GeneCopoeia™
Expressway to Discovery

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iQuant™ NGS-BR dsDNA Assay Kit
(2 – 1000 ng)
Catalog Number: N021-1, N021-2

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
iQuant™ NGS-BR dsDNA Assay Kit (Cat. No. N021-1, 250 assays)				**The product is stable for at least 6 months when stored as directed.**
iQuant™ NGS-BR Reagent (Component A)	50 mL	1X	2-8 °C Protect from light	
iQuant™ NGS-BR dsDNA Standard #1 (Component B)	0.5 mL	0 ng/μL in TE buffer		
iQuant™ NGS-BR dsDNA Standard #2 (Component C)	0.5 mL	100 ng/μL in TE buffer		
iQuant™ NGS-BR dsDNA Assay Kit (Cat. No. N021-2, 500 assays)				
iQuant™ NGS-BR Reagent (Component A)	100 mL	1X	2-8 °C Protect from light	
iQuant™ NGS-BR dsDNA Standard #1 (Component B)	1 mL	0 ng/μL in TE buffer		
iQuant™ NGS-BR dsDNA Standard #2 (Component C)	1 mL	100 ng/μL in TE buffer		

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

Product Description

The iQuant™ NGS-BR dsDNA Assay Kit provides a simple, sensitive, and accurate quantitation for dsDNA. The kit contains ready-to-use assay reagent, 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 add your sample (any volume between 1 µl and 50 µl is acceptable) to the assay reagent, 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™ NGS-BR reagent. Treat the iQuant™ NGS-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™ BR dsDNA 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™ NGS-BR reagent to 200 µL.)

1.1 Warm up the iQuant™ NGS-BR dsDNA Assay Kit to room temperature.

1.2 Add 190 µL of the iQuant™ NGS-BR reagent (Component A) 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.

1.3 Prepare a series of dsDNA standard dilutes from iQuant™ NGS-BR dsDNA Standard #2 (Component C) or your known dsDNA sample.

1.4 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.

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

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

1.7 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 ThermoFisher

(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 20 μL depending on the concentration of dsDNA sample, then adjust the volume of iQuant™ NGS-BR reagent to 200 μL.)

2.1. Warm up the iQuant™ NGS-BR dsDNA Assay Kit to room temperature.

2.2. Add 190 μL of the iQuant™ NGS-BR reagent (Component A) to each assay tube. (**Note:** Use only thin-wall, clear 0.5 mL PCR tubes. iQuant™ assay tubes (Cat No. N022)).

2.3. Add 10 μL of dsDNA standard #1 (Component B), dsDNA standard #2 (Component C), 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.

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

2.5. Measure the fluorescence on the Qubit® fluorometer using the **dsDNA Broad Range** program, according to the manufacture's recommendation.

