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iQuant™ dsDNA BR Assay Kit

(2 – 1000 ng)

Catalog Number: N012, N013

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
iQuant™ dsDNA BR Assay Kit (Cat. No. N012)				The product is stable for at least 6 months when stored as directed.
iQuant™ dsDNA BR Reagent (Component A)	200 µL	200X in DMSO	2-8 °C Protect from light	
iQuant™ dsDNA BR Buffer (Component B)	50 mL	1X		
dsDNA Standard #1 (Component C)	200 µL	0 ng/µL in TE buffer		
dsDNA Standard #2 (Component D)	200 µL	10 ng/µL in TE buffer		
iQuant™ dsDNA BR Assay Kit (Cat. No. N013)				
iQuant™ dsDNA BR Reagent (Component A)	1 mL	200X in DMSO	2-8 °C Protect from light	
iQuant™ dsDNA BR Buffer (Component B)	200 mL	1X		
dsDNA Standard #1 (Component C)	1 mL	0 ng/µL in TE buffer		
dsDNA Standard #2 (Component D)	1 mL	10 ng/µL in TE buffer		

Approximate fluorescence excitation/emission maxima, in nm: 500/530, bound to DNA.

Product Description

The iQuant™ dsDNA BR Assay Kit provides a simple, sensitive, and accurate quantitation for dsDNA. The kit contains concentrated assay reagent, dilution buffer, and pre-diluted dsDNA standards. The assay kit is highly selective for dsDNA, and highly reliable in detecting dsDNA ranging from 2 to 1000 ng, and offers advantages in stability, linear dynamic range, and sensitivity over other traditional of DNA quantitation. The assay is performed at room temperature. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µl and 50 µl is acceptable), and read the fluorescence using fluorescence plate reader or Fluorometer such as Qubit® or Quantus™ Fluorometer. The kit is well tolerated to common contaminants such as proteins, salts, solvents and detergents.

Handling and Disposal

There is no safety data available for iQuant™ dsDNA BR reagent. Treat the iQuant™ dsDNA BR reagent with the safety precautions as other potentially harmful reagents and to dispose of the reagent in accordance with local regulations. Centrifuge the iQuant™ dsDNA BR reagent and the dsDNA standards before opening vials to minimize loss on the cap. Use properly calibrated pipettes for best accuracy.

General Protocol

1. **Measure dsDNA samples using a Fluorescence Microplate Reader**

(Note: For simplicity, the following protocol is written using 10 µL of dsDNA sample volume. In practice, the volume of dsDNA sample could be ranging from 1 µL to 50 µL depending on the concentration of dsDNA sample, then adjust the volume of iQuant™ working solution to

200 μL.)

1.1 Warm up the iQuant™ dsDNA BR Assay Kit to room temperature. Check the iQuant™ dsDNA BR reagent for any precipitation. If precipitation is seen, warm up the vial in a water bath and vortex until dissolved.

1.2 Prepare the iQuant™ working solution by diluting the iQuant™ dsDNA BR reagent 1:200 in 1X iQuant™ dsDNA BR Buffer **IMMEDIATELY** before use. Use a clean plastic tube each time you make iQuant™ working solution. For example, to measure 8 samples in duplicate, add 20 μL of iQuant™ dsDNA BR reagent to 4 mL of 1X iQuant™ dsDNA BR Buffer. Mix well and use immediately.

1.3 Add 190 μL of the iQuant™ working solution to each well of a black 96-well microplate. Black plates such as Greiner or Corning black 96-well plates are recommended to minimize fluorescence bleed-through from other well.

1.4 Prepare a series of dsDNA standard dilutes from dsDNA Standard #2 (Component D) or your known dsDNA sample.

1.5 Add 10 μL of each dsDNA standard dilutes and the unknown dsDNA samples in duplicate or triplicates into separated wells and mix well by pipetting up and down.

1.6 Incubate the microplate at room temperature for 2 minutes in the dark.

1.7 Measure the fluorescence using a microplate reader with 485 nm excitation and 530 nm emission, with the appropriate cut-off.

1.8 Generate a linear standard curve by plotting fluorescence versus DNA concentration of the DNA standards. Use the standard curve and the fluorescence of the unknown DNA samples to determine the unknown DNA concentration.

2. Measure dsDNA samples using the Qubit® Fluorometer from Invitrogen or the Quantus® Fluorometer from Promega

(Note: For simplicity, the following protocol is written using 10 μL of dsDNA sample volume. In practice, the volume of dsDNA sample could be ranging from 1 μL to 50 μL depending on the concentration of dsDNA sample, then adjust the volume of iQuant™ working solution to 200 μL.)

