Quant-iT™ OliGreen™ ssDNA Reagent and Kit

Catalog Numbers O7582, O11492

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Quant-iT™ OliGreen™ ssDNA Assay Reagent is an ultrasensitive fluorescent nucleic acid stain for quantifying oligonucleotides and single-stranded DNA (ssDNA) in solution (Figure 1). Short, synthetic oligonucleotides are used in many molecular biology techniques, such as DNA sequencing, site-directed mutagenesis, DNA amplification, and *in situ* hybridization. Unfortunately, the classic methods for quantifying oligonucleotides are not very sensitive and often require a highly concentrated sample.

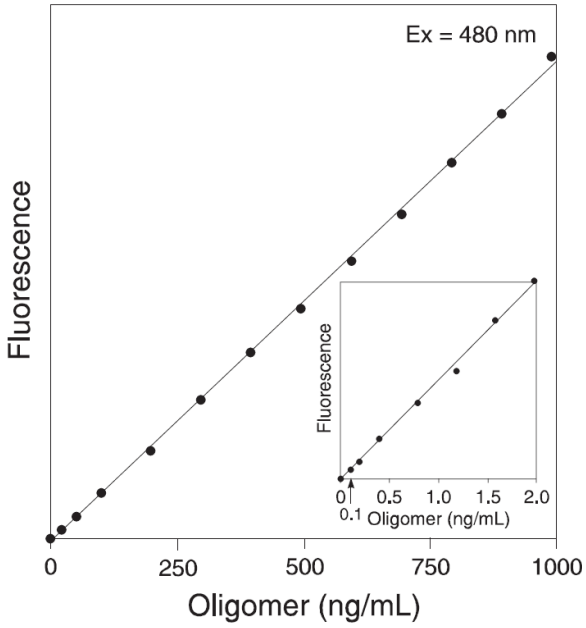

Figure 1 Linear quantification of a synthetic 24-mer (an M13 sequencing primer) from 0.1 to 1,000 ng/mL using the Quant-iT™ OliGreen™ ssDNA Assay Reagent.
Samples in 10 mm × 10 mm cuvettes were excited at 480 nm. The fluorescence emission intensity was measured at 520 nm using a spectrofluorometer and plotted as a function of oligonucleotide concentration. The inset shows an enlargement of the results obtained with oligonucleotide concentrations between 0 and 2.0 ng/mL.

The Quant-iT™ OliGreen™ ssDNA Assay Reagent enables researchers to quantify as little as 1 ng/mL oligonucleotide or ssDNA (200 pg in a 200 µL assay volume) with a fluorescence microplate reader using fluorescein excitation and emission wavelengths. We have also quantitated several ssDNAs with the Quant-iT™ OliGreen™ ssDNA Assay Reagent, including M13 and ϕX174 viral DNA and denatured calf thymus DNA, and obtained similar sensitivity. In addition, the Quant-iT™ OliGreen™ ssDNA Assay Reagent has been used to detect phosphodiester and phosphorothioate oligonucleotides in plasma and serum samples and to develop a sensitive fluorescence-based capillary electrophoresis method for detecting short, single-stranded oligonucleotides.

The linear detection range of the Quant-iT™ OliGreen™ assay in a standard fluorometer extends over 4 orders of magnitude in oligonucleotide concentration (100 pg/mL to 1 µg/mL) with a single dye concentration (Figure 1). Moreover, we have shown that this linearity is maintained in the presence of several compounds commonly found to contaminate nucleic acid preparations, including salts, urea, ethanol, chloroform, detergents, proteins, ATP, and agarose; however, many of these compounds do affect the signal intensity (see “Effects of common contaminants” on page 4).

Nucleotides and short oligonucleotides of six bases or less do not interfere in the quantitation assay; however, the Quant-iT™ OliGreen™ ssDNA Assay Reagent does exhibit fluorescence enhancement when bound to double-stranded DNA (dsDNA) and RNA.

