

# Qubit RNA IQ Assay: a fast and easy fluorometric RNA quality assessment

## Abstract

The quality of RNA samples is paramount to any downstream application involving this nucleic acid. The ability to quickly and easily measure RNA quality is enabled by chip-based electrophoresis approaches. However, these methods are time-consuming, expensive, and prone to errors in handling. To overcome these challenges, our expertise in nucleic acid dyes was leveraged to generate a solution-based, multiplexed assay for the Invitrogen™ Qubit™ 4 Fluorometer that enables fast and easy measurement of RNA quality.

## Introduction

Utilizing two dyes with two separate emission channels, one that selectively binds to degraded RNA and another that selectively binds to large and intact RNA, we have developed a ratiometric fluorescence-based method to quickly assess the integrity of RNA within a sample. To enable this assay, the Qubit platform was updated as the Qubit 4 Fluorometer, allowing multiplexed assays and new user interface features on the instrument, which already has an integral role in nucleic acid workflows. As a result, we offer an RNA assessment assay that enables the measurement of RNA quality in as little as 5 minutes.

## Results

### Assay overview

The RNA integrity and quality (IQ) assay utilizes three standards consisting of: a blank; a small, degraded RNA; and a large, intact RNA. Samples are interrogated using the multiplexed dye mixture, and the two emission signals are combined using a proprietary algorithm to yield a quality score representative of the ratio of small and large RNAs in the sample. The touchscreen interface of the Qubit 4 Fluorometer makes it easy to select, run, and interpret the RNA IQ assay (Figure 1).

