molecular probes
life technologies

Quant-iT™ OliGreen® ssDNA Reagent and Kit

Catalog nos. O7582, O11492

Table 1. Contents and storage

Material	Amount	Concentration	Storage*	Stability
Quant-iT OliGreen® ss DNA Reagent (Cat. no. O7582)				
Quant-iT OliGreen® ss DNA Reagent, solution in dimethylsulfoxide (DMSO)	1 mL	Not applicable	• Room temperature • Desiccate • Protect from light	When stored as directed, the product is stable for 1 year.
Quant-iT OliGreen® ss DNA Assay Kit (Cat. no. O11492)				
Quant-iT OliGreen® ss DNA Reagent (Component A), solution in dimethylsulfoxide (DMSO)	1 mL	Not applicable	• Room temperature • Desiccate • Protect from light	When stored as directed, the product is stable for 1 year.
20X TE (Component B)	25 mL	200 mM Tris-HCl, 20 mM EDTA, pH 7.5	• ≤25˚C*	
Oligonucleotide standard (Component C)	1 mL	100 µg/mL solution in TE	• 2–8˚C*	
*For long-term storage, store the Quant-iT OliGreen® ss DNA Reagent, 20XTE, and oligonucleotide standard at ≤–20˚C.				
Number of assays: Sufficient material is supplied for 200 assays using a 2 mL assay volume or 2,000 assays using a 200 µL assay volume.				
Approximate fluorescence excitation/emission maxima: 500/525 nm, bound to nucleic acid.				

Introduction

The Quant-iT™ OliGreen® ssDNA reagent is an ultra-sensitive fluorescent nucleic acid stain for quantifying oligonucleotides and single-stranded DNA (ssDNA) in solution (Figure 1, page 2). The Quant-iT™ OliGreen® ssDNA reagent is offered as a stand-alone product (Cat. no. O7582) or as part of a convenient kit (Cat. no. O11492), which also includes concentrated assay buffer and an oligonucleotide standard. Short, synthetic oligonucleotides are used in a number of 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.

The most commonly used technique for measuring oligonucleotide and ssDNA concentration is the determination of absorbance at 260 nm (A_{260}). The major disadvantages of the absorbance method are the large relative contribution of nucleotides to the signal, the interference caused by contaminants commonly found in nucleic acid preparations and the relative insensitivity of the assay (an A_{260} of 0.1 corresponds to ~3 µg/mL solution of a synthetic 24-mer M13 sequencing primer).

For Research Use Only. Not for use in diagnostic procedures.

In contrast, the Quant-iT™ OliGreen® ssDNA reagent enables researchers to quantify as little as 100 pg/mL oligonucleotide or ssDNA (200 pg in a 2 mL assay volume) with a standard spectrofluorometer and fluorescein excitation and emission wavelengths. This sensitivity exceeds that achieved with absorbance methods by 10,000-fold. Using a fluorescence microplate reader, we can detect as little as 1 ng/mL oligonucleotide or ssDNA (200 pg in a 200 µL assay volume). We have also quantitated several ssDNAs with the Quant-iT™ OliGreen® reagent, including M13 and φX174 viral DNA and denatured calf thymus DNA, and obtained similar sensitivity. In addition, Quant-iT™ OliGreen® reagent has been used to detect phosphodiester and phosphorothioate oligonucleotides in plasma and serum samples[1] and to develop a sensitive fluorescence-based capillary electrophoresis method for detecting short, single-stranded oligonucleotides.[2]

The linear detection range of the Quant-iT™ OliGreen® assay in a standard fluorometer extends over four orders of magnitude in oligonucleotide concentration—from 100 pg/mL to 1 µg/mL—with a single dye concentration (Figure 1, below). 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 *Oligonucleotide Standard Curve*, page 4).

Nucleotides and short oligonucleotides of six bases or less do not interfere in the quantitation assay; however, the Quant-iT™ OliGreen® 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® reagent exhibits significant base selectivity. Quant-iT™ OliGreen® dye 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.

