OligoGreen ssDNA Quantification Kit *2000T*

Cat #: B-CHK002

Size: 1mL

Storage: Store at -20°C

Product Introduction

OliGreen oligonucleotide quantitation reagent is an ultra-sensitive fluorescent nucleic acid stain used for quantifying oligonucleotides and single-stranded DNA (ssDNA) in solution. Short synthetic oligonucleotides are used in many molecular biology techniques such as DNA sequencing, site-directed mutagenesis, DNA amplification, and in situ hybridization. However, classical methods for quantifying oligonucleotides are not highly sensitive and often require highly concentrated samples. The most commonly used technique for measuring the concentration of oligonucleotides and ssDNA is by measuring the absorbance at 260 nm (A260). The main drawbacks of the absorbance method are the significant contribution of nucleotides to the signal, interference from common contaminants in nucleic acid preparations, and the relative insensitivity of the measurement (0.1 A260 corresponds to approximately 3 µg/mL of a synthetic 24-mer M13 sequencing primer). In contrast, our OliGreen ssDNA quantitation reagent enables researchers to quantitate oligonucleotides or ssDNA as low as 100 pg/mL using a standard fluorescence spectrophotometer and fluorescence excitation and emission wavelengths (200 pg in a 2 mL detection volume). This sensitivity is over 10,000 times higher than the absorbance method. Using a fluorescence microplate reader, we can detect oligonucleotides or ssDNA as low as 1 ng/mL (200 pg in a 200 µL detection volume). We have also quantitated several ssDNA samples, including M13 and fX174 viral DNA and denatured bovine thymus DNA, using the OliGreen reagent and obtained similar sensitivity. The OliGreen assay detects oligonucleotide concentrations over a wide range, from 100 pg/mL to 1 µg/mL. Furthermore, we have demonstrated that the linearity of the OliGreen assay remains robust in the presence of various potential compounds, including salts, urea, ethanol, chloroform, detergents, proteins, ATP, and agarose; short oligonucleotides of six bases or fewer do not interfere with the quantitation measurement.

Product information:

Name	Volume	Storage
OliGreen ssDNA Quantitation Reagent	1mL, in DMSO	-20°C protect from light

Note: This reagent is enough for 200 measurements when using a 2 mL assay volume(Cuvette) , or 2000 measurements when using a 200 µL assay volume(96 wells microplate).

Materials Needed But Not Supplied:

1. 20X TE buffer: 25mL, 200mM Tris-HCl, 20mM EDTA, pH 7.5
2. Oligonucleotide standard(for example 18 base M13 sequencing primer with sequence 5 '-TGTAAAACGACGGGCCAGT-3): 1 mL, 100ug/ml in TE Buffer

Recommended Protocol:

The assay protocol is designed for standard fluorescence cuvettes with a detection volume of 2 mL. To perform analysis in a microplate, please adjust the indicated volume accordingly. For example, it is recommended to use a 96-well microplate with a volume of 200 µL.

1. Preparation of TE buffer

Prepare TE Dilution buffer, which consists of 10 mM Tris-HCl, 1 mM EDTA, pH 7.5, for diluting the OliGreen reagent and oligonucleotide/ssDNA samples. Since the OliGreen reagent is a highly sensitive ssDNA detection reagent, it is essential to use TE solution free from contaminating nucleic acids. The 20X TE Buffer included in the OliGreen ssDNA Quantitation Kit is free from nucleases and nucleic acids. Prepare a 1X TE working solution by diluting the concentrated buffer 20-fold with sterile, distilled water free from DNAse.

2. Preparation of OliGreen working solution

Prior to the experiment, prepare an aqueous working solution of the OliGreen reagent by diluting the concentrated DMSO solution in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5 (TE buffer) at a 200-fold dilution. For example, to prepare enough working solution for analyzing 20 samples, add 100 µL of the OliGreen quantitation reagent to 19.9 mL of TE buffer. We recommend using plastic rather than glass for preparing this solution, as the reagent may adsorb onto glass surfaces. Due to the light-sensitive nature of the OliGreen reagent, protect the working solution from light by covering it with aluminum foil or storing it in a dark place. For optimal results, use this solution within a few hours of preparation.