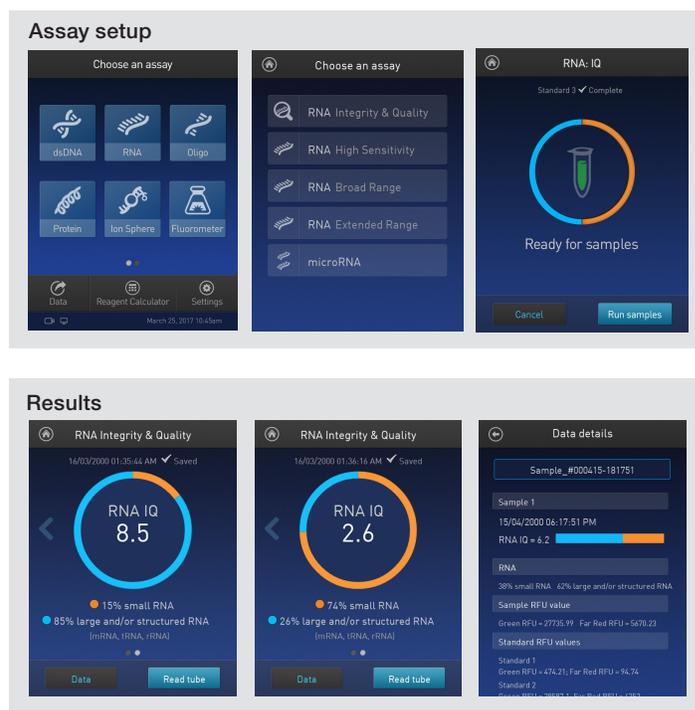
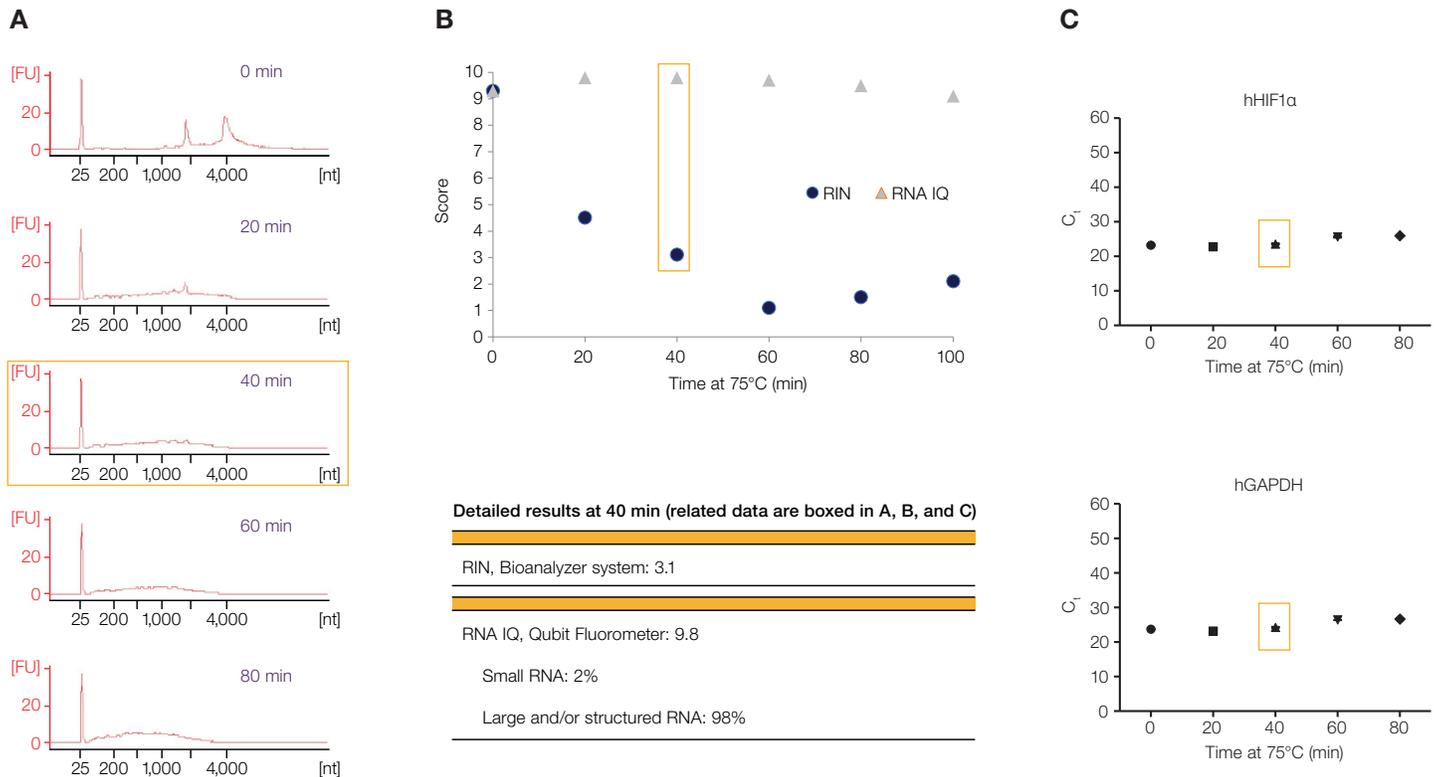


Figure 1. RNA IQ user interface on the Qubit 4 Fluorometer.

## Comparison of RNA IQ to RNA integrity number (RIN)

Analysis using the Agilent™ Bioanalyzer™ system, Qubit RNA IQ Assay, and RT-qPCR was performed on total RNA (isolated from human liver) that was heat-treated at 75°C for various amounts of time. RT-qPCR analysis was performed using Invitrogen™ RETROScript™ reverse transcriptase

and Applied Biosystems™ TaqMan® hHIF1α and hGAPDH assays. Of note is the rapidly decreasing RIN, while  $C_t$  and RNA IQ values remain largely consistent across the series (Figure 2).



**Figure 2. RNA IQ is a better predictor of RT-qPCR performance than RIN.** (A) Data from the Bioanalyzer system show rapidly decreasing rRNA peaks over time. (B) A comparison of RIN and RNA IQ values is shown, including more detailed results at the 40 min time point. (C) In agreement with the RNA IQ assay, RT-qPCR results are largely consistent over time.

