GeneCopoeia™
Expressway to Discovery

9620 Medical Center Drive, Suite 101
Rockville, MD 20850, USA
www.genecopoeia.com
1-866-360-9531

iQuant™ ssDNA Quantitation Kit
(1 – 200 ng)
Catalog Number: N014, N015

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
iQuant™ ssDNA Quantitation Reagent (Cat. No. N014)				
iQuant™ ssDNA Reagent	1 mL	200X in DMSO	2-8 °C Protect from light	The product is stable for at least 6 months when stored as directed.
iQuant™ ssDNA Quantitation Kit (Cat. No. N015)				
iQuant™ ssDNA Reagent (Component A)	1 mL	200X in DMSO	2-8 °C Protect from light	
iQuant™ ssDNA Buffer (Component B)	30 mL	10X		
iQuant™ ssDNA Standard #1 (Component C)	1 mL	0 ng/μL in TE buffer		
iQuant™ ssDNA Standard #2 (Component D)	1 mL	20 ng/μL in TE buffer		

Number of assays: 1000 assays.
Approximate fluorescence excitation/emission maxima, in nm: 500/530, bound to DNA.

Product Description

The iQuant™ 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.

Handling and Disposal

There is no safety data available for iQuant™ ssDNA reagent. Treat the iQuant™ ssDNA reagent with the safety precautions as other potentially harmful reagents and to dispose of the reagent in accordance with local regulations. Centrifuge the iQuant™ ssDNA reagent and the ssDNA 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™ ssDNA Quantitation Kit to room temperature. Check the iQuant™ ssDNA reagent for any precipitation. If precipitation is seen, warm up the vial in a water bath and vortex

until dissolved.

1.2 Make 1X iQuant™ ssDNA Buffer by diluting the 10X iQuant™ ssDNA Buffer 1:9 in DI water.

1.3 Prepare the iQuant™ working solution by diluting the iQuant™ ssDNA reagent 1:200 in 1X iQuant™ ssDNA Buffer (prepared from step 1.2) **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™ ssDNA reagent to 4 mL of 1X iQuant™ ssDNA Buffer. Mix well and use immediately.

1.4 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.

1.5 Prepare a series of ssDNA standard dilutes from iQuant™ ssDNA Standard #2 (Component D) or your known ssDNA sample.

1.6 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.

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

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

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

2. Measure ssDNA 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™ ssDNA Quantitation Kit to room temperature. Check the iQuant™ ssDNA reagent for any precipitation. If precipitation is seen, warm up the vial in a water bath and vortex until dissolved.

2.2. Make 1X iQuant™ ssDNA Buffer by diluting the 10X iQuant™ ssDNA Buffer 1:9 in DI water.

2.3. Prepare the iQuant™ working solution by diluting the iQuant™ ssDNA reagent 1:200 in 1X iQuant™ ssDNA Buffer (prepared from step 1.2) **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™ ssDNA reagent to 2 mL of 1X iQuant™ ssDNA Buffer. Mix well and use immediately.

2.4. 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)).

2.5. Add 10 μL of ssDNA standard #1 (Component C), ssDNA 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.

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

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

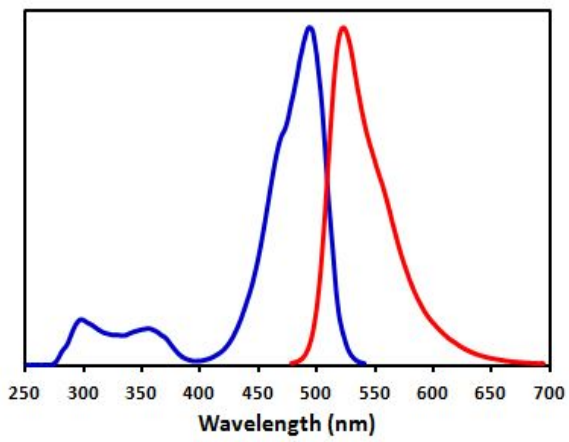

Figure 1. Excitation (blue) and emission spectra (red) of iQuant™ ssDNA reagent in the presence of ssDNA.