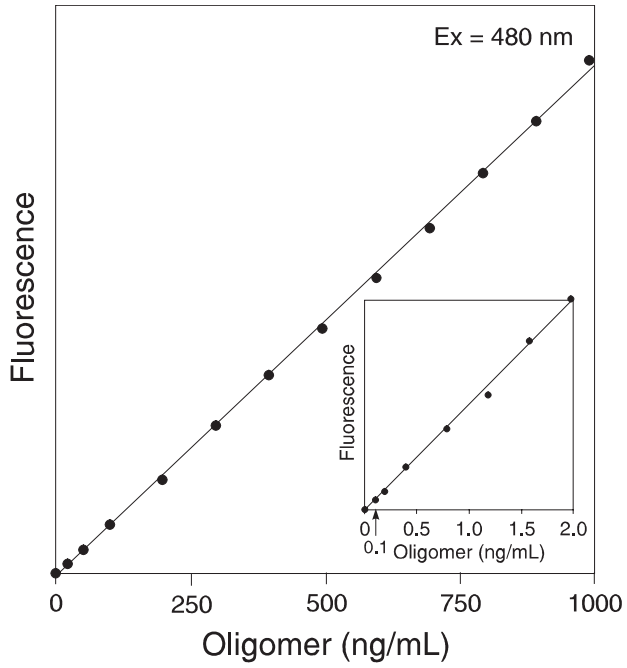

Figure 1. Linear quantification of a synthetic 24-mer (an M13 sequencing primer) from 0.1 to 1,000 ng/mL using the Quant-iT™ OliGreen® ssDNA reagent (Cat. no. O7582). Samples in 10 mm × 10 mm cuvettes were excited at 480 nm. The fluorescence emission intensity was measured at 520 nm using a spectrofluorometer and plotted as a function of oligonucleotide concentration. The inset shows an enlargement of the results obtained with oligonucleotide concentrations between zero and 2.0 ng/mL.

Before Starting

Materials required but not provided

- Sterile, distilled, DNase-free water
- Cuvettes or 96-well microplates

Caution

No data are available addressing the mutagenicity or toxicity of Quant-iT™ OliGreen® ssDNA reagent. Because this reagent binds nucleic acids, treat it as a potential mutagen and handle with appropriate care.

Handle the DMSO stock solution with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution.

As with all nucleic acid reagents, pour solutions of Quant-iT™ OliGreen® reagent through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dye.

Prepare assay buffer

TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) is used for diluting the Quant-iT™ OliGreen® reagent and for diluting oligonucleotide and ssDNA samples. Because the Quant-iT™ OliGreen® reagent is an extremely sensitive detection reagent for ssDNA, it is imperative that the TE solution used is 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 1X TE working solution by diluting the concentrated buffer 20-fold with sterile, distilled, DNase-free water.

Oligonucleotide standard

The oligonucleotide standard is an 18-base M13 sequencing primer, with the sequence 5'-TGTAAAACGACGGCCAGT-3'.

Experimental Protocols

The assay procedure is designed for use with standard fluorescence cuvettes and has 2 mL assay volumes. To perform microplate assays, reduce the indicated volumes appropriately. For example, 200 µL volumes are recommended for use with 96-well microplates.

Prepare 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® 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 example, to prepare enough working solution to assay 20 samples, add 100 µL Quant-iT™ OliGreen® reagent to 19.9 mL TE buffer. We recommend preparing this solution in plastic rather than glass, as the reagent may adsorb to glass surfaces. Protect the working solution from light by covering it with foil or placing it in the dark, as the Quant-iT™ OliGreen® reagent is susceptible to photodegradation. **For best results, use this solution within a few hours of preparation.**

Oligonucleotide standard curve

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. Prepare the standard curve with oligonucleotides or ssDNA that are similar in length and base composition to the type being assayed.

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® reagent exhibits significant base selectivity. Our results have also shown that 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 4, page 6). Thus, to serve as an effective control, 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 such compounds.

Dilute the oligonucleotide standard, provided at 100 µg/mL in the Quant-iT™ OliGreen® Assay Kit to 50-fold in TE buffer to make the 2 µg/mL working solution.

1.1 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.

To generate a five-point standard curve from 10 ng/mL to 1 µg/mL, proceed to step 1.2. For a low-range standard curve from 100 pg/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 and proceed to step 1.5.

1.2 For the **high-range** standard curve, dilute the 2 µg/mL oligonucleotide stock solution into disposable cuvettes (or plastic test tubes for transfer to quartz cuvettes) as shown in Table 2, below.

Add 1.0 mL of the aqueous working solution of Quant-iT™ OliGreen® reagent (prepared in *Preparing Reagent*) to each cuvette. Mix well and incubate for 2 to 5 minutes at room temperature, **protected from light**.

