Product Information

WonderBlue® High Sensitivity dsDNA Quantitation Kit

Catalog Number: W2011

Product Size: 200T, 1000T

Contents:

Component	200T	1000T
A (WonderBlue® High Sensitvity dsDNAQuanttaton Soluton)	50 mL	250 mL
B (WonderBlue® High Sensitvity dsDNA Enhancer, 100×)	0.5 mL	2.5 mL
C (dsDNA Standards in 10 mM Tris pH 7.5 , 1 mM EDTA , 2 mM sodium azide, 0 , 0.5, 1, 2 ,4,6, 8 and 10 ng/uL dsDNA from calf thymus)	0.1 mL per group (8 groups)	0.5 mL per group (8 groups)

Storage

Store at 4°C and protect from light. When stored as directed, product is stable for at least 6 months.

Parameters

Ex/Em: 485/530 nm (with dsDNA).

Figure 1 is the spectrum of WonderBlue®.

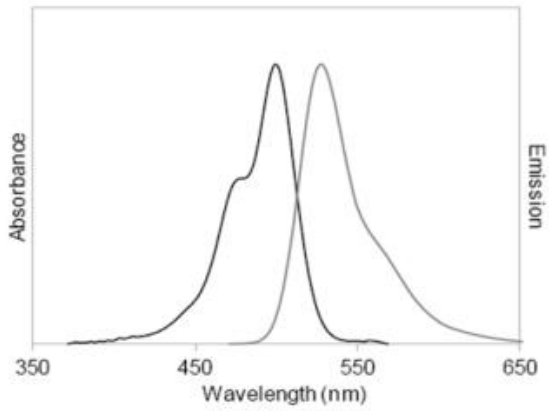

Figure 1. Excitation and emission spectra of WonderBlue® under conditions of dsDNA supersaturation.

Description

The WonderBlue® dsDNA Quantitation Kit (WonderBlue® High Sensitivity dsDNA Quantitation Kit) is different from conventional absorbance-based measurement. This kit can distinguish dsDNA, ssDNA or RNA, and selectively detect dsDNA (Figure 2). Compared with traditional DNA quantification methods, this product has the advantages of wide detection range, high sensitivity and high specificity. The kit contains three components: WonderBlue® High Sensitivity dsDNA Quantitation Buffer, Enhancer solution and pre-diluted dsDNA Standards. It can quantify 0.2-100 ng dsDNA in 200 µL system (Figure 3). In addition, the kit can minimize the effects of other pollutants, and can tolerate conventional pollutants such as proteins, salts, organic solvents and detergents (Table 1). The kit has no permeability, no cytotoxicity and no induced mutagenesis, and is harmless to human.

Protocol

1. For best results, please use the accurately calibrated pipette and RNase-free pipette tip, test tube, and test plate. It is recommended to set 3 duplicate wells for each DNA standard and unknown sample. If there is more than one 96-well plate, it is recommended to set a standard curve for each 96-well plate to minimize the error between the plates.

2. Before use, remove the product from storage conditions to room temperature. If precipitation occurs in 100× Enchancer storage solution, it can be dissolved in a 37℃ water bath. Each

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component should be thoroughly shaken or vortexed and centrifuged to avoid unnecessary reagent loss.

3. Each sample to be tested corresponds to 200 μL of WonderBlue working solution. For a 96-well plate, pipette 200 μL of 100 × Enchancer, add to 20 mL Quantitation Solution, mix by vortexing, and configure into WonderBlue® working solution. For best results, the working solution should be used within one hour. If the working solution is stored again and used within 24 hours, the accuracy of the results will be slightly lost. During storage, the enhancer may precipitate, which can be resuspended by vortexing.

4. For each sample, pipette 200 μL of working solution into a black 96-well plate microwell. To ensure accurate and reliable results, it is recommended to make 3 duplicate wells for each test sample and DNA standard. This process can also be performed using a pipette with a precise range. The black detection plate can reduce the fluorescence interference between the test samples.

5. In a 96-well plate microwell, add 10 μL of dsDNA standard or 1-20 μl of unknown sample to each well, and mix gently with a pipette.

6. The microwell is incubated at room temperature and away from light for 5-10 min. For best results, read the assay plate immediately after the incubation. The data can also be read within 6 hours, but the accuracy of the results will be slightly lost.

7. Measure fluorescence values using a microplate reader with excitation and emission wavelengths at 485 nm and 530 nm.

8. Make a standard curve and calculate the DNA concentration of the test sample (Figure 2).

Note: The standard curve in Figure 3 is for reference only. You can make the standard curve based on the actual measured data to calculate the concentration of the sample.

