LIFESCT
Life Scientists' Service Center

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LiQuant™ NGS dsDNA Assay Kit

Cat. #: M0066 Size: 200 rxns

Introduction

LiQuant™ NGS 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 0.2 to 100 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.

Package Information

Components	M0066
LiQuant™ NGS dsDNA Reagent	40 ml
dsDNA Standard #1	0.4 ml
dsDNA Standard #2	0.4 ml

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

Storage

Store at 2-8°C and protect from light.

Handling and Disposal

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

Protocol

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 20 µl depending on the concentration of dsDNA sample, then adjust the volume of LiQuant™ NGS dsDNA reagent to 200 µl.

1. Warm up the LiQuant™ NGS dsDNA Assay Kit to room temperature.

2. Add 190 µl of the LiQuant™ NGS dsDNA reagent 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.

3. Prepare a series of dsDNA standard dilutes from dsDNA Standard #2 or your known dsDNA sample.

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.

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

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

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.

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 LiQuant™ NGS dsDNA reagent to 200 µl.

1. Warm up the LiQuant™ NGS dsDNA Assay Kit to room temperature.

2. Add 190 µl of the LiQuant™ NGS dsDNA reagent to each assay tube.

Note: Use only thin-wall, clear 0.5 ml PCR tubes.

3. Add 10 µl of dsDNA standard #1, dsDNA standard #2, 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.

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

5. Measure the fluorescence on the Qubit® fluorometer using the dsDNA High Sensitivity program, according to the manufacture's recommendation.

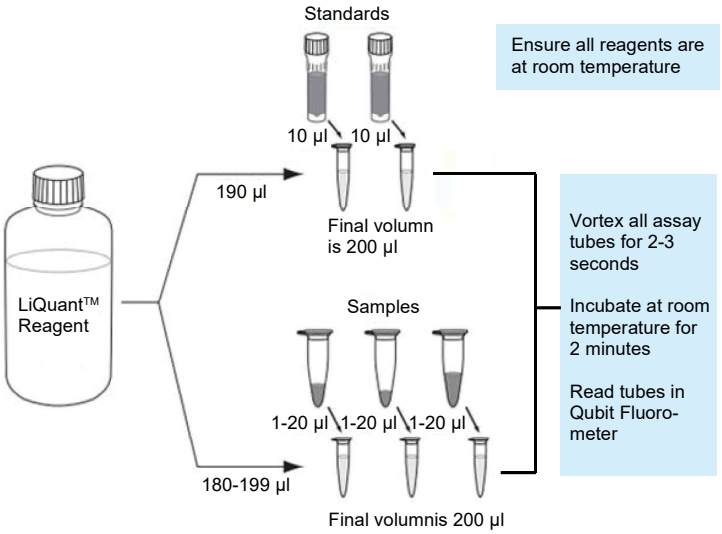

Figure 1. Qubit® assay workflow

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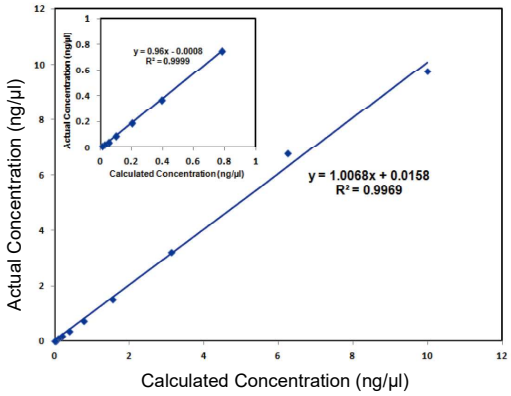

Figure 2. Quantitation of dsDNA using Qubit® Fluorometer

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 LiQuant™ NGS dsDNA reagent most linear dsDNA yield similar results. If the fluorescence of an unknown sample is higher than dsDNA standard #2, further dilute the sample and add 1~10 µl of diluted sample to perform the assay.

Effect of Contaminants in the LiQuant™ NGS 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