

## Introduction

**LiQuant™ dsDNA HS 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 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 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	M0065
LiQuant™ dsDNA HS Reagent	200 µL
LiQuant™ dsDNA HS Buffer	50 mL
dsDNA Standard #1	200 µL
dsDNA 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™ dsDNA HS reagent. Treat the LiQuant™ dsDNA HS reagent with the safety precautions as other potentially harmful reagents and to dispose of the reagent in accordance with local regulations. Centrifuge the LiQuant™ dsDNA HS 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 50 µL depending on the concentration of dsDNA sample, then adjust the volume of LiQuant™ working solution to 200 µL.

1. Warm up the LiQuant™ dsDNA HS Assay Kit to room temperature. Check the LiQuant™ dsDNA HS 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™ dsDNA HS reagent 1:200 in 1× LiQuant™ dsDNA HS 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™ dsDNA HS reagent to 4 mL of 1× LiQuant™ dsDNA HS 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 dsDNA standard dilutes from dsDNA Standard #2 or your known dsDNA sample.

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.

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 dsDNA samples using the Qubit® Fluorometer from ThermoFisher 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 LiQuant™ working solution to 200 µL.

1. Warm up the LiQuant™ dsDNA HS Assay Kit to room temperature. Check the LiQuant™ dsDNA HS 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™ dsDNA HS reagent 1:200 in 1× LiQuant™ dsDNA HS 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™ dsDNA HS reagent to 2 mL of 1× LiQuant™ dsDNA HS 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 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.

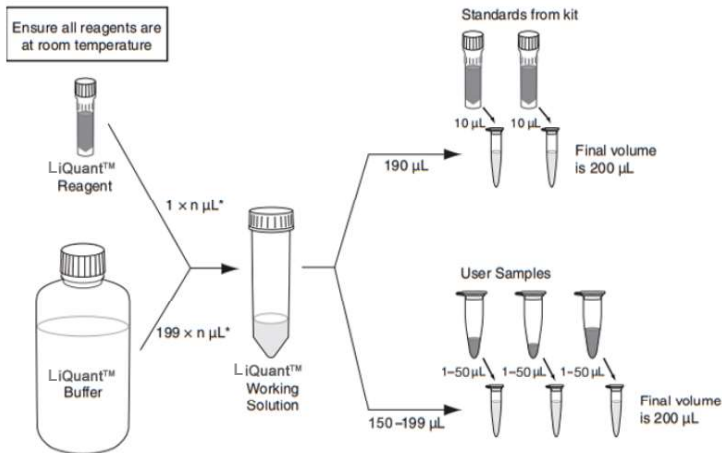


Figure 1. LiQuant™ dsDNA HS Assay workflow

### Effect of Contaminants in the LiQuant™ dsDNA HS Assay

Contaminant	Final Concentration in Assay	Concentration in 10 µL Sample	Result
<b>Proteins</b>			
Bovine Serum Albumin	10 mg/mL	200 mg/mL	OK
<b>Salts</b>			
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
<b>Organic Solvents</b>			
Ethanol	0.5%	10%	OK
Chloroform	0.5%	10%	OK
Phenol	0.1%	2%	OK
<b>Detergents</b>			
Sodium Dodecyl Sulfate	0.01%	0.2%	OK
Triton X-100	0.01%	0.2%	OK
<b>Other Compounds</b>			
dNTPs	100 µM	2 mM	OK
RNA	1X	1X	OK
Polyethylene Glycol	1%	20%	OK
Agarose	0.1%	2%	OK

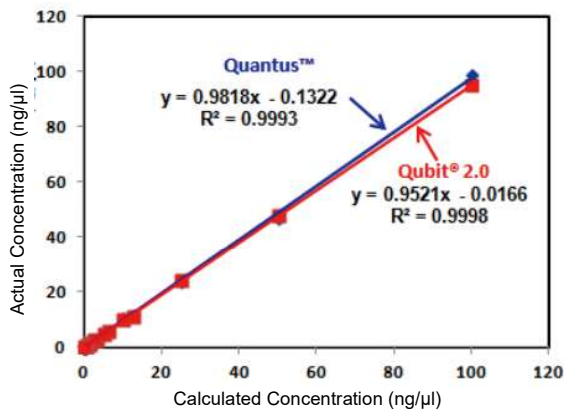


Figure 2. The quantitation of dsDNA with LiQuant™ dsDNA HS Assay Kit using Qubit® or Quantus™ Fluorometer.

### 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™ dsDNA HS 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 10 µL of diluted sample to perform the assay.