Qubit dsDNA assay specificity in the presence of single-stranded DNA

We offer two assays for quantitating double-stranded DNA (dsDNA) using the Invitrogen™ Qubit™ Fluorometer:

- Invitrogen™ Qubit™ dsDNA HS (High Sensitivity) Assay
- Invitrogen™ Qubit™ dsDNA BR (Broad Range) Assay

One advantage of these assays is their specificity for dsDNA. This study was carried out to investigate whether Qubit dsDNA quantitation assays accurately determine the concentration of dsDNA when the samples contain short or long single-stranded DNA (ssDNA).

Summary

The Qubit dsDNA HS Assay Kit exhibits high accuracy and precision for pure dsDNA samples in the range of 0.01–100 ng/µL. The Qubit dsDNA BR Assay Kit shows similarly high accuracy and precision for pure dsDNA samples in the range of 0.1–1,000 ng/µL. This study shows that both Qubit dsDNA assays detected only 2–10% of pure ssDNA for the majority of the range. Similarly, when an equal mass of ssDNA was added to dsDNA, the results generally changed by less than 10% from the results obtained with dsDNA alone.

Experimental method

We tested 2 types of ssDNA, long and short, in both assays. Specifically, we tested an 18-mer oligonucleotide (M13 sequencing primer, sequence 5′-TGTAAAACGACGGCCAGT-3′) and viral ssDNA isolated from M13mp18 phage (7,249 bases, New England Biolabs, Cat. No. N4040S). These were tested against, or in combination with, lambda dsDNA (Invitrogen™ λ DNA, Cat. No. 25250010) using the Qubit dsDNA BR Assay Kit and the Qubit dsDNA HS Assay Kit.

Qubit 4 Fluorometer

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

Key features include:

- Qubit assay dyes bind selectively to DNA or RNA, making them more sensitive than UV absorbance (Figure 1)
- Assays use as little as 1 µL of sample, even with very dilute samples
- Fast, reliable detection of degraded RNA with the new Invitrogen™ Qubit™ RNA IQ Assay
- New integrated reagent calculator to quickly generate working solution calculations

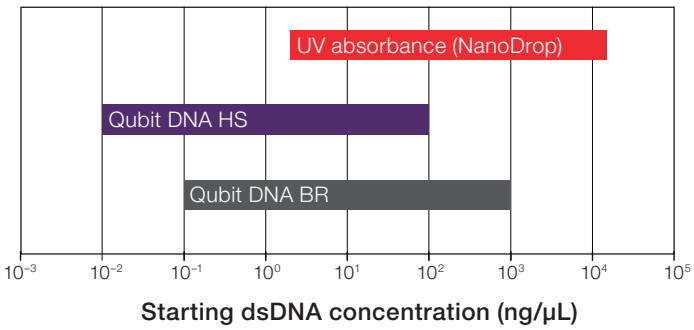

Figure 1. Comparison of dsDNA concentration ranges for the Qubit assays and UV absorbance measurements using the NanoDrop Spectrophotometer.

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Results

Assaying ssDNA alone

First we tested each type of DNA (oligo DNA, long ssDNA, and dsDNA) separately in each assay. For both the oligonucleotide and the long ssDNA, the Qubit dsDNA HS and BR Assays detected less than 10% of the actual concentration of ssDNA (calculated according to the manufacturer's reported concentration) in the sample (Figures 2A and 2B). These results show that the Qubit dsDNA assays are highly selective for dsDNA compared to ssDNA.