2.1. Warm up the iQuant™ dsDNA BR Assay Kit to room temperature. Check the iQuant™ dsDNA BR reagent for any precipitation. If precipitation is seen, warm up the vial in a water bath and vortex until dissolved.

2.2. Prepare the iQuant™ working solution by diluting the iQuant™ dsDNA BR reagent 1:200 in 1X iQuant™ dsDNA BR Buffer **IMMEDIATELY** before use. Use a clean plastic tube each time you make iQuant™ working solution. For example, to measure 8 samples in duplicate, add 10 μL of iQuant™ dsDNA BR reagent to 2 mL of 1X iQuant™ dsDNA BR Buffer. Mix well and use immediately.

2.3. Add 190 μL of the iQuant™ working solution to each assay tube. (**Note:** Use only thin-wall, clear 0.5 mL PCR tubes. Axygen PCR-05-C tubes (VWR, Cat No. 1011-830)).

2.4. Add 10 μL of dsDNA standard #1 (Component C), dsDNA standard #2 (Component D), and the unknown dsDNA samples to the appropriate tubes and mix by vortexing 2-3 seconds, and label the lids of each DNA standard tube and unknown sample tubes correctly.

2.5. Incubate all tubes at room temperature for 2 minutes in the dark.

2.6. Measure the fluorescence on the Qubit® fluorometer using the **dsDNA Broad Range** program, according to the manufacture's recommendation; or the Quantus® Fluorometer according to user manual.

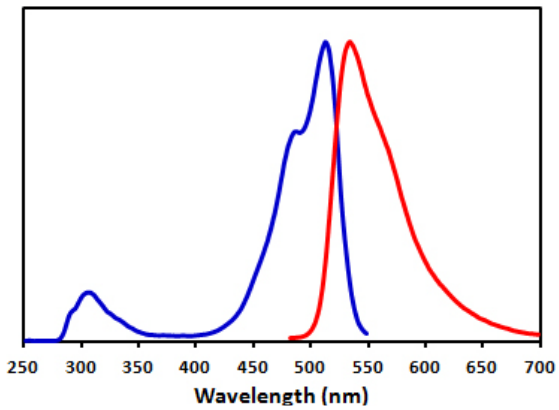

Figure 1. Excitation (blue) and emission spectra (red) of iQuant™ BR dsDNA reagent in the presence of dsDNA.

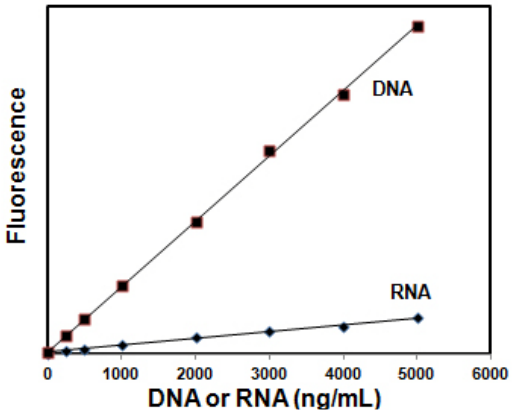

Figure 2. DNA selectivity and sensitivity of iQuant™ dsDNA BR assay kit.

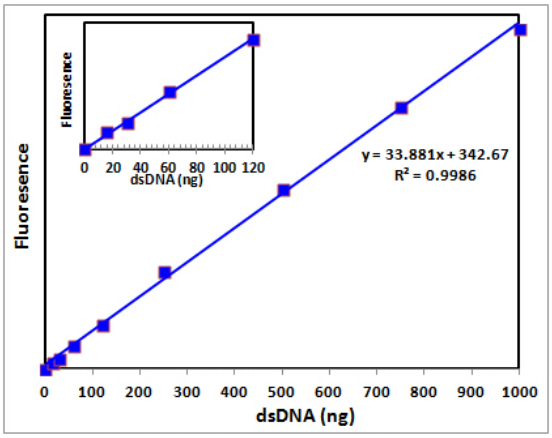

Figure 3. The quantitation of dsDNA with iQuant™ dsDNA BR Assay Kit using fluorescence plate reader.