## Qubit 4 Fluorometer

The Qubit 4 Fluorometer is designed to quickly and specifically quantitate DNA or RNA.

### Key features:

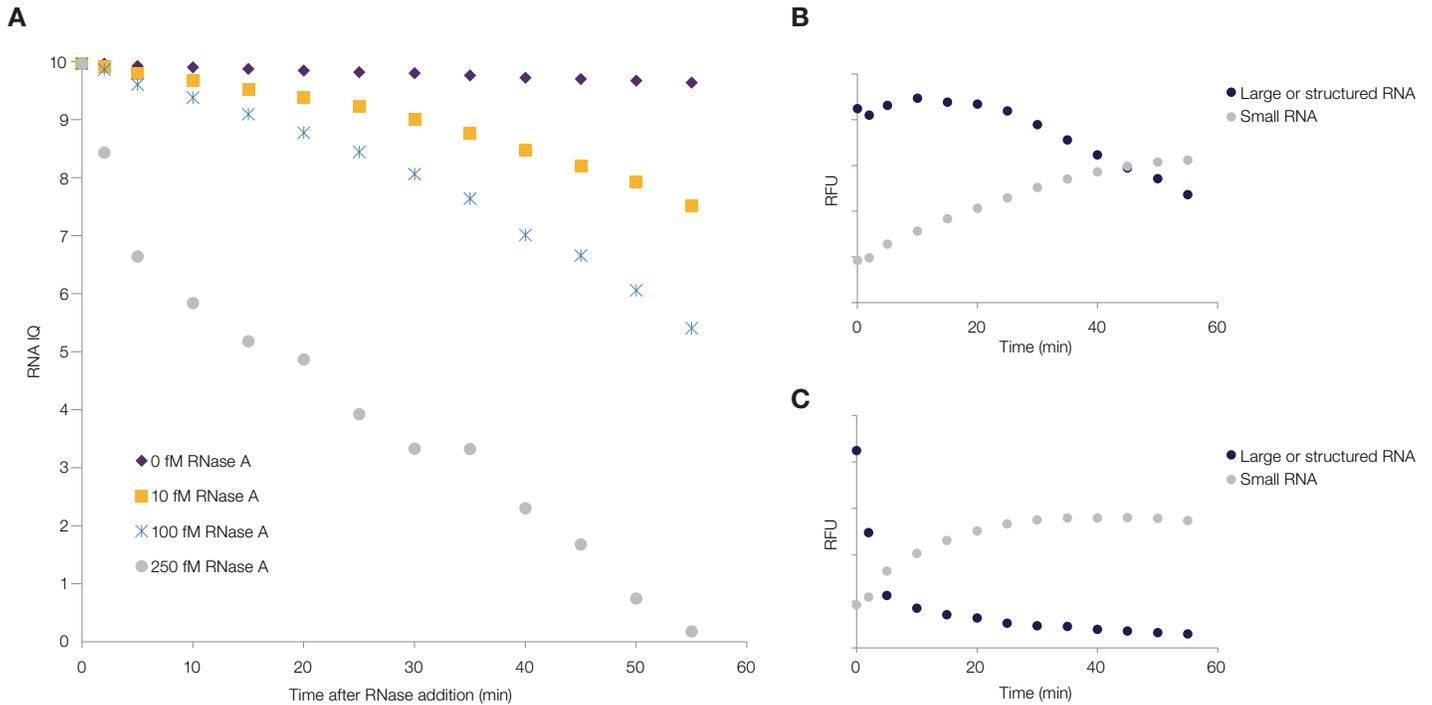
- Qubit assay dyes bind selectively to DNA or RNA, making it more sensitive than UV absorbance
- Uses as little as 1  $\mu$ L of sample, even with very dilute samples
- Fast, reliable detection of degraded RNA with the Qubit RNA IQ Assay
- New integrated reagent calculator to quickly generate working solution calculations