Also, our experiments with homopolymers have demonstrated that the Quant-iT™ OliGreen™ ssDNA Assay Reagent exhibits significant base selectivity. The Quant-iT™ OliGreen™ ssDNA Assay Reagent shows a large fluorescence enhancement when bound to poly(dT), but only a relatively small fluorescence enhancement when bound to poly(dG) and little signal with poly(dA) and poly(dC). Thus, it is important to use an oligonucleotide with similar base composition when generating the standard curve.

Contents and storage

Component	Quant-iT™ OliGreen™ ssDNA Reagent[1]	Quant-iT™ OliGreen™ ssDNA Assay Kit	Concentration	Storage[2]
	Cat. No. O7582	Cat. No. O11492		
Quant-iT™ OliGreen™ ssDNA Reagent (Component A)	1 mL	1 mL	200X in DMSO	2°C to 8°C[3] Desiccate Protect from light
20X TE (Component B)	Not applicable	25 mL	200 mM Tris-HCl, 20 mM EDTA, pH 7.5	≤30°C
Oligonucleotide standard (Component C)[4]	Not applicable	1 mL	100 µg/mL solution in TE	2°C to 8°C[3]
Number of labelings: 2,000 with an assay volume of 200 µL in a 96-well microplate format. The Quant-iT™ OliGreen™ ssDNA Assay can be adapted for use in cuvettes or 384-well microplates.				
Approximate fluorescence excitation/emission maxima: 500/525 nm, bound to nucleic acid.				

[1] Stand-alone reagent does not include Components B and C.
[2] When stored as directed, products are stable for at least 6 months.
[3] For long-term storage, the Quant-iT™ OliGreen™ ssDNA Reagent and oligonucleotide standard can be stored at ≤–20°C.
[4] The oligonucleotide standard is an 18-base M13 sequencing primer, with the sequence 5'-TGTAAAACGACGGCCAGT-3'.

Required materials not supplied

- Nuclease-free pipettors and tips
- Nuclease-free water
- Microplates for Fluorescence-based Assays, 96-well (Cat. No. M33089)

Prepare the assay buffer

Prepare the 1X TE working solution by diluting the concentrated buffer 20-fold with sterile, distilled, DNase-free water.

IMPORTANT! TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) is used to prepare the Quant-iT™ OliGreen™ ssDNA Assay Reagent and for diluting the ssDNA standards and samples. Because the Quant-iT™ OliGreen™ ssDNA Assay Reagent is an extremely sensitive detection reagent for ssDNA, the TE solution used must be free of contaminating nucleic acids. The 20X TE buffer that is included in the Quant-iT™ OliGreen™ ssDNA Assay Kit is nuclease-free and nucleic acid–free.

Prepare the reagent

Allow the reagent to warm to room temperature before opening the vial. Immediately before the experiment, prepare an aqueous working solution of the Quant-iT™ OliGreen™ ssDNA Assay Reagent by making a 200-fold dilution of the concentrated DMSO solution in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5 (TE buffer). For microplate assays of a total 200 µL assay volume, you need 100 µL of the Quant-iT™ OliGreen™ ssDNA Assay Reagent working solution per sample.

For example, to prepare enough working solution to assay 100 samples in 200 µL assay volumes , add 50 µL Quant-iT™ OliGreen™ ssDNA Assay Reagent to 9.95 mL TE buffer.

Note: We recommend preparing this solution in plastic rather than glass, as the reagent may adsorb to glass surfaces. Protect the working solution from light, as the Quant-iT™ OliGreen™ ssDNA Assay Reagent is susceptible to photodegradation. **For best results, use the working solution within a few hours of preparation.**

Prepare the oligonucleotide standard curve

1. Prepare a 2 µg/mL stock solution of oligonucleotide in TE buffer. Determine the oligonucleotide concentration on the basis of absorbance at 260 nm (A_{260}) in a cuvette with a 1-cm pathlength; an A_{260} of 1.0 corresponds to 30–35 µg/mL oligonucleotide solution.

   The oligonucleotide standard, provided at 100 µg/mL in the Quant-iT™ OliGreen™ ssDNA Assay Kit, is diluted 50-fold in TE buffer to make the 2 µg/mL working solution. For example, 4 µL of the oligonucleotide standard mixed with 196 µL of TE is sufficient for the standard curve described in step 2.

