminants such as free nucleotides, proteins, salts, solvents, and some detergents. The Qubit® MicroRNA Assay Kits are designed for assaying

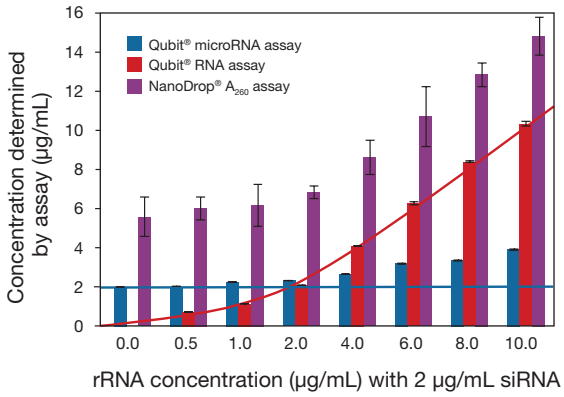

Figure 2. Selectivity of the Qubit® and Quant-iT™ microRNA assays for siRNA in the presence of ribosomal RNA (rRNA). rRNA at the concentrations listed on the x-axis was added to samples containing 2 µg/mL siRNA. The mixtures were then assayed using the Qubit® microRNA assay, the Qubit® RNA assay, and the NanoDrop® A_{260} assay. Results from eight replicates were averaged, with standard deviations shown. Total RNA concentration at the low end was below the NanoDrop® instrument's detection limit (1.5 µg/mL), so accuracy and precision were limited. The Qubit® microRNA assay read 100–196% of the 2 µg/mL siRNA concentration, even with a 5-fold excess of rRNA over siRNA. The Qubit® RNA assay read approximately 100% of the rRNA concentration, except at a 4-fold excess of siRNA over rRNA, where it read 150%. The blue and red trendlines indicate the actual concentrations of siRNA and rRNA, respectively, in the samples.

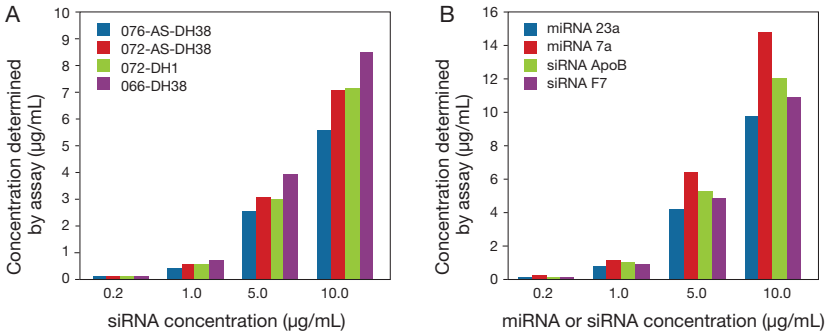

Figure 3. The Qubit® and Quant-iT™ microRNA assays can detect single-stranded and double-stranded siRNA and miRNA. Concentrations of pure siRNA and miRNA samples were determined by optical density on a PerkinElmer® spectrophotometer at concentrations yielding an A_{260} of 0.3–0.6. Samples were then diluted and tested in the Qubit® microRNA assay at four different concentrations. Four different single-stranded siRNA molecules **(A)** and two double-stranded siRNAs and two double-stranded miRNAs **(B)** were tested.

fewer than 20 samples at a time using the Qubit® Fluorometer (see "The Qubit 3.0 Fluorometer" box on page 34), whereas the Quant-iT™ MicroRNA Assay Kit is designed for high-throughput assays (20–2,000 samples) and a fluorescence microplate reader.

Quantify small RNA with superior accuracy...

The Qubit® and Quant-iT™ microRNA assays are designed to accurately detect miRNA and siRNA. Figure 1 shows that these assays are accurate and precise for initial siRNA concentrations from 100 ng/mL to 12 µg/mL, even at the lower concentrations for which absorption-based measurements are both inaccurate and imprecise. The Qubit® and Quant-iT™ microRNA assays do not detect nucleotides and can therefore distinguish small RNA molecules from completely degraded RNA. Furthermore, only about 20–30% of large RNAs (>1,000 nucleotides), including rRNA and mRNA, are detected by these assays. In contrast, the Qubit® and Quant-iT™ RNA assays detect large RNAs well but detect little of the small RNAs, and the NanoDrop® A_{260} assay detects total RNA concentration including free nucleotides. Figure 2 demonstrates these assay characteristics for a mixture of siRNA and rRNA.

Additionally, both single-stranded and double-stranded small RNA molecules are detected by the Qubit® and Quant-iT™ microRNA assays (Figure 3). These assays are intended for total RNA or enriched RNA extracts. The assay reagent detects DNA as well, so samples containing DNA should be treated with DNase prior to small RNA quantitation.

And with a remarkably easy protocol!

Importantly, the Qubit® and Quant-iT™ microRNA assays are extremely precise at low concentrations of small RNAs (Figures 1 and 2) and are designed to use very small sample volumes. We have been able to reproducibly measure as little as 0.5 ng of pure miRNA in a Qubit® assay tube or a microplate well. The Qubit® and Quant-iT™ microRNA assays have a core dynamic range of 5–500 ng/mL small RNA in the assay tube, and accurate results can be obtained for initial sample concentrations from 50 ng/mL to 100 µg/mL. Moreover, this sensitivity is achieved with a very easy protocol: simply dilute the Qubit® or Quant-iT™ microRNA assay reagent 1:200, load 200 µL into a Qubit® assay tube or a microplate well, add 1–20 µL of sample, mix, and read the fluorescence.

