TECHNICAL NOTE

Qubit Fluorometer

Comparison of the accuracy and precision of dsDNA quantitation using Qubit and competitor assays

Introduction

The Invitrogen™ Qubit™ Fluorometer is a popular benchtop instrument for quantifying nucleic acids and proteins. It is widely used for applications such as next-generation sequencing because of the simplicity, accuracy, and precision of its associated fluorescence-based quantitation assays. Invitrogen™ currently offers the following assays for quantifying double-stranded DNA (dsDNA) using the Qubit Fluorometer:

- Invitrogen™ Qubit™ dsDNA BR (Broad Range) Assay Kit (Cat. No. Q32850)
- Invitrogen™ Qubit™ dsDNA HS (High Sensitivity) Assay Kit (Cat. No. Q32851)
- Invitrogen™ Qubit™ 1X dsDNA HS Assay Kit (Cat. No. Q33230)
- Invitrogen™ Qubit™ 1X dsDNA BR Assay Kit (Cat. Nos. Q33265 and Q33266)

The Qubit 1X dsDNA HS and BR assay kits provide a reagent and buffer in a formulation that is stable as a ready-to-use solution that helps provide the same limit of detection and dynamic range as the original Qubit dsDNA HS and BR assay.

Established advantages of these assays include their selectivity and sensitivity for dsDNA, and their superior accuracy and precision (Figure 1) compared to other dsDNA quantitation kits on the market. In this technical note we compare the performance of the Qubit dsDNA HS and BR assays to the equivalent assays sold by a leading competitor. Our data show that contrary to claims published by the competitor, our assays exhibit superior accuracy and precision of measured dsDNA quantities.

Methods

Qubit dsDNA HS and BR assays on the Qubit Fluorometer were compared to a competitor's dsDNA Ultra High Sensitivity, High Sensitivity and Broad Range assays using their fluorometer and were tested in accordance with the manufacturer's protocols. Serial dilutions of calf thymus DNA for the competitor assays and phage lambda DNA for the Qubit assays were prepared in 1X TE buffer, pH 7.5 and added to the working solution in a thin-walled, clear, UV-transparent 0.5 mL microcentrifuge tube. Sample volumes between 1 and 20 µL were used and adjusted based on the total mass of the dsDNA so that the final concentration in the assay tube was within the range of the particular assay. The working solution volume was adjusted so that the final volume in each tube was 200 µL. At least three replicates were prepared and measured for each DNA sample concentration being tested. The precision of each set of measurements was determined using the coefficient of variation (CV = (standard deviation/mean) x 100%). The accuracy of each measurement was also assessed by calculating the relative error (RE = [(expected concentration – measured concentration)/(expected concentration)]x 100%).

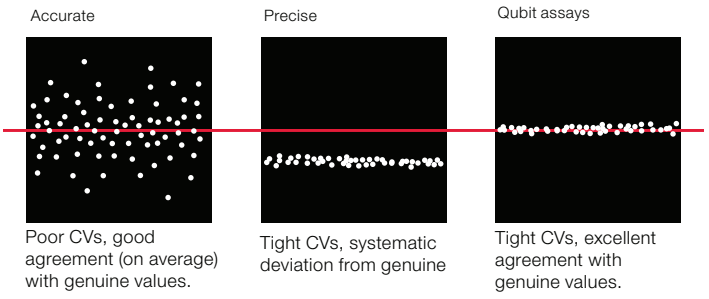

Figure 1. The need for both accurate and precise measurements of nucleic acid. Accurate but imprecise assays require replicate determinations. Precise but inaccurate assays cannot be trusted. Qubit assays are both accurate and precise, giving you results you can trust.

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Results

Published competitor data focuses on their high assay precision (low CV). Here, our evaluation of both the CV (precision) and RE (accuracy) gives a more complete assessment of the performance of the assays. For the competitor's dsDNA Broad Range Assay, our analysis shows measurement deviations and high CV throughout the concentration range (Figure 2 and Table 1). In contrast, the Qubit dsDNA BR Assay results in more accurate measurements across the range of dsDNA concentrations.