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

Volume of TE buffer	Volume of 2 µg/mL oligomer stock	Volume of diluted Quant-iT™ OliGreen® reagent	Final oligomer concentration in Quant-iT™ OliGreen® Assay
0 µL	1,000 µL	1,000 µL	1 µg/mL
500 µL	500 µL	1,000 µL	500 ng/mL
900 µL	100 µL	1,000 µL	100 ng/mL
990 µL	10 µL	1,000 µL	10 ng/mL
1,000 µL	0 µL	1,000 µL	blank

1.3 After incubation, measure the sample fluorescence using a spectrofluorometer or fluorescence microplate reader and standard fluorescein wavelengths (excitation ~480 nm, emission ~520 nm).

To ensure that the sample readings remain in the detection range of the fluorometer, set the instrument's gain so that the sample containing the highest oligonucleotide concentration yields a fluorescence intensity near the fluorometer's maximum. To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

1.4 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, page 2).

1.5 For the **low-range** standard curve—from 100 pg/mL to 50 ng/mL—dilute the 100 ng/mL oligonucleotide stock solution (prepared in step 1.1) into disposable cuvettes (or plastic test tubes for transfer to quartz cuvettes) as shown in Table 3, below.

Add 1.0 mL of the aqueous working solution of Quant-iT™ OliGreen® reagent (prepared in *Prepare Reagent*, page 3) to each cuvette. Mix well and incubate for 2 to 5 minutes at room temperature, **protected from light**. Continue with steps 1.3 and 1.4. If necessary, increase the fluorometer gain to accommodate the lower signals by amplifying the fluorescence signal of the low-range standard curve.

Table 3. Protocol for preparing a low-range standard curve.

Volume of TE buffer	Volume of 100 ng/mL oligomer stock	Volume of diluted Quant-iT™ OliGreen® reagent	Final oligomer concentration in Quant-iT™ OliGreen® Assay
0 µL	1,000 µL	1,000 µL	50 ng/mL
900 µL	100 µL	1,000 µL	5 ng/mL
990 µL	10 µL	1,000 µL	500 pg/mL
998 µL	2 µL	1,000 µL	100 pg/mL
1,000 µL	0 µL	1,000 µL	blank

Sample analysis

2.1 Dilute the experimental oligonucleotide solution in TE buffer to a final volume of 1.0 mL in disposable cuvettes or test tubes. You may wish to prepare more than one dilution of the experimental sample. 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.

2.2 Add 1.0 mL of the aqueous working solution of the Quant-iT™ OliGreen® reagent to each sample. Incubate for 2 to 5 minutes at room temperature, **protected from light**.

2.3 Measure the fluorescence of the sample using instrument parameters that correspond to those used when generating the standard curve (see steps 1.3 and 1.5). To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

2.4 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 *Oligonucleotide Standard Curve*, page 4.

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

References

1. J Membrane Biol 19, 1 (1974); **2.** J Biol Chem 265, 19543 (1990).

Appendix

Contaminating substances

A number of common contaminants have been tested in the Quant-iT™ OliGreen® assay, and most are well tolerated (Table 4, below). For untested contaminating substances, and, in general, for highest accuracy, the standards should be assayed under the same conditions as the unknowns.

Table 4. Effects of several compounds that commonly contaminate nucleic acid preparations on the signal intensity of the Quant-iT™ OliGreen® ssDNA assay.

Compound	Maximum acceptable concentration	% signal change*
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

* 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.

Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
O7582	Quant-iT™ OliGreen® ssDNA reagent *2000 assays*	1 mL
O11492	Quant-iT™ OliGreen® ssDNA Assay Kit *2000 assays*	1 kit
Related products		
Q33120	Quant-iT™ dsDNA Assay Kit, High Sensitivity, 1000 assays *0.2–100 ng*	1 kit
Q33130	Quant-iT™ dsDNA Assay Kit, Broad Range, 1000 assays *2–1000 ng*	1 kit
Q10213	Quant-iT™ RNA Assay Kit, Broad Range, 1000 assays *20–1000 ng*	1 kit
Q33140	Quant-iT™ RNA Assay Kit, 1000 assays *5–100 ng*	1 kit
Q32882	Quant-iT™ microRNA Assay Kit, 1000 assays *5–500 ng*	1 kit
Q33210	Quant-iT™ Protein Assay Kit, 1000 assays *0.25–5 µg*	1 kit

Purchaser Notification

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