Reference factors

1. For detection of DNA samples from plant or animal, calf thymus DNA is often used as a reference for making DNA standards. Because Calf thymus DNA has a double-stranded structure and is highly aggregated, bases are evenly distributed (content AT 58%, GC 42%). If the fluorescence value of the test sample exceeds the standard curve, the sample needs to be further diluted. In order to maintain consistent results, it is important to make sure that the sample is loaded equally in each well and does not contain other high concentrations of contaminants.

2. WonderBlue® High Sensitivity dsDNA Quantification Kit can measure dsDNA with a range of 0.2-100 ng. For some samples that do not require linear detection, the kit detection range can be extended to 200 ng. If you need to detect lower levels of samples, you can further dilute the DNA standard with 1 × TE buffer, the concentration can be diluted to 0.02 ng / μL, and then load 10 μL per well according to conventional procedures.

3. For different types of detection instruments, you can optimize the instrument settings to obtain the best linearity. Some factors that may affect the final linearity and relative fluorescence intensity are: (1) excitation and emission wavelengths and bandwidths; (2) cut-off filters; (3) sensitivity settings; (4) accuracy of pipetting; (5)) Microplate manufacturer.

4. Table 1 details several common DNA contaminants, such as salts, solvents, detergents, and proteins (page 3).

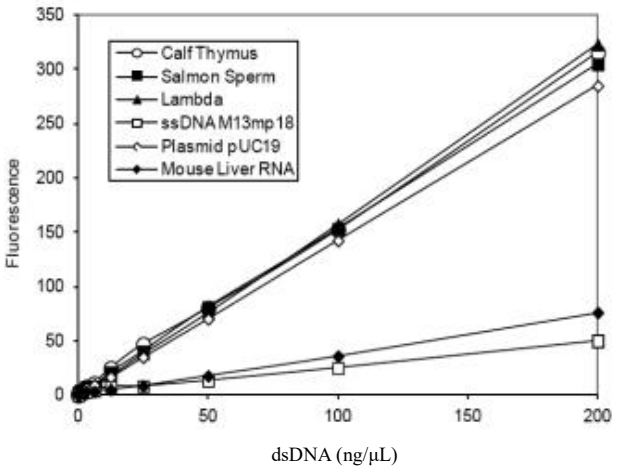

Figure 2. Relative fluorescence intensities from different types of nucleic acids detected using the WonderBlue® High Sensitivity dsDNA Quantitation Kit.

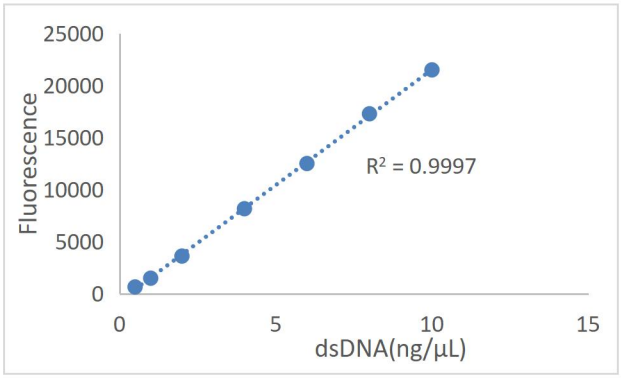

Figure 3. Calf thymus DNA standard curve using the WonderBlue® Hig Sensitivity dsDNA Quantification Kit (Ex / Em 485/530)

Table 1. Effects of common DNA contaminants on WonderBlue® High Sensitivity dsDNA Quantitation Kit

Compound	Initial Concentration	Final Concentration (200 µL)	Result
CH_3COONH_4	100 mM	5 mM	Pass
CH_3COONa	600 mM	30 mM	Pass
Nacl	200 mM	10 mM	Pass
Mgcl	25 mM	1.25 mM	Pass
C_6H_5OH	2 %	0.10 %	Pass
C_2H_5OH	10 %	0.5 %	Pass
$CHCl_3$	2 %	0.1 %	Pass
SDS	0.2 %	0.01 %	Pass
Triton X-100	0.2 %	0.01 %	Pass
BSA	200 mg/mL	10 mg/mL	Pass*
dNTPs**	2 mM	100 µM	Pass
$HO(CH_2CH_2O)_nH$	40 %	2 %	
Agarose	2 %	0.1 %	

The standard curve of three dsDNA parallel samples, respectively, under the above conditions containing the specified concentration of pollutants (the initial concentration is tested, "Pass" refers to the range of the experimental results <20% compared with the system without pollutants. All samples were excited at 485 nm with the Molecular Devices Gemini XS microplate reader and the fluorescence intensity was measured at 530 nm. "Pass *" refers to the slight variation of the standard curve measured under this condition. "DNTPs **" refers to the mixture of dATP, dCTP, dGTP and dTTP.

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