The use of glycogen and GlycoBlue reagent in Qubit DNA and RNA assays as measured on the Qubit Fluorometer

Glycogen (a branched-chain carbohydrate; Cat. No. 10814010) and Invitrogen™ GlycoBlue™ Coprecipitant (glycogen covalently linked to a blue dye; Cat. No. AM9515) are reagents commonly used to facilitate nucleic acid precipitation without adding extraneous nucleic acids to the sample. This study was carried out to determine whether glycogen or GlycoBlue Coprecipitant affects the accuracy of nucleic acid quantitation using Invitrogen™ Qubit™ kits.

Summary

We evaluated whether Invitrogen™ Qubit™ DNA and RNA quantitation assays can deliver accurate quantitation results when the nucleic acid samples contain high concentrations of glycogen or GlycoBlue Coprecipitant. We found that nucleic acid samples containing 500 ng/µL of glycogen (25 ng/µL in the assay tube) were quantified accurately (within 5% of controls) in all four assays tested: the Qubit dsDNA HS, dsDNA BR, RNA BR, and RNA HS assays. Similarly, nucleic acid samples containing 500 ng/µL of GlycoBlue Coprecipitant (25 ng/µL in the assay tube) were quantified accurately in three of the assays tested: the Qubit dsDNA HS, dsDNA BR, and RNA BR assays. The Qubit RNA HS assay, however, produced accurate quantitation results only when GlycoBlue Coprecipitant was present at the lower concentration of 100 ng/µL (5 ng/µL in the assay tube). This means that for all of the Qubit DNA and RNA assays tested, glycogen and GlycoBlue Coprecipitant are tolerated in the DNA or RNA sample at concentrations above the manufacturers' recommended concentrations. Table 1 gives a summary of the concentrations tested, manufacturers' recommended concentrations, and results for each assay.

Table 1. Concentrations of additives tested in this investigation.

Additive	Concentration recommended (in sample)*	Concentration tested (in sample)†	Concentration tolerated in each Qubit assay (in assay tube)§
Glycogen	200–400 ng/µL (10–20 ng/µL in the assay tube**)	500 ng/µL (25 ng/µL in the assay tube**)	dsDNA HS: 25 ng/µL dsDNA BR: 25 ng/µL RNA BR: 25 ng/µL RNA HS: 25 ng/µL
GlycoBlue Coprecipitant	50 ng/µL (2.5 ng/µL in the assay tube**)	50–500 ng/µL (2.5–25 ng/µL in the assay tube**)	dsDNA HS: 25 ng/µL dsDNA BR: 25 ng/µL RNA BR: 25 ng/µL RNA HS: 5 ng/µL

* Concentration recommended by the manufacturer in the literature that accompanies the product.
† Concentration tested in our evaluation.
§ Final concentration after diluting 1:20 for the assay.
** The nucleic acid sample is diluted 1:20 for the assays. 10 µL of the nucleic acid sample is added to the Qubit™ reagent in the assay tube.

Assay methods and results

Qubit dsDNA HS assay

Core range of the assay: 1–500 ng/mL of double-stranded DNA

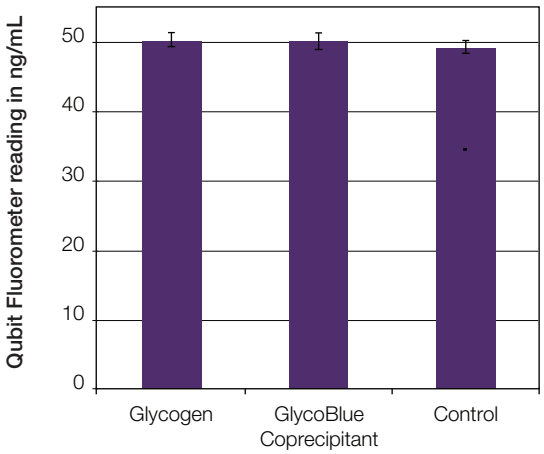

Figure 1. Results from the Qubit dsDNA HS assay for samples containing DNA plus 25 ng/µL glycogen or GlycoBlue Coprecipitant (in the assay tube) compared to samples containing DNA only. The DNA concentration in the assay tubes was 50 ng/mL (near the low end of the Qubit dsDNA HS assay range). Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of three replicates.