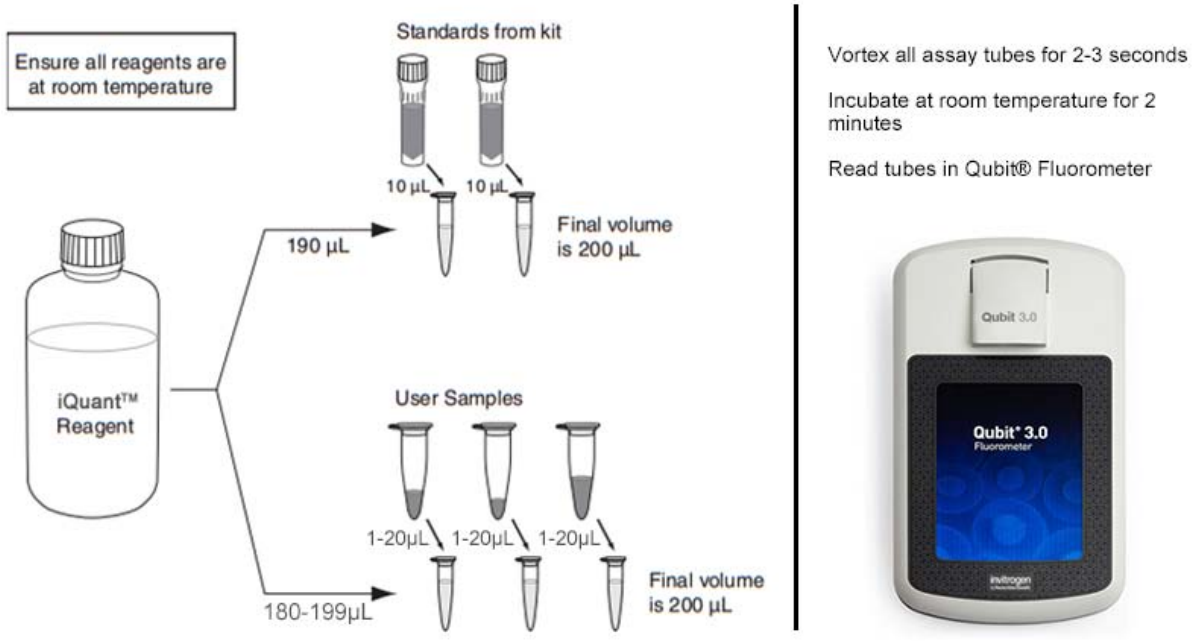

Figure 1. Qubit® assay workflow

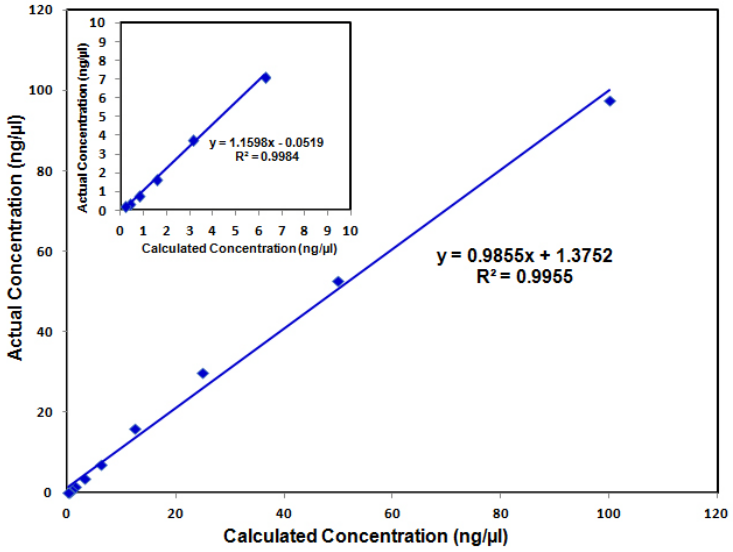

Figure 2. Quantitation of dsDNA using Qubit® Fluorometer.

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™ NGS-BR reagent most linear dsDNA yield similar results. If the fluorescence of an unknown sample is higher than dsDNA standard #2 (Component C), further dilute the sample and add 1~10 µL of diluted sample to perform the assay.

Appendix

Table 2. Effect of Contaminants in the iQuant™ NGS-BR dsDNA Assay

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

Table 3. iQuant™ assay kits compatible with the Qubit® Fluorometer

Product	Cat. No.	Unit	Target	Notes
iQuant™ NGS-HS dsDNA Assay Kit	N020-1	250T	dsDNA	Detection range: 10 pg/µL – 100 ng/µL; Useful for quantitation of PCR products, viral DNA, and samples for subcloning.
	N020-2	500T		
iQuant™ NGS-BR dsDNA Assay Kit	N021-1	250T	dsDNA	Detection range: 100 pg/µL – 1000 ng/µL; Useful for quantitation of genomic and miniprep DNA samples.
	N021-2	500T		
iQuant™ HS dsDNA Quantitation Kit	N011	1000T	dsDNA	Detection range: 10 pg/µL – 100 ng/µL; Useful for quantitation of PCR products, viral DNA, and samples for subcloning.
iQuant™ BR dsDNA Quantitation Kit	N013	1000T	dsDNA	Detection range: 100 pg/µL – 1000 ng/µL; Useful for quantitation of genomic and miniprep DNA samples.
iQuant™ ssDNA Quantitation Kit	N015	1000T	ssDNA	Detection range: 50 pg/µL – 200 ng/µL; Useful for quantitation of oligos, primers, denatured DNA, PCR products.
iQuant™ HS RNA Quantitation Kit	N017	1000T	RNA	Detection range: 250 pg/µL – 100 ng/µL; Useful for quantitation of samples of microarray and RT-PCR.
iQuant™ BR RNA Quantitation Kit	N019	1000T	RNA	Detection range: 1 ng/µL – 1000 ng/µL; Useful for quantitation of samples of microarray and RT-PCR.

Order information

Cat. No.	Product Name	Unit Size
N010	iQuant™ High Sensitivity dsDNA Reagent (200 X)	1 mL
N011	iQuant™ High Sensitivity dsDNA Quantitation Kit	1000 assays
N012	iQuant™ Broad Range dsDNA Reagent (200 X)	1 mL
N013	iQuant™ Broad Range dsDNA Quantitation Kit	1000 assays
N014	iQuant™ ssDNA Reagent (200 X)	1 mL
N015	iQuant™ ssDNA Quantitation Kit	1000 assays
N016	iQuant™ High Sensitivity RNA Reagent (200 X)	1 mL
N017	iQuant™ High Sensitivity RNA Quantitation Kit	1000 assays
N018	iQuant™ Broad Range RNA Reagent (200 X)	1 mL
N019	iQuant™ Broad Range RNA Quantitation Kit	1000 assays
N020-1	iQuant™ NGS-HS dsDNA Assay Kit	250 assays
N020-2	iQuant™ NGS-HS dsDNA Assay Kit	500 assays
N021-1	iQuant™ NGS-BR dsDNA Assay Kit	250 assays
N021-2	iQuant™ NGS-BR dsDNA Assay Kit	500 assays
N022	iQuant™ Assay Tubes	500 tubes