3. Preparation of Oligonucleotide Standard

3.1 Prepare a stock solution of oligonucleotide at a concentration of 2 µg/mL in TE beffer. Measure the concentration of the oligonucleotide in a cuvette with a 1 cm path length based on the absorbance at 260 nm (A260). An A260 of 1.0 corresponds to a concentration of 30-35 µg/mL for the oligonucleotide solution. For the standard curve, we typically use the 18-mer M13 sequencing primer (with a 2 µg/mL solution having an A260 of 0.065), although any purified oligonucleotide or ssDNA preparation can be used. It is preferable to prepare the standard curve using oligonucleotides or ssDNA with similar lengths and base compositions as the ones being analyzed. We have found that oligonucleotides with random sequences of 10 or more bases produce approximately equivalent signals regardless of the fragment length; however, OliGreen exhibits significant base selectivity. Our results also indicate that the OliGreen assay remains linear in the presence of several compounds typically found in nucleic acid preparations, although the signal intensity may be affected (**Table 1**). Therefore, oligonucleotide or ssDNA solutions used for preparing the standard should be treated in the same manner as the test samples and should contain similar levels of such compounds. To generate a five-point standard curve ranging from 10 ng/mL ~ 1 µg/mL, turn 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 generate a 100 ng/mL oligonucleotide stock solution and turn to step 1.5.

Table 1. Effects of Common Compounds on OliGreen ssDNA Assay

Compound	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% increase
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 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.

3.2 For a high-range standard curve, as shown in **Table 2**, dilute the 2 µg/mL oligonucleotide stock solution into disposable cuvettes (or plastic tubes for transfer to quartz cuvettes). Then add 1.0 mL of the aqueous OliGreen reagent solution to each cuvette. Mix thoroughly and incubate at room temperature for 2 to 5 minutes, protected from light.

Table 2. High-range standard curve

Volume (μL) of TE	Volume (μL) of 2 μg/mL Oligomer Stock	Volume (μL) of Diluted OliGreen Reagent	Final Oligomer Concentration in OliGreen Assay
0	1000	1000 μL	1 μg/mL
500	500	1000 μL	500 ng/mL
900	100	1000 μL	100 ng/mL
990	10	1000 μL	10 ng/mL
1000	0	1000 μL	blank

3.3 After incubation, measure the fluorescence of the samples using a spectrophotofluorometer or a fluorescence microplate reader at the standard fluorescence wavelength (excitation ~480 nm, emission ~520 nm). Set the instrument gain to ensure that the standard with the highest oligonucleotide concentration produces fluorescence intensity near the maximum value of the fluorometer, to ensure that the sample readings remain within the detection range of the fluorometer. Keep the fluorescence measurement time consistent for all samples to minimize photobleaching effects.

3.4 Subtract the fluorescence value of the blank from the fluorescence values of each sample. Use the corrected data to generate a standard curve relating fluorescence to oligonucleotide concentration (Figure 1).

3.5 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 tubes for transfer to quartz cuvettes) as shown in **Table 3**. Then add 1.0 mL of OliGreen working solution (prepared in the section 2) to each cuvette. Mix thoroughly and incubate at room temperature for 2 to 5 minutes, protected from light. Proceed with steps 1.3 and 1.4. If necessary, increase the gain of the fluorometer to accommodate lower signals by amplifying the fluorescence signal of the low-range standard curve.

Table 3. Low-range standard curve

Volume (μL) of TE	Volume (μL) of 100 ng/mL Oligomer Stock	Volume (μL) of Diluted OliGreen Reagent	Final Oligomer Concentration in OliGreen Assay
0	1000	1000 μL	50 ng/mL
900	100	1000 μL	5 ng/mL
990	10	1000 μL	500 pg/mL
998	2	1000 μL	100 pg/mL
1000	0	1000 μL	blank

4. Sample Analysis

4.1 Dilute the experimental oligonucleotide solution in TE buffer to a final volume of 1.0 mL in disposable cuvettes or test tubes. You may want to prepare dilutions for multiple experimental samples. Highly diluting the samples can help reduce interference from certain contaminants. However, avoid using extremely small sample volumes as they can be difficult to accurately pipette.

4.2 Add 1.0 mL of OliGreen working solution to each sample. Incubate at room temperature for 2 to 5 minutes, avoiding exposure to light.

4.3 Measure the fluorescence of the samples using instrument parameters that correspond to those used for generating the standard curve (refer to steps 3.3 and 3.5). To minimize photobleaching effects, keep the fluorescence measurement time consistent for all samples.

4.4 Subtract the fluorescence value of the blank from the fluorescence value of each sample. Determine the concentration of the oligonucleotide from the standard curve.

4.5 Repeat the measurement of the samples using different dilutions of the reagent to confirm the quantitative results.

References

1. Antisense Nucleic Acid Drug Dev 7,133（1997）; J Chromatogr A 755,271（1996）

Note:

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.