Version 2

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Qant-iT™ PicoGreen® dsDNA Quantification V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bftfjnjn

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ABSTRACT

The following protocol is intended for the quantification of double-stranded DNA using Quant-iT™PicoGreen® dsDNA Assay Kit (ThermoFisher). This protocol is a simplified and condensed version of the full protocol from the manufacturer. The procedure described here is for 96 reactions. If samples are run in duplicates, then this should allow quantifying 40 samples.

EXTERNAL LINK

https://www.thermofisher.com/order/catalog/product/P11496

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Angel, R., Claus, P., and Conrad, R. (2012). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. ISME J 6, 847–862. doi:10.1038/ismej.2011.141.

ATTACHMENTS

mp07581.pdf

MATERIALS

NAME	CATALOG #	VENDOR
Quant-iT™ PicoGreen™ dsDNA Assay Kit	P11496	Invitrogen - Thermo Fisher

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Quant-iT™ PicoGreen™ dsDNA Assay Kit	P11496	Invitrogen - Thermo Fisher

EQUIPMENT

NAME	CATALOG #	VENDOR
Multipette E3	4987000010	Eppendorf
Synergy 2	Synergy2	Agilent Technologies
96-well microtiter plate	265301	Thermo Fisher Scientific

SAFETY WARNINGS

Quant-iT™ PicoGreen® reagent is classified as Not Hazardous. Nevertheless, the user should always consult the MSDS accompanying any of the reagents and apparatus described in this protocol.

BEFORE STARTING

1. This protocol is optimised for measuring an entire 96-well plate. It assumes that 16 wells will be used for measuring the standards and the blank samples (in duplicates) and 80 wells will be used for measuring unknown DNA samples (typically in duplicates).
2. The protocol can be easily adjusted for a lower number of samples by reducing the volume of the working solutions of the reagents. Note though that enough TE should be retained for diluting the standard stock solution (490 or 680 µl), for potentially diluting the unknown samples, if their concentration is too high, and for accounting for pipetting errors. To fill the plate, 19.2 ml of TE is needed. So if only 40 wells are to be used for

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measuring unknown samples prepare about $50/96 \times 22 \approx 11.5$ ml of TE buffer.

3. The dynamic range of the assay is between 50 pg ml^{-1} to 1000 ng ml^{-1}. This translates into DNA sample concentrations of 0.05-5 ng μl^{-1} and 1-200 ng μl^{-1} in the low-range and high-range assays, respectively. Samples with higher DNA concentration need to be diluted (e.g. in DNase-free water or TE buffer).
4. Note that some compounds that can be present as DNA contaminations (e.g. salts, ethanol, detergents, proteins) are claimed by the manufacturer to not interfere with the measurement. Please refer to the full protocol for a list of these compounds and their effect on the measurement. Also, equimolar presence of ssDNA and RNA in the sample should have only minimal effect on the quantitation results.

Prepare reaction 53m

1 Take out all reagents from the fridge and bring them to room temperature. 20m
Take out the DNA samples from the freezer. DNA samples should be slowly thawed on ice.

Quant-iT™PicoGreen® dsDNA reagent is dissolved in dimethylsulfoxide (DMSO), which freezes below 19 °C. The reagent must be completely thawed before using it by bringing it to room temperature. After the reagent thawed, it is advisable to briefly vortex the tube to make sure it is adequately mixed and to spin it down in a centrifuge or a mini centrifuge.

Quant-iT™PicoGreen® dsDNA reagent is light sensitive and should be protected from light at all times.

Quant-iT™ PicoGreen™ dsDNA Assay Kit
by Invitrogen - Thermo Fisher
Catalog #: P11496

2 Prepare 22 ml 1X TE buffer by pipetting 1.1 ml of 20X TE buffer into 20.9 ml of nuclease-free water into a sterile and nuclease-free 50 ml tube. 2m
Mix by inverting the tube several times.

1.1 ml 20X TE buffer

20 ml nuclease-free water

3 **For high-range quantification:** 2m

Dilute the DNA-standard stock solution (λ DNA 100 ng μl^{-1}) to a final concentration of 2 ng μl^{-1} by mixing 10 µl λ DNA-standard stock solution with 490 µl 1X TE buffer.

10 µl λ DNA-standard stock solution

490 µl 1X TE buffer

For low-range quantification:

Prepare a 40-fold dilution of the 2 ng μl^{-1} DNA-standard work solution by mixing 5 µl of the 2 ng μl^{-1} DNA-standard work solution with 195 µl 1X TE buffer to yield a 0.05 ng μl^{-1} DNA-standard work solution.