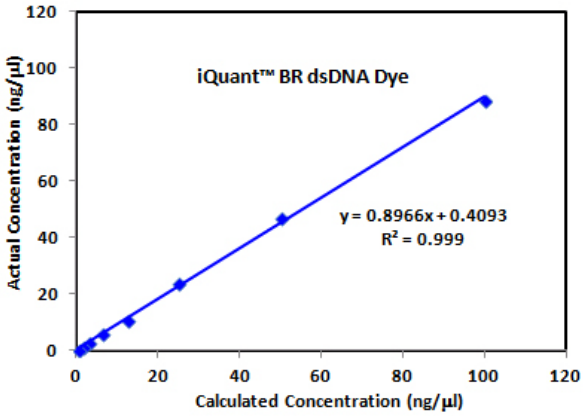

Figure 4. The quantitation of dsDNA with iQuant™ dsDNA BR Assay Kit using Qubit® Fluorometer.

Considerations for Data Analysis

It is more prefer to use a dsDNA standard similar to the unknown samples (i.e. similar in size, linear vs circular). We found using the iQuant™ dsDNA BR reagent most linear dsDNA yield similar results. If the fluorescence of an unknown sample is higher than dsDNA standard #2 (Component D), further dilute the sample and add 10 µL of diluted sample to perform the assay.

Appendix

Table 2. Effect of Contaminants in the iQuant™ dsDNA HS Assay Kit

Contaminant	Final Concentration in Assay	Concentration in 10 µL Sample	Result
Proteins			
Bovine Serum Albumin	10 mg/mL	200 mg/mL	OK
Salts			
Sodium Chloride	20 mM	400 mM	OK

Magnesium Chloride	5 mM	100 mM	OK
Sodium Acetate	20 mM	400 mM	OK
Ammonium Acetate	20 mM	400 mM	OK
Organic Solvents			
Ethanol	0.5%	10%	OK
Chloroform	0.5%	10%	OK
Phenol	0.1%	2%	OK
Detergents			
Sodium Dodecyl Sulfate	0.01%	0.2%	OK
Triton X-100	0.01%	0.2%	OK
Other Compounds			
dNTPs	100 μM	2 mM	OK
RNA	1X	1X	OK
Polyethylene Glycol	1%	20%	OK
Agarose	0.1%	2%	OK

Related Products

Cat. No.	Product Name	Unit Size
N010	iQuant™ dsDNA HS Assay Kit	200 assays
N011	iQuant™ dsDNA HS Assay Kit	1000 assays
N014	iQuant™ ssDNA Assay Kit	200 assays
N015	iQuant™ ssDNA Assay Kit	1000 assays

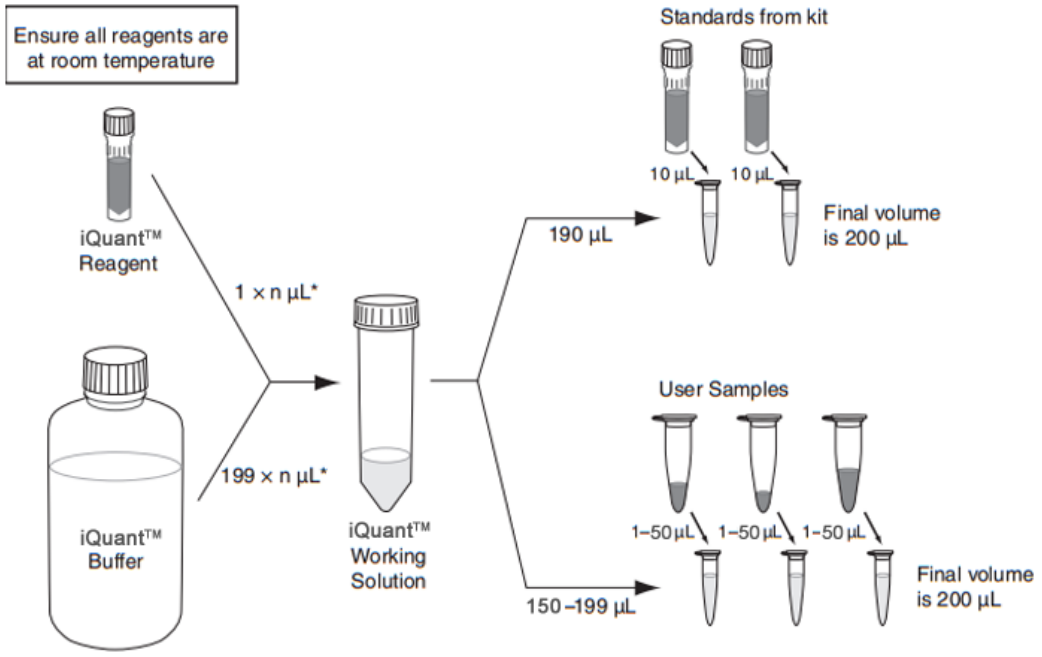

Figure 5. iQuant™ dsDNA BR Assay workflow