The competitor claims that their assays allow increased detection sensitivity compared to Qubit assays. However, our analysis shows that the competitor's dsDNA High Sensitivity Assay has reduced accuracy at low assay concentrations (Figure 3). The Qubit Fluorometer does not report values for the low or high end of the concentration range when the accuracy and precision are low, increasing confidence in the results (Table 1, concentrations in bold).

Even though there is no Qubit assay directly equivalent to the competitor's dsDNA Ultra High Sensitivity Assay, a significant portion of the detection range is covered by the Qubit dsDNA HS Assay while maintaining high precision and accuracy (Figure 4). This more detailed analysis highlights gaps in the performance claims of the competitor's assay (Table 2).

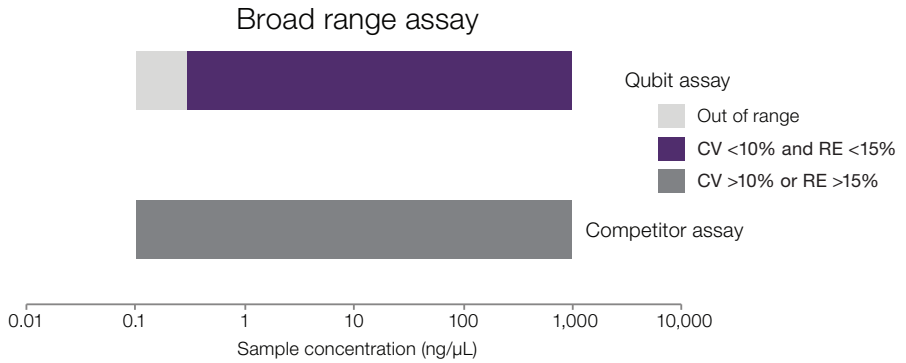

Figure 2. Comparison of Qubit and competitor assays over the broad dsDNA detection range of 0.1–1,000 ng/µL. The competitor's dsDNA Broad Range Assay has either low precision or low accuracy (CV >10% or RE >15%) for most dsDNA concentrations, while the Qubit dsDNA BR Assay has high precision (CV <10%) and accuracy (RE <15%) for most dsDNA concentrations from 0.2 to 1,000 ng/µL.

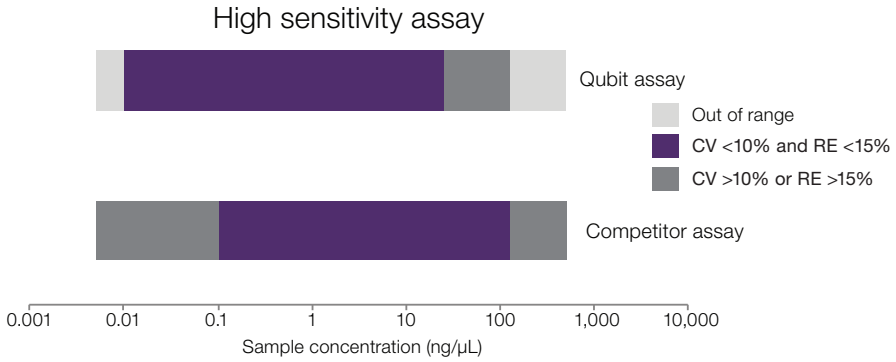

Figure 3. Comparison of Qubit and competitor assays over the high sensitivity dsDNA detection range of 5 pg/µL–250 ng/µL. The Qubit dsDNA HS Assay has higher accuracy and precision for low sample concentrations, compared to the competitor's dsDNA High Sensitivity Assay.