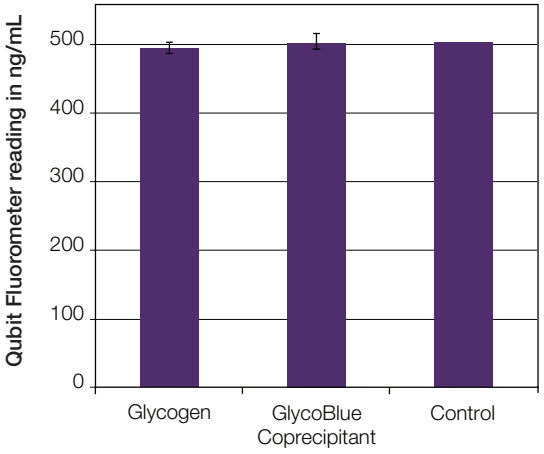

Figure 2. Results from the Qubit dsDNA HS assay for samples containing DNA plus 25 ng/µL glycogen or GlycoBlue Coprecipitant (in the assay tube) compared to samples containing DNA only. The DNA concentration in the assay tubes was 500 ng/mL (near the high end of the Qubit dsDNA HS assay range). Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of three replicates.

Qubit dsDNA BR assay

Core range of the assay: 0.01–5 µg/mL of double-stranded DNA

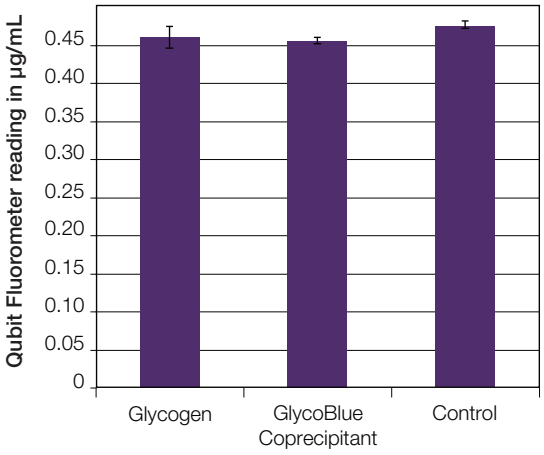

Figure 3. Results from the Qubit dsDNA BR assay for samples containing DNA plus 25 ng/µL glycogen or GlycoBlue Coprecipitant (in the assay tube) compared to samples containing DNA only. The DNA concentration in the assay tubes was 0.5 µg/mL (near the low end of the Qubit dsDNA BR assay range). Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of two or three replicates.

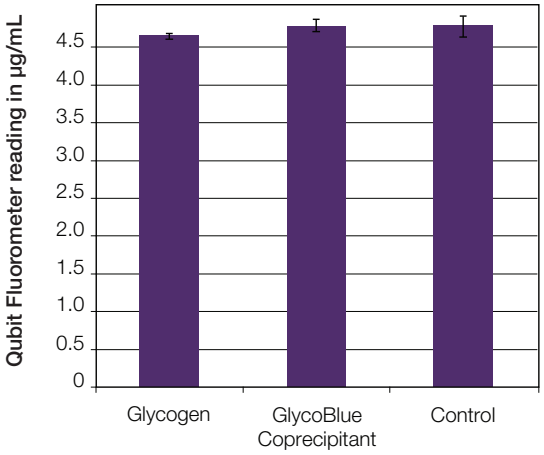

Figure 4. Results from the Qubit dsDNA BR assay for samples containing DNA plus 25 ng/µL glycogen or GlycoBlue Coprecipitant (in the assay tube) compared to samples containing DNA only. The DNA concentration in the assay tubes was 5 µg/mL (near the high end of the Qubit dsDNA BR assay range). Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of three replicates.