Assaying dsDNA in the presence of ssDNA with the Qubit dsDNA HS Assay Kit

We also tested dsDNA in the presence of ssDNA in the Qubit dsDNA assays to see if ssDNA would interfere with dsDNA quantitation. For the Qubit dsDNA HS Assay, at a 1:1 mass ratio of oligo DNA to dsDNA, the dsDNA concentration determined by the assay was within 11% of the value obtained without any oligo DNA, for all concentrations tested (Figure 3). At a 1:1 mass ratio of long ssDNA to dsDNA for low concentrations of DNA, the dsDNA concentration determined by the assay was within 8% of the value obtained without any ssDNA (Figure 3A and 3B). However, at higher concentrations of nucleic acid in an assay that contained a 1:1 mass ratio of long ssDNA to dsDNA, the concentration of dsDNA determined by the assay was lower than the value obtained without ssDNA, by 21% (Figure 3C). Therefore, for samples containing at least 400 ng/mL dsDNA (final concentration in the assay tube) where ssDNA contamination is a possibility, we recommend the Qubit dsDNA BR Assay.

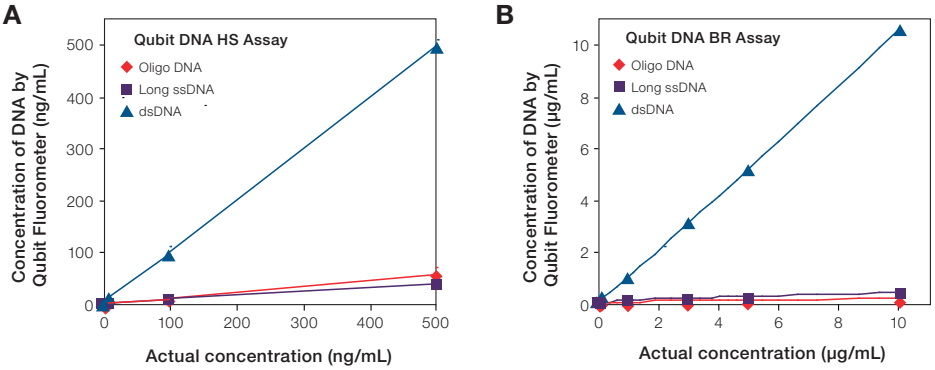

Figure 2. Detection of single-stranded DNA by the Qubit dsDNA HS (A) and BR (B) Assays. Duplicate samples of long ssDNA, oligo DNA, or lambda dsDNA were added to the Qubit dsDNA HS Assay at concentrations of 0.5 to 500 ng/mL in the assay tube and to the Qubit dsDNA BR Assay at concentrations of 0.01 to 10 µg/mL in the assay tube, according to kit protocols.

