PicaGreen dsDNA Quantification Kit *2000T*

Cat #: B-CHK001

Size: 1mL

Storage: Store at 4°C.

Product Introduction

The PicaGreen dsDNA quantification kit is used for the quantification of double-stranded DNA if its concentration cannot be determined by measuring absorbance at 260 nm. PicaGreen selectively binds to double-stranded DNA, so nucleotides, single-stranded DNA, RNA, proteins and other impurities do not impede the measurements.

Kit components:

Kit component	Volume	Storage	Shelf life
Component A: PicaGreen Quantification Reagent	1ml, 200× in DMSO	4°C protect from light	6 months
Component B: TE buffer	50mL	RT	
Component c: dsDNA quantitative standard	1 mL, 1ug/ml in TE Buffer	4°C protect from light	

Protocol:

1. **Preparation of the PicaGreen working solution**

PicaGreen dsDNA quantification reagent(Component A) is stored as a concentrated solution in anhydrous DMSO. Prepare the working solution of 2X PicoGreen reagent by diluting the concentrated solution with 1x TE (Component B) at a ratio of 1:200 at the day of the experiment. If you need to prepare enough working solution to detect 20 samples, add 100 µL of PicaGreen dsDNA quantification reagent (Component A) to 20 mL of 1x TE (Component B). Since the reagent can easily adsorb to glass surfaces, it should be prepared in plastic containers. PicaGreen reagent (Component A) is light-sensitive and prone to degradation, so the prepared solution should be covered with foil or stored in a dark. It is recommended to use the solution within a few hours of preparation to ensure optimal results.

2. **Preparation of DNA Standard**

2.1 Prepare a 1 µg/mL dsDNA solution (Component C) stock solution in 1x TE. Determine the DNA concentration by measuring the absorbance at A260nm in a 1 cm path length cuvette; A260=0.02 corresponds to a 1 µg/mL dsDNA solution. Although any purified dsDNA can be used to produce the standard curve, bovine thymus DNA is commonly used. It is preferable to use DNA similar to the samples being tested as the standard. However, most linear dsDNA molecules, regardless of their fragment length, produce roughly equal signals. The PicaGreen assay can maintain linearity even in the presence of various complexes commonly found in contaminated nucleic acid, although the signal intensity may be affected. Therefore, to obtain effective control, the buffer used to prepare the standard curve should be the same as the samples.

2.2 To generate a standard curve 8 points ranging from 0.5 ng-500 ng/mL (see Table 1), prepare a series dilution of DNA standards at 2X final concentration by mixing equal volumes of 2X DNA and 2X PicaGreen working solutions. Add the mixture in a 10x10 mm cuvette. Prepare a blank control by mixing equal volumes of 1x TE and 2X PicaGreen working solution. Keep the cuvettes in the dark and incubate at room temperature for 2-5 minutes.

Table 1. DNA standard curve when uses 10 × 10mm cuvette

Concentration of 2X DNA(ng/mL)	Volume of 2X DNA(mL)	Volume of 2X PicaGreen (mL)	Final Concentration of DNA(ng/mL)
1000	1	1	500
200	1	1	100
50	1	1	25
20	1	1	10
5	1	1	2.5
2	1	1	1
1	1	1	0.5
0	1	1	Blank