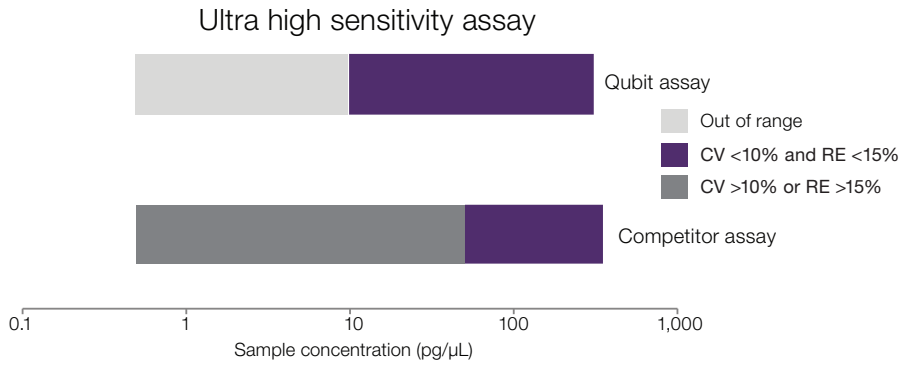

Figure 4. Comparison of Qubit and competitor assays over the ultra high sensitivity dsDNA detection range of 0.5–300 pg/µL. In contrast to claims from the competitor that their ultra high sensitivity assay can quantitate dsDNA concentrations as low as 0.5 pg/µL, we show that the measured concentrations in the range of 0.5–50 pg/µL have low accuracy and precision. For concentrations higher than 10 pg/µL, the Qubit dsDNA HS Assay offers better precision (CV <10%) and accuracy (RE <15%) than that of the competitor's dsDNA Ultra High Sensitivity Assay.

Table 1. Performance data for assays tested in this study.

Broad range

	Competitor assay			Qubit assay		
Concentration (ng/µL)	Mean (ng/µL)	CV (%)	RE (%)	Mean (ng/µL)	CV (%)	RE (%)
1,000*	1,198.70	3.3	–19.8	984.00	3.1	1.6
400*	328.67	18.4	17.8	382.00	12.3	4.5
200	209.94	8.1	–4.9	185.33	6.2	7.3
100	114.64	2.5	–14.6	93.27	2.3	6.7
50	58.32	4.2	–16.6	47.15	4.5	5.7
25	24.98	17.9	0.0	24.20	3.7	3.2
12.5	14.61	3.7	–16.8	11.78	2.2	5.8
6.25	6.53	3.6	–4.5	5.70	0.6	8.8
2	2.06	18.0	–3.2	1.82	3.7	8.8
1	0.87	13.5	12.9	0.98	7.5	2.2
0.5	0.46	25.3	7.3	0.43	8.7	13.1
0.2**	0.19	38.3	3.1	0.19	6.5	3.0
0.1**	0.12	6.8	–18.67	Out of range (too low)†		

High sensitivity

	Competitor assay			Qubit assay		
Concentration (ng/µL)	Mean (ng/µL)	CV (%)	RE (%)	Mean (ng/µL)	CV (%)	RE (%)
250*	227.73	15.1	8.9	Out of range (too high)†		
100*	95.87	2.6	4.1	82.70	6.6	17.3
25	25.23	2.8	–0.9	26.94	9.2	–7.7
10	9.79	2.6	2.1	9.79	3.3	2.1
3	2.78	1.6	7.3	2.94	3.6	2.1
1	0.96	1.4	4.0	0.97	6.1	3.1
0.3	0.28	2.3	6.6	0.31	8.4	–1.6
0.1	0.086	1.7	13.7	0.101	4.5	–1.0
0.03	0.024	2.4	21.0	0.027	8.2	10.0
0.01	0.0067	8.6	33.0	0.009	7.9	10.5
0.005	0.0047	12.3	6.0	Out of range (too low)†		