5 µl diluted DNA-standard solution

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195 µl 1X TE buffer

4 If needed, prepare a dilution of each sample in 1X TE buffer so that the reading will be within the dynamic range.

It is advisable to run samples in duplicates for a more accurate quantification

5 2m
Prepare PicoGreen® work solution: 9950 µL 1X TE buffer + 50 µL PicoGreen® into a sterile and nucleic-acids free 50 ml tube. Mix and protect from light.

9950 µl 1X TE buffer

50 µl PicoGreen®

6 10m
Prepare the following standard mixture in the first two columns of the black, sterile, 96-well plate:

Assay version	Diluted DNA std. (µl)	1X TE buffer (µl)	Final DNA amount (ng)
High-range (1-200 ng µl-1)	100	0	200
Use 2 ng µl-1 standard	50	50	100
	10	90	20
	1	99	1
	0	100	0
Low-range (50 pg µl-1 - 5 ng µl-1)	100	0	5
Use 0.05 ng µl-1 standard	50	50	2.5
	10	90	0.5
	1	99	0.05
	0	100	0

96-well microtiter plate

Nunc 265301

black, flat bottom

7 5m
Pipette 99 µl of 1X TE buffer in the remaining wells.

99 µl 1X TE buffer

Tip: use a mechanical or electronic dispenser during this step and step no. 9 to speed up the work.

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Multipette E3

Eppendorf 4987000010

electronic dispenser

8 Pipette 1 µl of the unknown DNA samples in the remaining wells. 10m

1 µl of DNA sample

Use either a diluted sample in case the concentration is expected to be higher than the dynamic range limit or larger volume in case the concentration is expected to be below the detection limit.

9 Pipette 100 µL of the PicoGreen® work solution in each well, including the standard and unknown sample wells. 2m

100 µl PicoGreen work solution

10 Protect the 96-well plate from light and incubate for 2-5 min at room temperature. 5m

00:02:00

Measure samples 5m

11 Place the plate in a plate reader and measure the fluorescence according to the following parameters: 5m

Excitation	~480 nm
Emission	~520 nm
Integration time	40 s
Lag time	0 s
Gain	Optimal
Number of flashes	10
Calculated well	highest standard
Shaking	5 s

It is also possible to set the gain to a fixed value (e.g. 100). If the fluorescence values of the standard drop over time this could indicate damage to the reagents or the DNA standard.

Synergy 2

absorbance microplate reader

BioTek Synergy2

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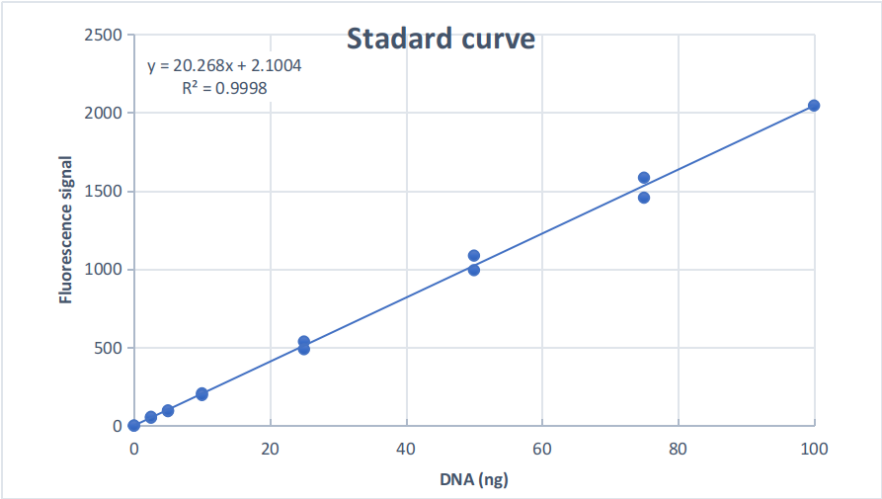
12 Plot the measured fluorescent values of the standard samples against their known concentrations and fit a linear curve using linear regression. Make sure that the coefficient of determination (R2) is close to 1 (typically > 0.99). Calculate the DNA concentrations in the unknown samples using the slope and intercept parameters of the linear equation. Output values you obtained are in ng μl^{-1}, assuming 1 µl of each sample was used.
See attached example spreadsheet. 10m



Do not forget to account for any dilutions when calculating the concentration of the DNA in the unknown samples.

PicoGreen_example.xlsx

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