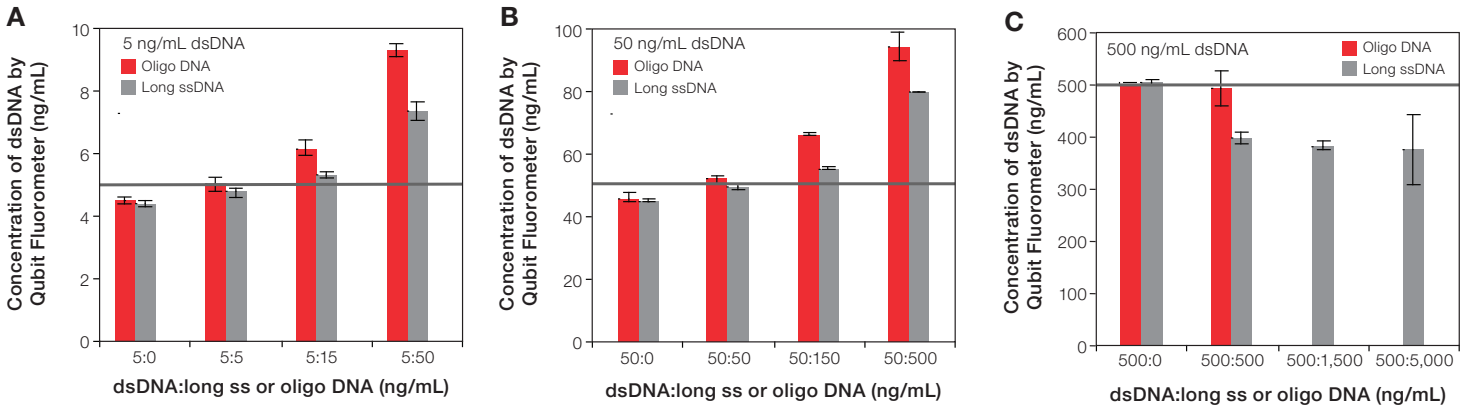

Figure 3. Double-stranded DNA in the presence of oligo DNA or long ssDNA in the Qubit dsDNA HS Assay on the Qubit Fluorometer. Duplicate samples of dsDNA were mixed with 0, 1X, 3X, or 10X concentrations (relative to the dsDNA concentration) of oligo DNA or ssDNA and added to the Qubit dsDNA HS Assay at dsDNA concentrations of **(A)** 5 ng/mL, **(B)** 50 ng/mL, and **(C)** 500 ng/mL in the assay tube. The horizontal lines indicate the actual concentrations of dsDNA.

Assaying dsDNA in the presence of ssDNA with the Qubit dsDNA BR Assay Kit

For the Qubit dsDNA BR Assay, at a 1:1 mass ratio of oligo DNA to dsDNA, the dsDNA concentration determined by the assay was within 3% of the value obtained without oligo DNA, for all concentrations tested. At a 1:1 mass ratio of long ssDNA to dsDNA, the dsDNA concentration determined by the assay was within 11% of the value obtained without ssDNA, for all concentrations tested (Figure 4).