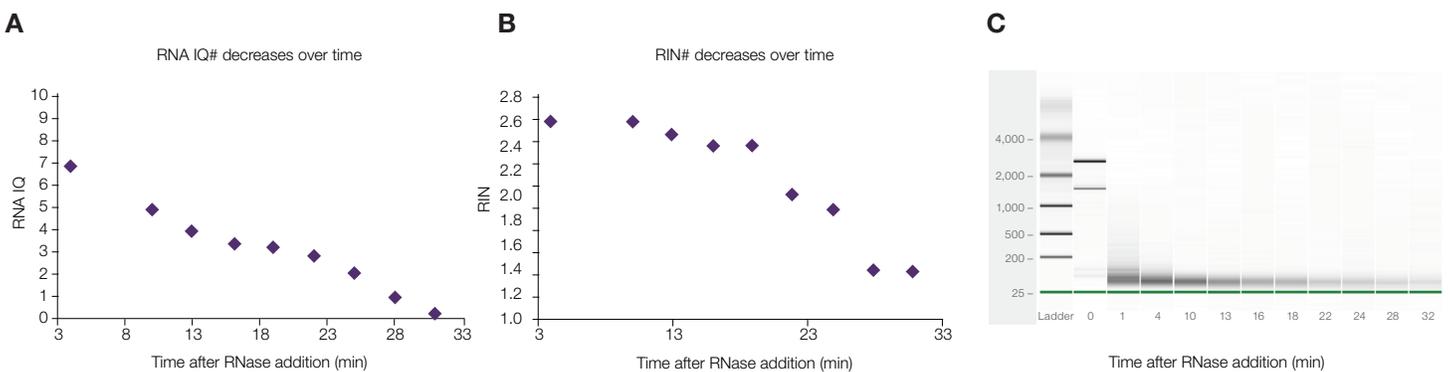
### Measurement of RNA degradation

Triplicate samples of 100 ng/mL rRNA solutions were incubated with RNase A in the final assay solution containing multiplexed dyes and assay buffer. rRNA degradation by RNase A was measured in real time using the RNA IQ assay via the two fluorescence channels (Figure 3).

To compare RNA IQ and RIN measurements, various amounts of RNase A were added to aliquots of a 100 ng/mL solution of rRNA and at various time points treated with Invitrogen™ RNaseOUT™ Recombinant Ribonuclease Inhibitor to stop the reaction. Results were measured using either the Qubit RNA IQ Assay or Agilent™ RNA 6000 Nano Kit (Figure 4).



**Figure 3. Real-time measurement of rRNA degradation by RNase A, using the RNA IQ assay.** Results are plotted for (A) various concentrations of RNase A, (B) 10 fM RNase A, and (C) 100 fM RNase A.



**Figure 4. RNA assessment by either RNA IQ or RIN following RNase treatment.** Both (A) RNA IQ and (B) RIN values decrease over time. (C) RNA size rapidly decreases, as shown with the Bioanalyzer electropherogram.

### Correlation to RNA sequencing (RNA-Seq) results

RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue was subjected to RNA-Seq on the Ion Torrent™ Oncomine™ platform, and the results compared to RNA IQ results. Sufficiently mapped reads (>50% mappable reads) were found to correlate to RNA IQ >4. With this guideline, only 4 out of 60 samples resulted in a false-negative result, a 6.7% failure rate (Figure 5).

### Demonstration of dye selectivity

Triplicate samples containing *E. coli* rRNA (100 ng/μL) and varying amounts of siRNA (0 to 50 ng/μL) were assayed with the Qubit RNA IQ Assay on the Qubit 4 Fluorometer. The results show the selectivity of the two dyes in binding to different RNAs (Figure 6).

