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DNA Quantification using the Qubit Fluorometer

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ARTICLE



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We use this protocol and it's working

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Abstract

Step by step guide to utilising the QubitTM dsDNA HS (High Sensitivity) Assay Kit for dsDNA quantification.

Guidelines

This protocol utilises the QubitTM dsDNA HS assay on the QubitTM 4 Fluorometer. The QubitTM 3.0 Fluorometer is also compatible with this protocol. If utilising the QubitTM Flex, the protocol will differ slightly in reading the standards and samples, and the respective tubes utilised, but the same working solution can be made.

Materials

Equipment:

	Equipment	Supplier	Catalogue Number
	Qubit TM 4 Fluorometer	ThermoFisher Scientific	Q33226
	Vortex		
	Microcentrifuge		

Consumables and Reagents:

	Consumables/Reagent	Supplier	Catalogue Number
	Qubit TM dsDNA Quantification Assay	ThermoFisher Scientific	Q32851/Q32854
	Qubit TM Assay Tubes	ThermoFisher Scientific	Q32856



DNA Quantification using the Qubit Fluorometer

- 1 Remove the Qubit™ Standards from the fridge and allow to come to room temperature.

Note

The Qubit™ dsDNA HS Assay delivers optimal performance when all solutions are at room temperature ( 22 °C –  28 °C).

- 2 Prepare the Qubit™ working solution in a fresh tube by diluting the Qubit™ dsDNA HS Reagent 1:200 in Qubit™ dsDNA HS Buffer.

Note

Each standard tube requires  190 µL of Qubit™ working solution, and each sample tube requires  199 µL . Prepare sufficient Qubit™ working solution to accommodate all standards and samples.

Note

Qubit™ dsDNA HS Buffer and Qubit™ dsDNA HS Reagent can be stored at the room temperature but the Qubit™ dsDNA HS Reagent should be protected from light.

- 3 Set up two Qubit™  0.5 mL tubes for calibration, labelling them 'Standard #1' and 'Standard #2' on the lid.

Note

Avoid labelling the tubes on the side as this may interfere with the instrument readings.

- 4 Add  190 µL of Qubit™ working solution to each of the tubes used for standards.

5 Add  10 μL of each QubitTM standard to the appropriate tube.

Note

Ensure the QubitTM standards have been sufficiently vortexed before use.

6 Add  199 μL QubitTM working solution to the a new QubitTM sample tube and add  1 μL of your sample to the appropriate tube.

7 Vortex all QubitTM tubes for  00:00:03 , then spin them down. Allow all tubes to incubate at room temperature for  00:02:00 .

8 On the Home screen of the QubitTM Fluorometer select the dsDNA High Sensitivity as the assay type. Next, calibrate the instrument by reading the standards.

9 Insert the tube containing Standard #1 into the sample chamber, close the lid, and then press Read standard. When the reading is complete, remove Standard #1. The instrument displays the results on the Read standard screen.

10 Repeat the previous step but utilise Standard #2.

11 Once calibrated, any samples utilising the calibrated QubitTM working solution can be ran. Select the sample volume ( 1 μL) and units (ng/ μL). Insert a sample tube into the sample chamber, close the lid, and then press Read tube.

12 When the reading is complete (~  00:00:03) remove the sample tube. The instrument displays the results on the assay screen. The top value is the concentration of the original sample. The bottom value is the dilution concentration.

13 Repeat for any remaining samples.

Protocol references

This protocol utilises the QubitTM dsDNA HS Assay Kit User Guide supplied by ThermoFisher Scientific (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_dsDNA_HS_Assay_UG.pdf).