LIFESCT
Life Scientists' Service Center

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LiQuant™ ssDNA Assay Kit

Cat. #: M0067 Size: 200 rxns

Introduction

LiQuant™ ssDNA Assay Kit provides an easy and accurate quantitation for ssDNA or oligonucleotides. The kit is not selective for ssDNA over dsDNA or RNA, but it will not detect contaminating protein or nucleotides. The assay kit is highly reliable in detecting ssDNA ranging from 1 to 200 ng, and offers advantages in stability, linear dynamic range, and sensitivity over other traditional of DNA quantitation.The kit contains concentrated assay reagent, dilution buffer, and pre-diluted ssDNA standards. 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.

Package Information

Components	M0067
LiQuant™ ssDNA Reagent	200 µL
LiQuant™ ssDNA Buffer	50 mL
ssDNA Standard #1	200 µL
ssDNA Standard #2	200 µL

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™ ssDNA reagent. Treat the LiQuant™ ssDNA reagent with the safety precautions as other potentially harmful reagents and to dispose of the reagent in accordance with local regulations. Centrifuge the LiQuant™ ssDNA reagent and the ssDNA standards before opening vials to minimize loss on the cap. Use properly calibrated pipettes for best accuracy.

Protocol

Measure ssDNA samples using a Fluorescence Microplate Reader

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

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

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

3. Add 190 µL of the LiQuant™ 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.

4. Prepare a series of ssDNA standard dilutes from ssDNA Standard #2 or your known ssDNA sample.

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

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

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

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.

Measure ssDNA samples using the Qubit® Fluorometer from ThermoFisher

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

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

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

3. Add 190 µL of the LiQuant™ working solution to each assay tube.

Note: Use only thin-wall, clear 0.5 mL PCR tubes. Axygen PCR-05-C tubes.

4. Add 10 µL of ssDNA standard #1, ssDNA standard #2, and the unknown ssDNA 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.

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

6. Measure the fluorescence on the Qubit® fluorometer using the ssDNA program, according to the manufacture's recommendation.

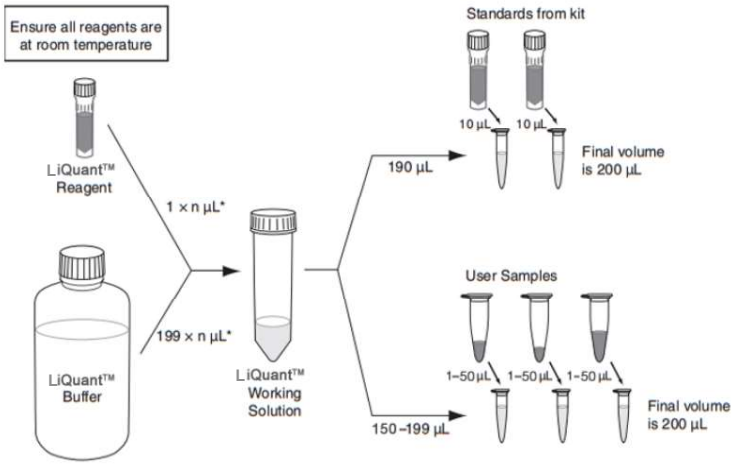

Figure 1. LiQuant™ ssDNA Assay workflow

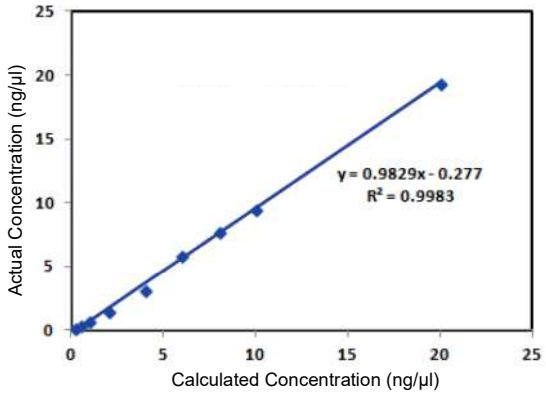

Figure 2. The quantitation of dsDNA with LiQuant™ ssDNA Assay Kit using Qubit® Fluorometer.

Considerations for Data Analysis

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

Effect of Contaminants in the LiQuant™ ssDNA Assay Kit

Contaminant	Final Concentration in Assay	Concentration in 10 µL Sample	Result
Proteins			
Bovine Serum Albumin	50 µg/mL	1 mg/mL	OK
Salts			
Sodium Chloride	2.5 mM	50 mM	OK
Magnesium Chloride	0.1 mM	2 mM	OK
Sodium Acetate	1 mM	20 mM	OK
Ammonium Acetate	1 mM	20 mM	OK
Organic Solvents			
Ethanol	0.5%	10%	OK
Chloroform	0.1%	2%	OK
Phenol	0.01%	0.2%	OK
Detergents			
Triton X-100	0.005%	0.1%	OK

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