Ultra high sensitivity

	Competitor assay			Qubit assay		
Concentration (pg/µL)	Mean (pg/µL)	CV (%)	RE (%)	Mean (pg/µL)	CV (%)	RE (%)
300	298.08	4.5	0.6	274.67	1.8	8.4
150	146.22	4.5	2.5	138.00	2.6	8.0
50	46.20	6.9	7.6	50.43	1.4	–0.8
10	8.92	11.2	10.8	11.37	5.0	–13.6
2	1.74	21.3	14.9	Out of range (too low)†		
1	0.82	33.2	22.3			
0.5	0.39	79.7	27.8			

* Measured with 1 µL of sample, all other samples tested used 10 µL of sample in accordance with manufacturer's protocols.
** Measured with 20 ng/µL, all other samples tested used 10 µL of sample, in accordance with manufacturer's protocols.
† The Qubit Fluorometer does not report values with low accuracy or precision at the concentrations in bold. This prevents compounding errors in downstream experiments.

Table 2. Comparison between claims by competitor and results of in-house testing by Thermo Fisher Scientific for dsDNA quantitation with competitor and Qubit assays.

dsDNA assay	Claim by competitor	In-house analysis by Thermo Fisher Scientific
Broad range	The dsDNA Broad Range Assay has a broader quantitation range than the Qubit dsDNA BR Assay.	The Qubit dsDNA BR Assay has better accuracy and precision than the competitor's dsDNA Broad Range Assay (Figure 2).
High sensitivity	The dsDNA High Sensitivity Assay has a broader quantitation range than the Qubit dsDNA HS Assay.	The Qubit dsDNA HS Assay has better accuracy and precision at the low end of the concentration range (0.01–0.1 ng/µL) than the competitor's dsDNA High Sensitivity Assay (Figure 3).
Ultra high sensitivity	The dsDNA Ultra High Sensitivity Assay can quantify dsDNA concentrations as low as 0.5 pg/µL. There is no equivalent Qubit assay.	The low end (0.5–50 pg/µL) of the competitor's dsDNA Ultra High Sensitivity Assay has low accuracy and precision (Figure 4). The Qubit dsDNA HS Assay offers comparable precision and accuracy over the same concentration range as the competitor's dsDNA Ultra High Sensitivity Assay (Fig. 4)

Conclusions

There are many quantification assays available, but selecting the right one at the right time can be challenging. It's important to differentiate between detection ranges and accuracy claims. While some assays boast wide detection ranges and high precision, they may lack accuracy. Qubit systems, however, provide both accuracy and precision. Accurate and reproducible measurements are crucial for confidence in downstream applications. Qubit and Quant-iT assays deliver top-tier nucleic acid quantification for various throughput levels and automated lab settings. Qubit assays ensure high precision (CV <10%) and accuracy (RE <15%) across the core dynamic range.

In contrast, competitor assays often emphasize sensitivity or broad dynamic ranges but may lack accuracy or precision data. When accuracy and precision are compromised, the Qubit Fluorometer doesn't report values at the extremes of the concentration range, ensuring reliable data and minimizing errors in downstream experiments.

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Ordering information

Product	Quantity	Cat. No.
Qubit 1X dsDNA HS Assay Kit, 100 assays	1 kit	Q33230
Qubit 1X dsDNA HS Assay Kit, 500 assays	1 kit	Q33231
Qubit dsDNA BR Assay Kit, 100 assays	1 kit	Q32850
Qubit dsDNA BR Assay Kit, 500 assays	1 kit	Q32853
Qubit dsDNA HS Assay Kit, 100 assays	1 kit	Q32851
Qubit dsDNA HS Assay Kit, 500 assays	1 kit	Q32854
Qubit Assay Tubes, set of 500	1 set	Q32856
Qubit 4 Fluorometer with WiFi	1 instrument	Q33238
Qubit 4 Quantitation Starter Kit (with WiFi)	1 kit	Q33239
Qubit 4 NGS Starter Kit (with WiFi)	1 kit	Q33240
Qubit 4 RNA IQ Starter Kit (with WiFi)	1 kit	Q33241

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