   Note: For a standard curve, we commonly use an 18-mer M13 sequencing primer (a 2 µg/mL solution has an A_{260} of 0.065), although any purified oligonucleotide or ssDNA preparation may be used. We have found that random-sequence oligonucleotides of ten bases or longer yield approximately equivalent signals, regardless of fragment length; however, the Quant-iT™ OliGreen™ ssDNA Assay Reagent exhibits significant base selectivity. Prepare the standard curve with oligonucleotides or ssDNA that are similar in length and base composition to the type being assayed.

   Note: The oligonucleotide or ssDNA solution used to prepare the standard curve should be treated the same way as the experimental samples and should contain similar levels of contaminants. See "Effects of common contaminants" on page 4 for a list of contaminants tested in the Quant-iT™ OliGreen™ assay.

2. For the **high-range** standard curve from 10 ng/mL to 1 µg/mL, dilute the 2 µg/mL oligonucleotide stock solution into microplate wells as shown in Table 1. For the **low-range** standard curve from 1 ng/mL to 50 ng/mL, prepare a 20-fold dilution of the 2 µg/mL oligonucleotide solution to yield a 100 ng/mL oligonucleotide stock solution, then prepare the dilution series shown in Table 2.

   Table 1 Protocol for preparing a high-range standard curve.

Volume of TE buffer	Volume of 2 µg/mL oligonucleotide stock	Volume of diluted Quant-iT™ OliGreen™ ssDNA Assay Reagent	Final oligonucleotide concentration in the assay
0 µL	100 µL	100 µL	1 µg/mL
50 µL	50 µL	100 µL	500 ng/mL
90 µL	10 µL	100 µL	100 ng/mL
99 µL	1 µL	100 µL	10 ng/mL
100 µL	0 µL	100 µL	blank

   Table 2 Protocol for preparing a low-range standard curve.

Volume of TE buffer	Volume of 100 ng/mL oligonucleotide stock	Volume of diluted Quant-iT™ OliGreen™ ssDNA Assay Reagent	Final oligonucleotide concentration in the assay
0 µL	100 µL	100 µL	50 ng/mL
90 µL	10 µL	100 µL	5 ng/mL
98 µL	2 µL	100 µL	1 ng/mL
100 µL	0 µL	100 µL	blank

3. Add 100 µL of the aqueous working solution of the Quant-iT™ OliGreen™ ssDNA Assay Reagent (prepared in "Prepare the reagent" on page 2) to each microplate well. Mix well and incubate for 2–5 minutes at room temperature, **protected from light**.

4. Measure the sample fluorescence using a fluorescence microplate reader and standard fluorescein wavelengths (excitation ~480 nm, emission ~520 nm).

   Note: To ensure that the sample readings remain in the detection range, set the instrument's gain so that the sample containing the highest oligonucleotide concentration yields a fluorescence intensity near the microplate reader's maximum. For optimal detection sensitivity, the instrument gain can be increased for the low-range assay relative to the high-range assay. To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

5. Subtract the fluorescence value of the reagent blank from that of each of the samples. Use corrected data to generate a standard curve of fluorescence versus oligonucleotide concentration (Figure 1).

Analyze samples

1. Dilute the experimental oligonucleotide solution in TE buffer to a final volume of 100 µL in microplate wells.

   Note: You can alter the amount of sample diluted, provided that the final volume remains 100 µL. High dilutions of the experimental sample may serve to diminish the interfering effect of certain contaminants. However, extremely small sample volumes should be avoided because they are difficult to pipet accurately.

2. Add 100 µL of the aqueous working solution of the Quant-iT™ OliGreen™ ssDNA Assay Reagent to each sample. Incubate for 2–5 minutes at room temperature, **protected from light**.

3. Measure the fluorescence of the samples using the same instrument parameters used to generate the standard curve (see step 4). To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

4. Subtract the fluorescence value of the reagent blank from that of each of the samples. Determine the oligonucleotide concentration of the sample from the standard curve generated in “Prepare the oligonucleotide standard curve” on page 3.