Accurate small RNA quantitation for downstream applications

The sensitivity and accuracy of the Qubit® and Quant-iT™ microRNA assays allow you to better determine the concentration of small RNAs in precious RNA samples, leading to greater success in subsequent applications such as sequencing and qRT-PCR. The Qubit® MicroRNA Assay Kits (available in 100- and 500-assay sizes) are designed for use with the Qubit® Fluorometer; they provide assay reagent, dilution buffer, and prediluted standards. The microRNA assay file is now preloaded on the Qubit® Fluorometer and may also be downloaded at **lifetechnologies.com/qubit** and permanently uploaded to earlier versions of the Qubit® Fluorometer. The Quant-iT™ MicroRNA Assay Kit is designed for high-throughput assays (20–2,000 samples) and detection on a fluorescence microplate reader. If you are assaying more than 2,000 samples, we recommend using the cost-effective Quant-iT™ MicroRNA Reagent along with the protocol provided. Learn more at **lifetechnologies.com/mirnabp70**. ■

Product	Quantity	Cat. No.
Qubit® MicroRNA Assay Kit, 100 assays	1 kit	Q32880
Qubit® MicroRNA Assay Kit, 500 assays	1 kit	Q32881
Quant-iT™ MicroRNA Assay Kit, 1,000 assays	1 kit	Q32882
Quant-iT™ MicroRNA Reagent	1 mL	Q32883
Qubit 3.0 Fluorometer	1 each	Q33216

The Qubit® 3.0 Fluorometer

The Qubit® 3.0 Fluorometer (Cat. No. Q33216) is a benchtop fluorometer that can be used to quantify DNA, RNA, microRNA, and protein using the highly sensitive and accurate fluorescence-based Qubit® quantitation assays (Table 1). In addition, the Qubit® 3.0 Fluorometer can be used to directly measure the fluorescence of samples, as well as to evaluate Ion Sphere™ Particle quality using the Ion Sphere™ Quality Control Kit prior to performing a sequencing run on the Ion Personal Genome Machine® (PGM™) Sequencer. Key features of this third-generation fluorometer include:

- Powerful dual-core processor that quickly and accurately quantifies DNA, RNA, and protein in less than 5 seconds per sample
- Compatibility with Qubit® quantitation assays, which use as little as 1 µL of sample and rely on dyes selective for dsDNA, RNA, or protein, minimizing the effects of contaminants such as degraded DNA or RNA
- Effective data management, with the ability to store up to 1,000 sample results
- Large 5.7-inch, state-of-the-art color touch screen for easy workflow navigation
- Small footprint, saving valuable space on your lab bench
- Ability to personalize your Qubit® fluorometer with the assays you run most often, to add new assays, and even to create your own assays with the MyQubit software and web tool, available at **lifetechnologies.com/qubit**

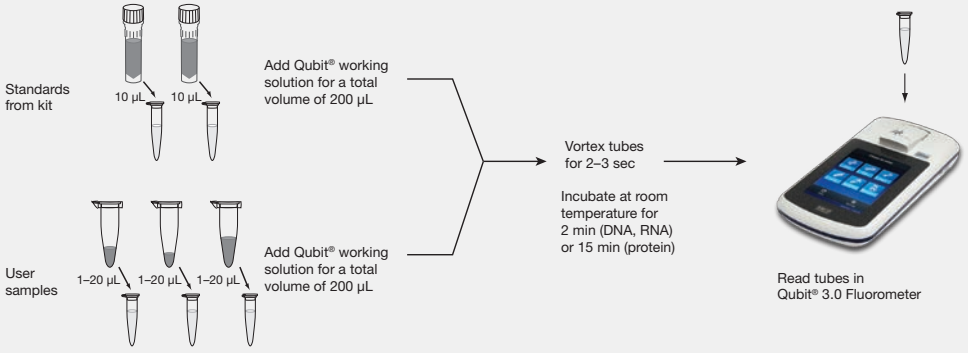

Figure 1. The Qubit® quantitation assay workflow. When paired with the Qubit® 3.0 Fluorometer, the Qubit® Quantitation Assay Kits provide a seamless workflow and easy data collection. Each Qubit® Assay Kit provides concentrated assay reagent, dilution buffer, and prediluted standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration on the Qubit® 3.0 Fluorometer. The assays are performed at room temperature, and the signal is stable for 3 hours.

Table 1. Qubit® assay kit selection guide.

Qubit® assay kit	Assay range	Sample starting concentration	100-assay size *	500-assay size *
Qubit® dsDNA BR Assay Kit	2–1,000 ng	100 pg/µL–1 µg/µL	Q32850	Q32853
Qubit® dsDNA HS Assay Kit	0.2–100 ng	10 pg/µL–100 ng/µL	Q32851	Q32854
Qubit® ssDNA Assay Kit	1–200 ng	50 pg/µL–200 ng/µL	Q10212	NA
Qubit® RNA HS Assay Kit	5–100 ng	250 pg/µL–100 ng/µL	Q32852	Q32855
Qubit® RNA BR Assay Kit	20–1,000 ng	1 ng/µL–1 µg/µL	Q10210	Q10211
Qubit® MicroRNA Assay Kit	1–500 ng	0.05–100 ng/µL	Q32880	Q32881
Qubit® Protein Assay Kit	0.25–5 µg	12.5 µg/mL–5 mg/mL	Q33211	Q33212

* Based on an assay volume of 200 µL. Qubit® assays are ideal when you need to process 1–20 samples; for 20–2,000+ samples, we recommend Quant-iT™ assay kits and reagents, which are designed for use with fluorescence microplate readers. NA = Not available.