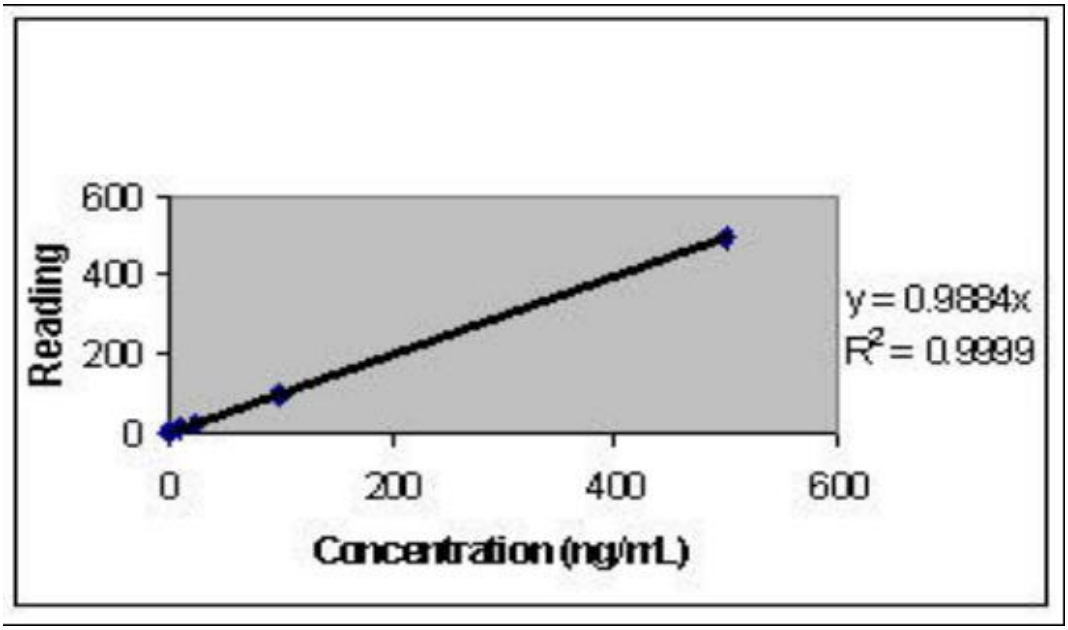
2.3 When using a microplate adapter, the PicaGreen assay can also be performed at lower assay volumes (50 µL to 200 µL). To generate a standard curve ranging from 1 ng-100 ng/mL (see Table 2), prepare a series dilution of DNA standards at 2X final concentration by mixing equal volumes of 2X DNA and 2X PicaGreen working solutions. Add at least 50 µL of the solution to a microplate well. Ensure that no bubbles are introduced into the sample, gently tapping the outside of the microplate well can often disperse bubbles. Keep the plate in the dark and incubate at room temperature for 2-5 minutes.

Table 2. DNA standard curve when using microplate

Concentration of 2X DNA(ng/mL)	Volume of 2X DNA(mL)	Volume of 2X PicaGreen (mL)	Final Concentration of DNA(ng/mL)
200	30	30	100
50	30	30	25
20	30	30	10
5	30	30	2.5
2	30	30	1
0	30	30	Blank

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

2.5 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 DNA concentration. The following example uses the TBS-380 microplate fluorometer.



3. **Sample Analysis**

3.1 Dilute the unknown DNA samples in 1x TE buffer to the desired volume (1.0 mL for 10x10 mm cuvettes, 25-100 µL for microplate wells). Highly diluting the samples is recommended to ensures maximum dilution of any contaminants. However, avoid using sample volumes that are too small to accurately pipette. Remove RNA and ssDNA from the samples as described in section 4.

3.2 Add the working solution of 2X PicaGreen (prepared in step 3.1) to each sample, mix well, and transfer the mixture to the appropriate cuvette. Keep the cuvettes in the dark and incubate at room temperature for 2-5 minutes.

3.3 Measure the fluorescence of the samples using the same instrument parameters used to generate the standard curve (see step 2.4).

To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

3.4. Subtract the fluorescence value of the reagent blank from that of each of the samples. Determine the DNA

concentration of the sample from the standard curve generated in in step 2.5.

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

4. Removal of RNA and ssDNA in the sample

When the molar concentration of ssDNA is similar to that of dsDNA, the interference of the former on the measurement of the latter is minimal. When the concentration of ssDNA is 10 times higher than that of dsDNA, the interference on the fluorescence signal is also only around 10%. However, at low DNA concentrations, ssDNA can cause significant interference. At high concentrations, the fluorescence values generated by RNA binding to the PicaGreen reagent can be eliminated by treating the samples with RNAse. RNAse A, RNAse T1, and S1 nuclease can remove all single-stranded nucleic acids, ensuring that the fluorescence values of the samples are generated by dsDNA. (For specific methods, please refer to relevant references.)

References

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[6] Molecular Cloning: A Laboratory Manual, Second Edition, J. Sambrook, E.F. Fritsch and T. Maniatis, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)