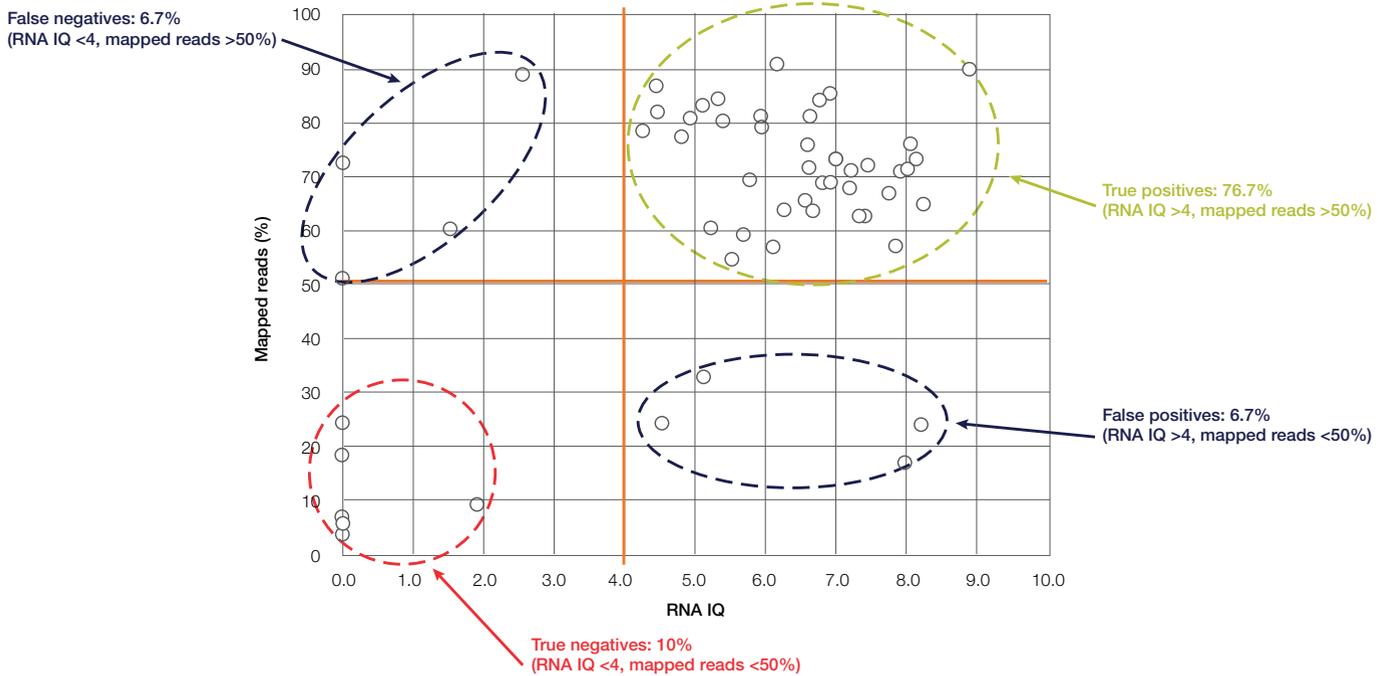


Figure 5. Correlation of RNA IQ values and RNA-Seq mappable reads from FFPE clinical research samples.

