

FS-QHS-02

Qubit dsDNA High Sensitivity Assay Kit

Introduction

The Qubit dsDNA HS (High sensitivity) Assay Kit, when used with the Qubit Fluorometer, provides an accurate and selective method for the quantitation of DNA samples. Is suitable for all the Qubit Fluorometers including Qubit 2.0 and Qubit 3.0., and Qubit 4.0

The conventional method for quantifying DNA content is to measure absorbance at 260 nm (A260).

The major drawbacks of this method are that nucleotides, single-stranded nucleic acids and proteins strongly affect the signal, and the measurement is subject to interference from contaminants introduced during nucleic acid preparation; moreover, it cannot distinguish DNA from RNA, and it is insensitive (a 5 µg/mL dsDNA solution gives A260 = 0.1).

The Qubit dye quantitation method is simple and convenient, has become the standard for residual DNA testing in biological products.

For example, the Nanodrop series of microvolume spectrophotometers is currently the most common instrument used to measure sample concentration and purity by UV absorbance.

Because it measures the absorbance of all substances at 260 nm (including DNA/RNA, degraded nucleic acids, free nucleotides and other impurities), the readings are not very accurate.

In contrast, a fluorometer uses fluorescent dyes that bind specifically to the target molecule, thereby significantly improving the accuracy and sensitivity of detection.

Importantly, accurate DNA quantitation is critical for downstream applications.

The Nanodrop series of full-wavelength microvolume spectrophotometers cannot accurately determine DNA concentrations below 5 ng/µL; yet many DNA samples fall exactly within this range.

When measuring double-stranded DNA, the fluorometer achieves a detection limit of 0.2 ng/µL.

This reagent is intended for use in costly downstream experiments such as qPCR, PCR cloning, transfection and next-generation sequencing that require precise quantitation, with an accurate quantitation range of 10 pg/µL to 10 ng/µL.

Principle of the Assay

Qubit dye emits fluorescence only upon binding to double-stranded DNA; no fluorescence is produced in the absence of DNA, and the fluorescence intensity is proportional to the DNA concentration.

The detection limit of the Qubit dye method for DNA quantitation is approximately 10 pg/µL, and linearity is good ($R^2 > 0.99$) within a DNA concentration range of **10 pg/µL to 10 ng/µL**.

This kit utilizes the principle that *Qubit dye specifically binds double-stranded DNA* without binding to proteins, RNA or salt ions. Upon excitation, the Qubit fluorometer collects the emitted light and specifically analyzes the actual dsDNA content of the sample, eliminating interference from proteins, RNA, salt ions and other substances in the sample, thereby achieving accurate quantitation of double-stranded DNA.

Advantages

- ✓ This method can quantify double-stranded DNA from samples derived from any expression host.
- ✓ PCR amplification products can be quantified directly without purifying DNA from the reaction mixture.
- ✓ It far exceeds the sensitivity of the traditional UV A260 method and Hoechst 33258.
- ✓ The specific assay shows no cross-reactivity with interfering substances such as Mg^{2+} , sodium chloride, sodium acetate, ammonium acetate, ethanol, phenol, chloroform, SDS, RNA, protein, Triton X-100, dNTPs, BSA, etc.
- ✓ The determination of dsDNA is minimally affected in the presence of equimolar concentrations of ssDNA and RNA.

Required Materials and Equipment

Disposable powder-free gloves; Qubit® assay tubes (500 tubes, Life Technologies, Cat. No. Q32856 0,5ml, thin-walled tubes) or Axygen® PCR-05-C tubes 0,5 ml (VWR, part no. 10011)

Precautions

1. Assay temperature: All assays must be performed at room temperature (22–28 °C).
2. Incubation time: After mixing DNA with the working solution, incubate for 3 min; the fluorescence signal remains stable for up to 3 hours.
3. Fluorescence calibration: Calibrate by lot before each use to ensure the consistency of real-time assay data.

Kit Components

Components	100 assays	500 assays	Concentration	Storage and Stability
Qu-bit dye dsDNA HS Reagent (Component A)	250µL	1.25mL	200X in DMSO	4°C Protect from light
dsDNA HS Buffer (Component B)	50mL	250mL	N/A	
dsDNA HS Standard #1 (Component C)	1mL	5mL	0 ng/uL in TE Buffer	
dsDNA HS Standard #2 (Component D)	1mL	5mL	10 ng/uL in TE Buffer	

*Note: Reagents from different lots must not be mixed.

Assay Procedure

1. Preparation before the assay:

- 1) Before use, allow all components of the at room temperature at least ½ hour before the experiment.
- 2) Prepare and label a sufficient number of 0.5 mL PCR thin-wall tubes. Do not label the side wall of the PCR tubes, as this may interfere with fluorescence signal acquisition. Qubit® assay tubes or Axygen® PCR-05-C tubes are recommended.

2. Preparation of the working solution

In a plastic container, dilute an appropriate amount of dsDNA Reagent to 1× with dsDNA HS Buffer at the recommended ratio [e.g., mix 1 µL of dsDNA HS Reagent (Component A) with 199 µL of dsDNA HS Buffer (Component B)]. Prepare fresh before use and use within 3 hours.