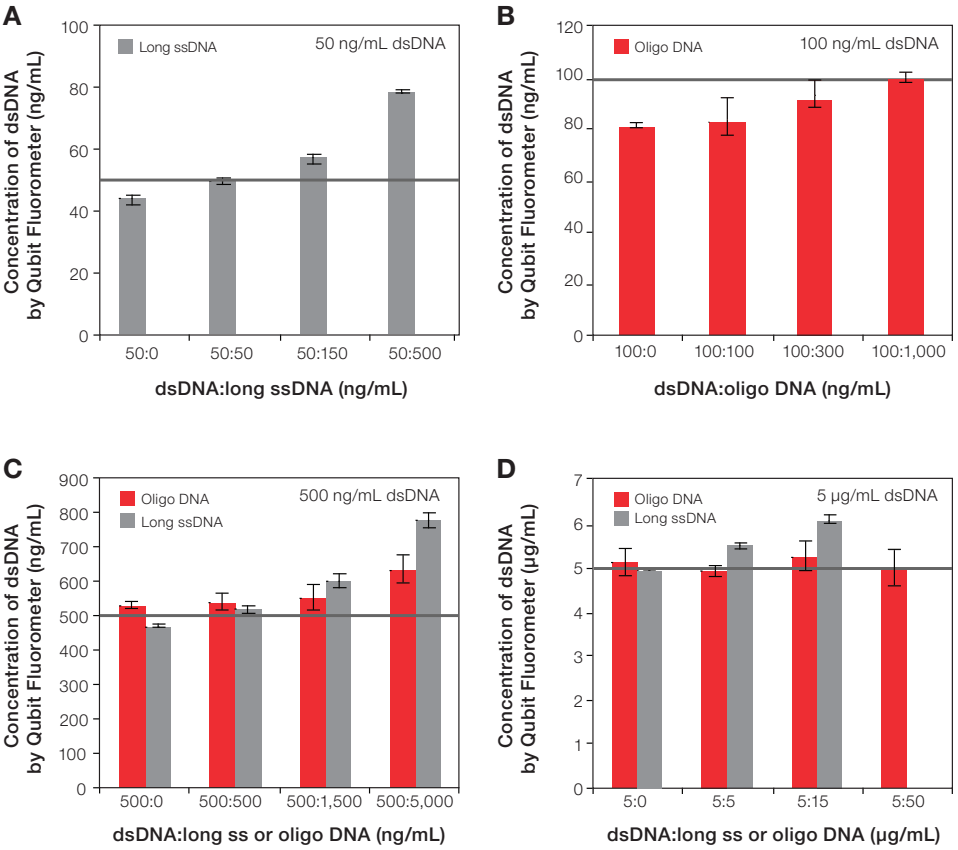

Figure 4. Double-stranded DNA in the presence of oligo or long ssDNA in the Qubit dsDNA BR Assay on the Qubit Fluorometer. Duplicate samples of dsDNA were mixed with 0, 1X, 3X, or 10X concentrations (relative to the dsDNA concentration) of oligo DNA or long ssDNA and added to the Qubit dsDNA BR Assay at dsDNA concentrations of **(A)** 50 ng/mL, **(B)** 100 ng/mL, **(C)** 500 ng/mL, and **(D)** 5 µg/mL in the assay tube. The horizontal lines indicate the actual concentrations of dsDNA.

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Conclusion

The concentration detected for a mixture of equal masses of dsDNA and ssDNA was generally within 10% of the concentration determined for dsDNA alone. For example, a mixture of 500 ng/mL dsDNA and 500 ng/mL long ssDNA was measured as 520 ng/mL dsDNA using the Qubit dsDNA BR Assay (Figure 4C). However, at the high end of the dsDNA concentration range for the Qubit dsDNA HS Assay, with equal masses of double-stranded and long single-stranded DNA, the concentration determined by the assay was only 80% of the actual dsDNA concentration (Figure 3C). For this reason, for concentrations of dsDNA above 400 ng/mL (in the assay tube), when long ssDNA contamination is suspected, we recommend using the Qubit dsDNA BR Assay. When concentrations of oligo or long ssDNA exceeded the concentration of dsDNA by three-fold or more, the concentration of dsDNA determined by either assay was in most cases more than 10% higher than the actual dsDNA concentration. Table 1 summarizes the results.

Table 1. Summary of ssDNA contamination results using the Qubit dsDNA HS and BR Assays.

Amount of ssDNA tolerated in the assay*		
	Qubit dsDNA HS Assay	**Qubit dsDNA BR Assay**
18-mer oligo ssDNA	1:1 ssDNA:dsDNA, across the full assay concentration range	1:1 ssDNA:dsDNA, across the full assay concentration range
M13mp18 phage (long) ssDNA	1:1 ssDNA:dsDNA, up to 400 ng/mL**	1:1 ssDNA:dsDNA, across the full assay concentration range

* Level of ssDNA contamination tolerated by the assay with <11% perturbation of results.

** For dsDNA concentrations above 400 ng/mL in the assay tube, where long ssDNA contamination is suspected, the Qubit dsDNA HS Assay is not recommended; for these we recommend the Qubit dsDNA BR Assay.

Ordering information

Product	Initial sample concentration	Quantitation range	Quantity	Cat. No.
DNA kits				
Qubit ssDNA Assay Kit	50 pg/µL to 200 ng/µL	1–200 ng	100 assays	Q10212
Qubit dsDNA BR Assay Kit	100 pg/µL to 1,000 ng/µL	2–1,000 ng	100 assays	Q32850
			500 assays	Q32853
Qubit dsDNA HS Assay Kit	10 pg/µL to 100 ng/µL	0.2–100 ng	100 assays	Q32851
			500 assays	Q32854
Qubit 1X dsDNA HS Assay Kit†	10 pg/µL to 100 ng/µL	0.2–100 ng	100 assays	Q33230
			500 assays	Q33231
Instrument and accessories				
Qubit 4 Quantitation Starter Kit			1 kit	Q33227
Qubit 4 NGS Starter Kit			1 kit	Q33228
Qubit 4 RNA IQ Starter Kit			1 kit	Q33229
Qubit 4 Fluorometer			1	Q33226

† This kit contains a ready-to-use formulation that does not require mixing of buffer and reagent.

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