5. The assay can be repeated using a different dilution of the sample to confirm the quantitation results.

Effects of common contaminants

The Quant-iT™ OliGreen™ Assay remains linear in the presence of several compounds that commonly contaminate nucleic acid preparations, although the signal intensity may be affected (Table 3). For the highest accuracy, the standards should be prepared under the same conditions as the experimental samples and contain similar levels of contaminants.

Table 3 Effects of common contaminants on the signal intensity of the assay.

Compound	Maximum acceptable concentration	% Signal change[1]
Salts		
Ammonium acetate	50 mM	13% decrease
Sodium acetate	30 mM	3% decrease
Sodium chloride	100 mM	25% decrease
Zinc chloride	1 mM	43% decrease
Magnesium chloride	5 mM	34% decrease
Urea	2 M	47% increase
Organic solvents		
Phenol	0.2%	19% decrease
Ethanol	10%	19% increase
Chloroform	2%	2% increase
Detergents		
Sodium dodecyl sulfate	0.01%	73% increase
Triton™ X-100	0.1%	11% increase
Proteins		
Bovine serum albumin	2%	20% increase
IgG	0.1%	37% decrease
Other compounds		
Polyethylene glycol	1%	29% increase
Agarose	0.1%	8% increase
ATP	0.1%	30% increase

[1] The compounds were incubated at the indicated concentrations with Quant-iT™ OliGreen™ Reagent in the presence of 660 ng/mL of a 24-mer M13 sequencing primer. All samples were assayed in a final volume of 200 µL in 96-well microplates. Samples were excited at 485 nm and fluorescence intensity was measured at 520 nm.

Related products

Table 4 Bulk Reagents and Kits

Product	Quantity	Cat. No.
Quant-iT™ PicoGreen™ dsDNA Assay Kit	1 mL assay kit 10 x 100 µL	P7589 P11496
Quant-iT™ PicoGreen™ dsDNA Reagent	1 mL reagent 10 x 100 µL	P7581 P11495
TE Buffer (20X), RNase-free	100 mL	T11493
Quant-iT™ RiboGreen™ RNA Assay Kit	1 mL assay kit	R11490
Quant-iT™ RiboGreen™ RNA Reagent	1 mL reagent	R11491
Quant-iT™ RediPlate™ 96 RiboGreen™ RNA Quantitation Kit	1 plate	R32700
Quant-iT™ OliGreen™ ssDNA Assay Kit	1 mL assay kit	O11492
Quant-iT™ OliGreen™ ssDNA Assay Reagent	1 mL reagent	O7582

Table 5 Microplate Reader Assays

Product	Dynamic Range	Quantity	Cat. No.
Quant-iT™ 1X dsDNA Assay Kit, High Sensitivity	200 pg–100 ng	1,000 reactions	Q33232
Quant-iT™ 1X dsDNA Assay Kit, Broad-Range	4 ng–2 µg	1,000 reactions	Q33267
Quant-iT™ DNA Assay Kit, High Sensitivity	200 pg–100 ng	1,000 reactions	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	4 ng–1 µg	1,000 reactions	Q33130
Quant-iT™ RNA Assay Kit	5–100 ng	1,000 reactions	Q33140
Quant-iT™ RNA Reagent	5–100 ng	1,000 reactions	Q32884
Quant-iT™ RNA Assay Kit, Broad Range	20 ng–1 µg	1,000 reactions	Q10213
Quant-iT™ RNA XR Assay Kit	200 ng–10 µg	1,000 reactions	Q33225
Quant-iT™ microRNA Assay Kit	1–100 ng	1,000 reactions	Q32882
Quant-iT™ Protein Assay Kit	250 ng–5 µg	1,000 reactions	Q33210
Microplates for Fluorescence-based Assays, 96-well	—	10 plates	M33089

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0001932

Revision	Date	Description
B.0	15 March 2022	The format and content were updated.
A.0	16 February 2015	New document for the Quant-iT™ OliGreen™ ssDNA Assay Kit and Quant-iT™ OliGreen™ ssDNA Assay Reagent.