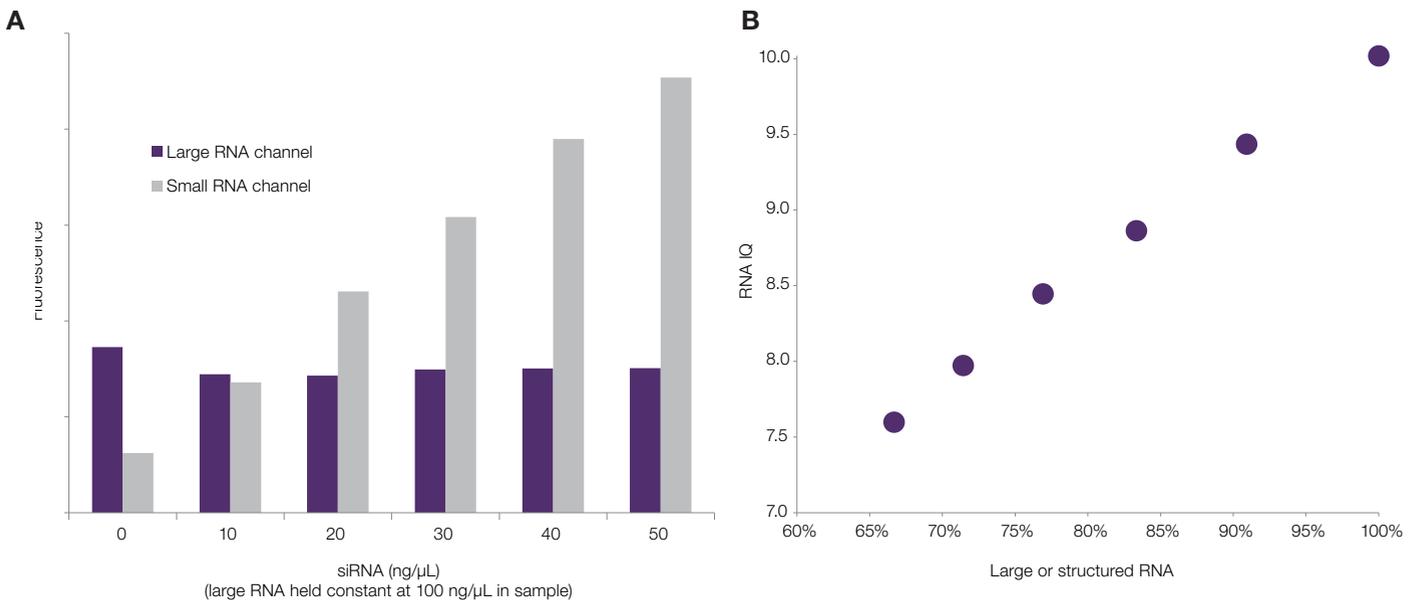


Figure 6. RNA IQ selectivity for large and small RNAs. (A) Fluorescence values obtained by the Qubit 4 Fluorometer are plotted for each type of RNA. (B) As expected, RNA IQ value increases with increasing percentage of large RNA.

## Conclusion

The Qubit RNA IQ Assay is a fast and easy method to measure RNA quality in under 5 minutes on the Qubit 4 Fluorometer. We have shown correlation to performance in RNA-Seq and RT-qPCR applications, and the ability to assess RNA degradation via enzymatic and thermodynamic processes. This assay allows assessment of RNA quality at a lower cost and with an easier, simpler, and faster workflow than with other solutions currently on the market.

- **Easy assessment of RNA integrity**—two unique dyes, one for large RNA and one for small, degraded RNA
- **Simple protocol**—add RNA sample to Qubit RNA IQ Buffer and measure on the Qubit 4 Fluorometer
- **Rapid time-to-results**—about 5 minutes for sample preparation and 4 seconds for sample measurement

## Ordering information

Product	Initial sample concentration	Quantitation range	Quantity	Cat. No.
<b>RNA integrity and quality kit</b>				
Qubit RNA IQ Assay Kit*	NA	NA	75 assays	Q33221
			275 assays	Q33222
<b>RNA quantitation kits</b>				
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	1–1,00 ng	100 assays	Q32880
			500 assays	Q32881
<b>Instrument and accessories</b>				
Qubit 4 Fluorometer			1	Q33226
Qubit 4 RNA IQ Starter Kit			1 kit	Q33229
Qubit 4 Quantitation Starter Kit			1 kit	Q33227
Qubit 4 NGS Starter Kit			1 kit	Q33228
Qubit Assay Tubes			500 tubes	Q32856

\* Note: The Qubit RNA IQ Assay for the detection of degraded RNA can only be run on the Qubit 4 Fluorometer and cannot be performed on the original Qubit, Qubit 2.0, or Qubit 3.0 Fluorometers.

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