Product Information

X-Green II dsDNA Quantitation Kit Plus

Catalog Number: X2040

Product Size: 2000T

Detection Instrument: Fluorescence microplate reader, fluorometer

Contents:

Component	Amount	Concentration	Storage
X-Green II (component A)	1 mL	Soluble in organic solvent	2-6 ℃ Protect from light
20 × Buffer (component B)	25 mL	20× Buffer	2-6 ℃
dsDNA standard （component C）	1 mL	100 μg/mL	2-6 ℃

Storage

Store at 4 °C and protect from light. When stored as directed, product is stable for at least 6 months. For long-term storage, both the 20× Buffer and dsDNA standard can be stored at ≤–20ºC.

Parameters

Ex/Em: 480/520 nm (with dsDNA)

Description

X-Green II is an ultra sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution. Detecting and quantitating small amounts of DNA is extremely important in a wide variety of biological applications. These include standard molecular biology techniques, such as synthesizing cDNA for library production and purifying DNA fragments for subcloning, as well as diagnostic techniques, such as quantitating DNA amplification products and detecting primers. The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm (A260). The major disadvantages of the absorbance method are the large relative contribution of nucleotides and single-stranded nucleic acids to the signal, the interference caused by contaminants commonly found in nucleic acid preparations, the inability to distinguish between DNA and RNA, and the relative insensitivity of the assay (an A260 of 0.1 corresponds to a 5 μg/mL dsDNA solution). The quantitative detection of X-Green II is simple and convenient, becoming the standard for the detection of residual DNA in biological products.

X-Green II only emits fluorescence when it binds to dsDNA, and the intensity of the fluorescence is proportional to the DNA concentration. X-Green II dsDNA Quantitstion Kit Plus can detect dsDNA in the range of 25 pg / mL-1000 ng / mL with a linear relationship (R^2> 0.99).

Protocol

Preparing the Reagent

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The X-Green II dsDNA Quantitation Reagent is stored as 1 mL stock solution in organic solvent. In the experiment, prepare a 2× X-Green II reagent by making a 200-fold dilution of the concentrated solution in 1× Buffer obtained by diluting 20× buffer 20 times. For example, to prepare enough working solution to assay 20 samples in a 2 mL final volume, add 100 µL X-Green II reagent to 19.9 mL 1× Buffer. We recommend preparing this solution in a plastic container 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 X-Green II is susceptible to photodegradation.

For best results, this solution should be used within a few hours of its preparation.

Method

1. Dilute the component B stock solution provided in the kit into 1 × Buffer. For the preparation of 50 mL 1 × Buffer, 2.5 mL of component B needs to be added to 47.5 mL of sterile water.

2. Dilute the DNA standard to make a standard curve. Dilute component C from 100 µg / mL to 2 µg / mL with 1 × Buffer. For example, take 40 µL of component C and add 1.96 mL of sterile water. For a low-concentration standard curve, a 2 µg / mL DNA standard can be diluted 40-fold to prepare 50 ng / mL DNA stock solution, and then further diluted. Specific dilution method can refer to Table 1, Table 2.

3. For each unknown sample, mix 1-10 µL of sample with 99-90 µL of 1 × Buffer, mix well, and add it to the well of the microplate for detection.

4. Dilute component A by making a 200-fold dilution of the concentrated solution in 1× Buffer to prepare a 2× X-Green II working solution. A volume of 100 µL is required for each standard and each unknown sample. Determine the total number of samples, and multiply this value by 100 µL to obtain the total volume of X-Green II reagent to be diluted. X-Green II is sensitive to light. Be careful when melting and diluting.

5. Add 100 µL of diluted X-Green II working solution to each standard and sample well. Mix by pipetting three times.

6. Cover the microplate with tin foil and incubate for 5 min at room temperature.

7. Read the data, take the average to generate a standard curve, and determine the DNA concentration in the unknown sample.

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

Volume(µL) of Buffer	Volume(µL) of 2µg/ mL DNA stock	Final DNA Concentration in X-Green II Assay
0	1000	1 µg/mL
900	100	100 ng/mL
990	10	10 ng/mL
999	1	1 ng/mL
1000	0	blank

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

Volume(µL) of Buffer	Volume(µL) of 50 ng/ mL DNA stock	Final DNA Concentration in X-Green II Assay
0	1000	25 ng/mL
900	100	2.5 ng/mL
990	10	250 pg/mL
999	1	25 pg/mL
1000	0	blank

Notes

1. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

2. For your safety and health, please wear lab coats and disposable gloves.

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