Testing for bias in the Quant-iT RNA assay using single- and double-stranded RNA homopolymers

The Invitrogen™ Quant-iT™ RNA assay measures RNA concentration in samples containing 250 pg/µL to 100 ng/µL of RNA. To test for the presence and extent of any bias in the Quant-iT RNA assay for any of the bases (A, C, G, or U), or for single- or double-stranded RNA, the response of single- and double-stranded RNA homopolymers in the Quant-iT RNA assay has been examined.

Table 1. Relative fluorescence enhancement of single- and double-stranded RNA homopolymers compared to *E. coli* ribosomal RNA.

ssRNA							
Identity	Poly(A)	Poly(U)	Poly(C)	Poly(G)	Poly(I)	Poly(A,U)	Poly(C,I)
% rRNA signal	4.88	1.20	0.78	49.42	23.82	26.72	55.99
dsRNA							
Identity	Poly(A)·Poly(U)			Poly(C)·Poly(G)		Poly(I)·Poly(C)	
% rRNA signal	17.96			8.06		6.50	

Bias analysis

Triplicate samples of homopolymer solutions in 10 mM Tris, 1 mM EDTA (pH 7.5) were added in 10 µL aliquots to the Quant-iT RNA assay (Cat. No. Q33140) in a standard microplate assay protocol, and their response was compared to that from the *E. coli* rRNA standard included in the assay kit. The final concentrations of nucleic acid in the assay wells ranged from 25 ng/mL to 500 ng/mL. Single-stranded homopolymers tested were: Poly(A), Poly(U), and Poly(C) (GE, Cat. No. 27-4110-01, 27-4440-02, and 27-

4220-02, respectively); Poly(G), Poly(I), Poly(A,U), and Poly(C,I) (Sigma-Aldrich, Cat. No. P-4404, P-4154, P-8662, and P-1038, respectively). Double-stranded homopolymers tested were: Poly(A)·Poly(U), Poly(C)·Poly(G), and Poly(I)·Poly(C) (Sigma, Cat. No. P-1537, P-4038, and P-9582, respectively). The results indicate a general base selectivity towards guanine and an overall preference for single-stranded over double-stranded species (Figure 1, Table 1).
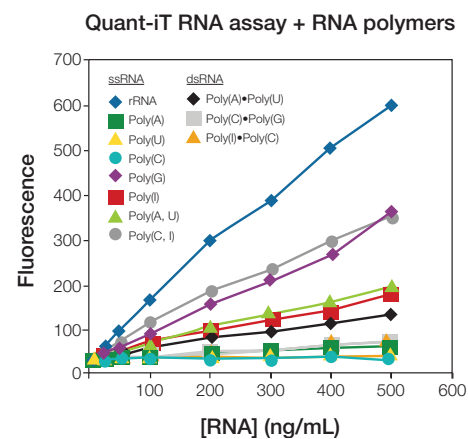
Figure 1. Quant-iT RNA assay response to single- and double-stranded polymers.

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Homopolymer length

Using S20,W (sedimentation) values listed on the supplied Certificates of Analysis, we determined the approximate average length of Poly(C), Poly(A), and Poly(U) (GE) to be 555, 535, and 321 bases, respectively. Average lengths of the remaining polymers, all obtained from Sigma-Aldrich, could not be determined, as key information was not available. According to a Sigma-Aldrich technical service representative, "This product is a mixture of molecules with a wide range of molecular sizes; therefore, we do not specify the chain length or number of bases it contains. This heterogeneity exists since there is no size-limiting or selection step during the production process."

Conclusions

The Quant-iT RNA assay showed some bias in response to different single-stranded homopolymers, with significant fluorescence increases observed in response to guanine and inosine. In addition, the assay generally gave larger responses to single-stranded polymers than to double-stranded polymers, even with similar base composition. Therefore, if the base composition and strandedness of the RNA being quantitated are known, we recommend using a similar type of RNA as the standard in the assay.

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