Qubit RNA BR assay

Core range of the assay: 0.1–5 µg/mL of RNA

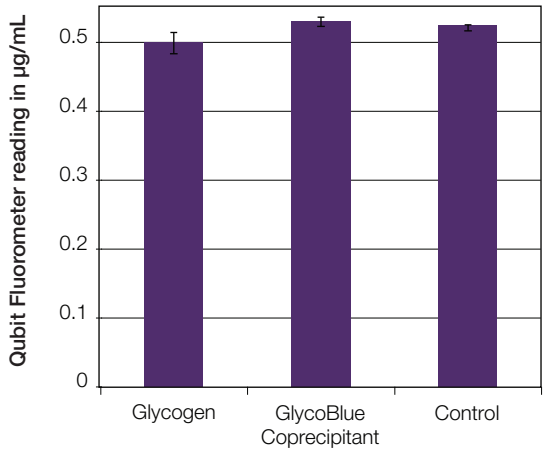

Figure 5. Results from the Qubit RNA BR assay for samples containing RNA plus 25 ng/µL glycogen or GlycoBlue Coprecipitant (in the assay tube) compared to samples containing RNA only. The RNA concentration in the assay tubes was 0.5 µg/mL (near the low end of the Qubit RNA BR assay range). Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of three replicates.

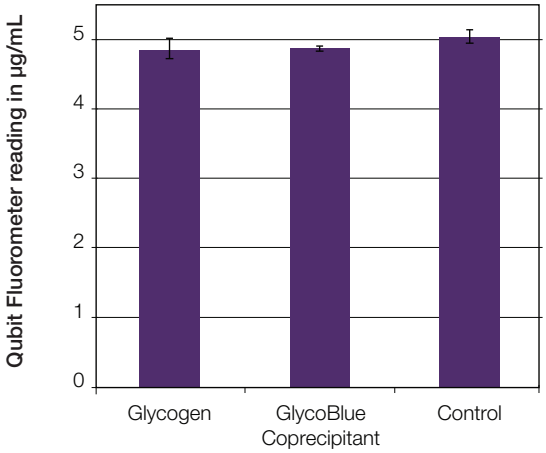

Figure 6. Results from the Qubit RNA BR assay for samples containing RNA plus 25 ng/µL glycogen or GlycoBlue Coprecipitant (in the assay tube) compared to samples containing RNA only. The RNA concentration in the assay tubes was 5 µg/mL (near the high end of the Qubit RNA BR assay range). Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of three replicates.

Qubit RNA HS assay

Core range of the assay: 25–500 ng/mL of RNA

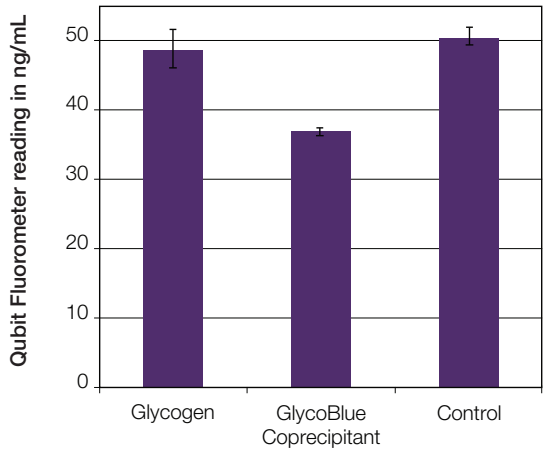

Figure 7. Results from the Qubit RNA HS assay for samples containing RNA plus 25 ng/µL glycogen or GlycoBlue Coprecipitant (in the assay tube) compared to samples containing RNA only. The RNA concentration in the assay tubes was 50 ng/mL (near the low end of the Qubit RNA HS assay range). Note that the results obtained with 25 ng/µL GlycoBlue Coprecipitant are not within 5% of the control readings. Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of three replicates.