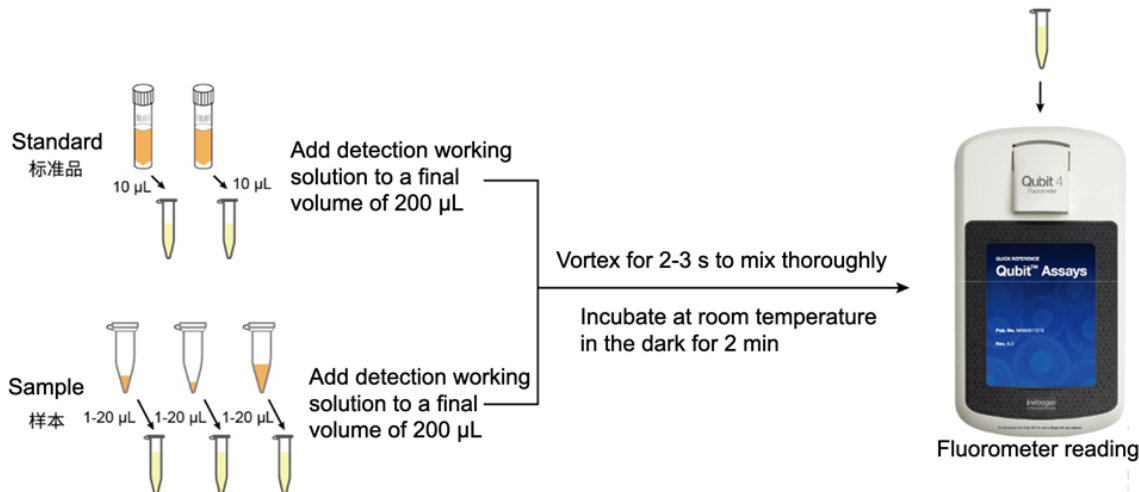
3. Preparation of samples to be tested

- 1) Prepare the standards. Aliquot 190 µL of working solution into each standard PCR tube, add 10 µL of dsDNA Standard 1 (Component C) and 10 µL dsDNA Standard 2 (Component D) to the corresponding standard PCR tubes, respectively, and vortex gently for 2–3 seconds, taking care to avoid bubble formation.
- 2) Prepare the test samples. Aliquot 180–199 µL of working solution into each sample PCR tube, add 1–20 µL of each test sample to bring the final volume in each tube to 200 µL, and vortex gently for 2–3 seconds, taking care to avoid bubble formation.

4. Detection

- 1) Place all the PCR tubes at room temperature, and incubate in the dark for 2 minutes.
- 2) According to the operating instructions of the Qubit® fluorometer below, select the dsDNA HS detection program to measure the fluorescence signal value.

1. Qubit operating flow chart:



2. Qubit® 3.0 operating steps:

- 2.1 On the Qubit® 3.0 home screen, select DNA, then select the assay type dsDNA High Sensitivity. When "Read standards" is displayed, select Read Standards to begin calibration.
- 2.2 Insert the tube containing Standard #1 into the sample chamber, close the lid, and press Read standard. When the reading is complete (~3 seconds), remove Standard #1.
- 2.3 Insert the tube containing Standard #2 into the sample chamber, close the lid, and press Read standard. When the reading is complete (~3 seconds), remove Standard #2.
- 2.4 Press Run samples.
- 2.5 On the reading screen, select the appropriate units and volume:
 - 2.5.1 Use the + or – buttons on the wheel to select the volume of sample added to the tube (from 1 to 20 µL);
 - 2.5.2 From the drop-down menu, select the concentration unit for the sample measurement.
- 2.6 Insert the tube containing the test sample into the sample chamber, close the lid, and press Read. When the reading is complete (~3 seconds), remove the sample and repeat until all samples have been measured.

3. Qubit® 2.0 operating steps:

- 3.1 On the Qubit® 2.0 home screen, select DNA, then select the assay type dsDNA High Sensitivity. When "Read standards" is displayed, select Read Standards to begin calibration.
- 3.2 Insert the tube containing Standard #1 into the sample chamber, close the lid, and press Read standard. When the reading is complete (~3 seconds), remove Standard #1.
- 3.3 Insert the tube containing Standard #2 into the sample chamber, close the lid, and press Read standard. When the reading is complete (~3 seconds), remove Standard #2.
- 3.4 Press Run samples.
- 3.5 Insert the tube containing the test sample into the sample chamber, close the lid, and press Read. When the reading is complete (~3 seconds), remove the sample and repeat until all samples have been measured.
- 3.6 Calculate the DNA concentration in the tube using the formula $200/X$.
 Sample concentration = QF value $\times$ 200 / X (QF value: the value directly measured by the Qubit® 2.0 for the sample; X: volume of sample added)

Effect of Contaminants on dsDNA Quantitation Kit Assay Results

Effect of contaminants in the Qubit dsDNA HS Assay, tested over a range of 1–500 ng/mL

Contaminant	Concentration in 10 μ L Sample	Final Concentration in the assay	Result
Salts			
Ammonium acetate	200 mM	10 mM	OK
Sodium acetate	200 mM	10 mM	OK
Sodium chloride	200 mM	10 mM	OK
Magnesium chloride	40 mM	2 mM	OK
Organic Solvents			
Phenol	2%	0.1%	OK
Ethanol	20%	1%	OK
Chloroform	4%	0.2%	OK
Detergents			
Sodium dodecyl sulfate	0.2%	0.01%	OK
Triton X-100	0.02%	0.001%	OK
Other Compounds			
Bovine serum albumin	400 μ g/mL	20 μ g/mL	OK
RNA	1 $\times$ *	1 $\times$ *	OK
dNTPs	2 mM	100 μ M	OK
Polyethylene glycol	20%	1%	OK
Agarose	2%	0.1%	OK

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