Sep 09, 2020

DNA quantification using the Quantus fluorometer

DOI
dx.doi.org/10.17504/protocols.io.7pzhmp6

Josh Quick[1]

[1]University of Birmingham

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Josh Quick
University of Birmingham

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.7pzhmp6

External link: https://doi.org/10.1016/j.remle.2020.05.007

Protocol Citation: Josh Quick 2020. DNA quantification using the Quantus fluorometer . **protocols.io** **https://dx.doi.org/10.17504/protocols.io.7pzhmp6**

Manuscript citation:
Cool K, Gaudreault NN, Morozov I, Trujillo JD, Meekins DA, McDowell C, Carossino M, Bold D, Kwon T, Balaraman V, Madden DW, Artiaga BL, Pogranichniy RM, Sosa GR, Henningson J, Wilson WC, Balasuriya UBR, García-Sastre A, Richt JA, Infection and transmission of SARS-CoV-2 and its alpha variant in pregnant white-tailed deer. bioRxiv doi: 10.1101/2021.08.15.456341

Protocol status: Working
We use this protocol and it's working

Created: September 26, 2019

Last Modified: September 09, 2020

Protocol Integer ID: 28121

Materials

STEP MATERIALS

QuantiFluor(R) ONE dsDNA System, 500rxn **Promega** **Catalog** #E4870

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1 Remove Lambda DNA 400 ng/µL standard from the freezer and leave on ice to thaw. Remove ONE dsDNA dye solution from the fridge and allow to come to room temperature.

QuantiFluor(R) ONE dsDNA System, 500rxn **Promega Catalog #**E4870

2 Set up two 0.5 mL tubes for the calibration and label them 'Blank' and 'Standard'

3 Add 200 µL ONE dsDNA Dye solution to each tube.

4 Mix the Lambda DNA standard 400 ng/µL standard by pipetting then add 1 µL to one of the standard tube.

5 Mix each sample vigorously by vortexing for 00:00:05 and pulse centrifuge to collect the liquid.

6 Allow both tubes to incubate at room temperature for 00:02:00 before proceeding.

7 Selection 'Calibrate' then 'ONE DNA' then place the blank sample in the reader then select 'Read Blank'. Now place the standard in the reader and select 'Read Std'.

8 Set up the required number of 0.5 mL tubes for the number of DNA samples to be quantified.

Note

Use only thin-wall, clear, 0.5mL PCR tubes such as Axygen #PCR-05-C

9 Label the tubes on the lids, avoid marking the sides of the tube as this could interfere with the sample reading.

10 Add 199 µL ONE dsDNA dye solution to each tube.

11 Add 1 µL of each user sample to the appropriate tube.

Note

Use a P2 pipette for highest accuracy.

12 Mix each sample vigorously by vortexing for 00:00:05 and pulse centrifuge to collect the liquid.

13 Allow all tubes to incubate at room temperature for 00:02:00 before proceeding.

14 On the Home screen of the Quantus Fluorometer, select `Protocol`, then select `ONE DNA` as the assay type.

Note

If you have already performed a calibration for the selected assay you can continue, there is no need to perform repeat calibrations when using ONE DNA pre diluted dye solution. If you want to use the previous calibration, skip to step 11. Otherwise, continue with step 9.

15 On the home screen navigate to 'Sample Volume' and set it to 1 µL then 'Units' and set it to ng/µL.

16 Load the first sample into the reader and close the lid. The sample concentration is automatically read when you close the lid.

17 Repeat step 16 until all samples have been read.

18 The value displayed on the screen is the dsDNA concentration in ng/µL, carefully record all results in a spreadsheet or laboratory notebook.