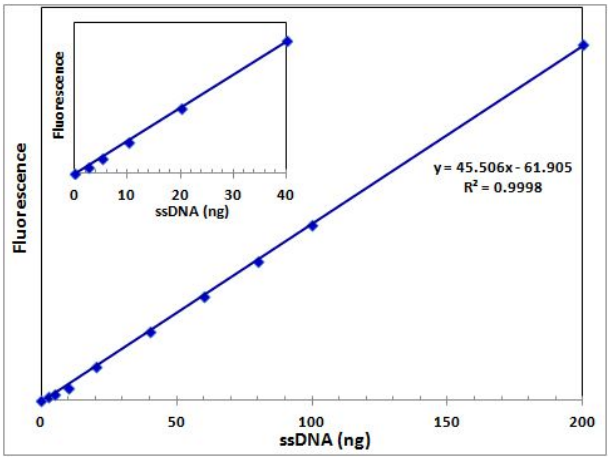

Figure 2. The quantitation of ssDNA with iQuant™ ssDNA Quantitation Kit using fluorescence plate reader.

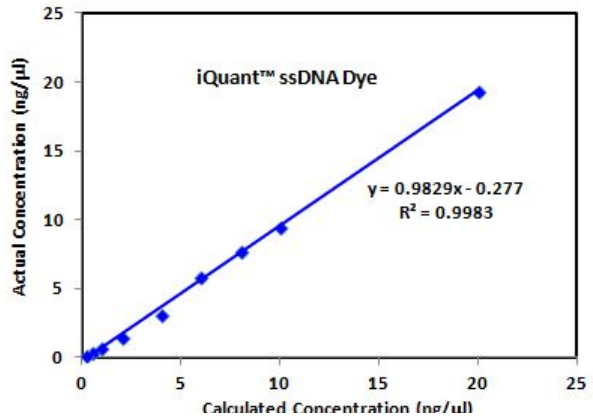

Figure 3. The quantitation of ssDNA with iQuant™ ssDNA Quantitation Kit using Qubit ® Fluorometer.

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 iQuant™ ssDNA reagent most ssDNA yield similar results. If the fluorescence of an unknown sample is higher than ssDNA 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™ ssDNA Assay

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

Related Products

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

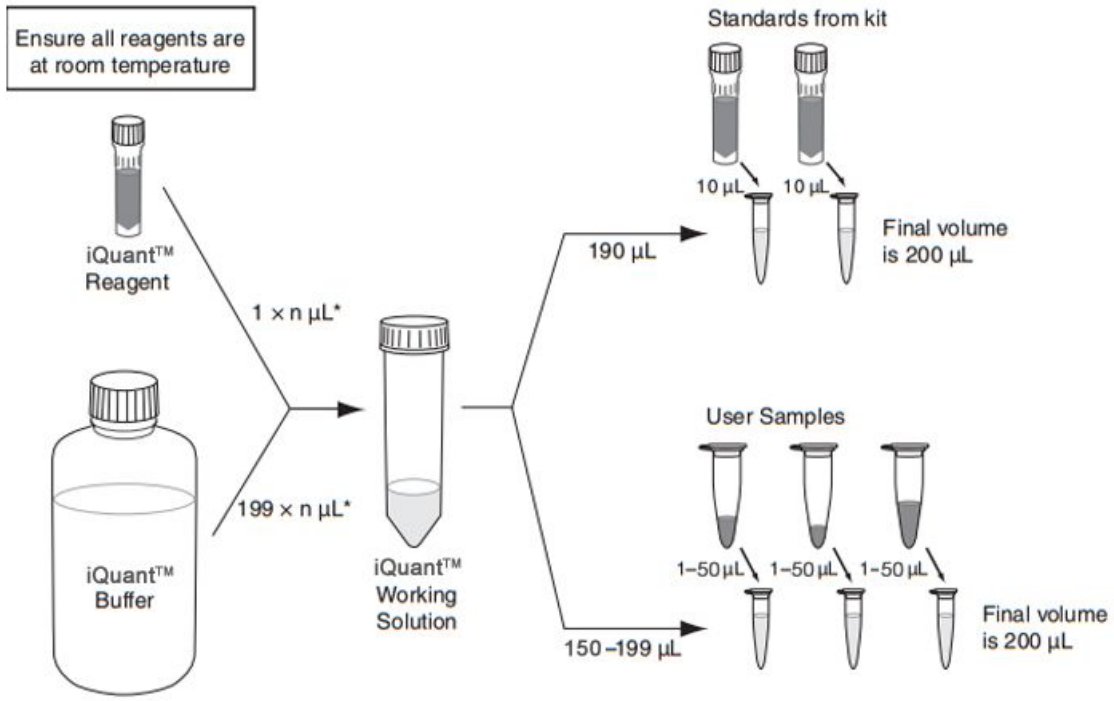

Figure 4. iQuant™ ssDNA quantitation assay workflow