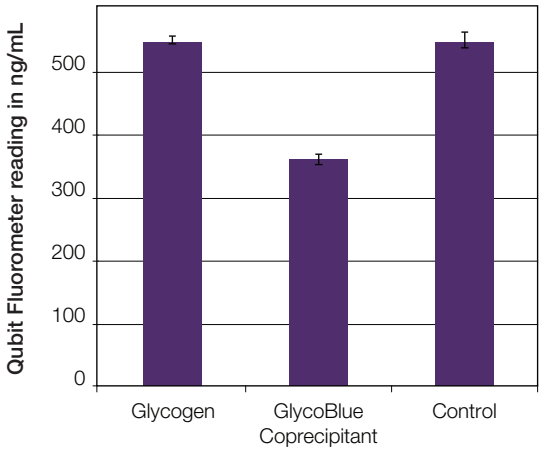

Figure 8. Results from the Qubit RNA HS assay for samples containing RNA plus 25 ng/µL glycogen or GlycoBlue Coprecipitant (in the assay tube) compared to samples containing RNA only. The RNA concentration in the assay tubes was 500 ng/mL (near the high end of the Qubit RNA HS assay range). Note that the results obtained with 25 ng/µL GlycoBlue Coprecipitant are not within 5% of the control readings. Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of three replicates.

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Qubit RNA HS assay

Core range of the assay: 25–500 ng/mL of RNA

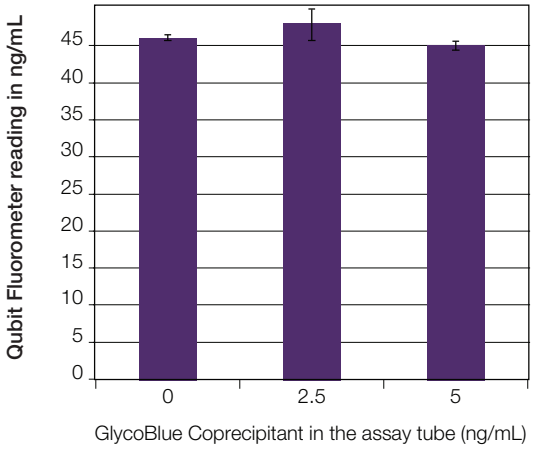

Figure 9. Results from the Qubit RNA HS assay for samples containing RNA plus 0–5 ng/µL GlycoBlue Coprecipitant (in the assay tube). The RNA concentration in the assay tubes was 50 ng/mL (near the low end of the Qubit RNA HS assay range). Note that the results obtained in the presence of 0 to 5 ng/µL GlycoBlue Coprecipitant are within 5% of the control readings. Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of two replicates.

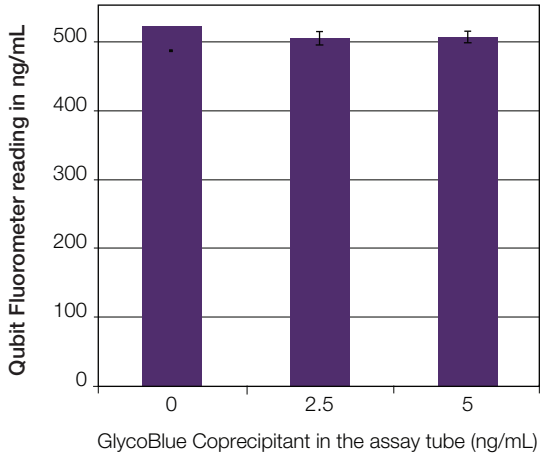

Figure 10. Results from the Qubit RNA HS assay for samples containing RNA plus 0–5 ng/µL GlycoBlue Coprecipitant (in the assay tube). The RNA concentration in the assay tubes was 500 ng/mL (near the high end of the Qubit RNA HS assay range). Note that the results obtained in the presence of 0 to 5 ng/µL GlycoBlue Coprecipitant are within 5% of the control readings. Assay tubes contained between 10 µL and 15 µL of sample; results are the averages of two replicates.

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