Quanti-iT™ Pico Green dsDNA Assay (Invitrogen P7589)

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Working

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Earth Microbiome Project

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Ul-Hasan S, Bowers RM, Figueroa-Montiel A, Licea-Navarro AF, Beman JM, Woyke T, Nobile CJ (2019) Community ecology across bacteria, archaea and microbial eukaryotes in the sediment and seawater of coastal Puerto Nuevo, Baja California. PLoS ONE 14(2): e0212355. doi: 10.1371/journal.pone.0212355

GUIDELINES

DNA Standard	Vol. µl DNA	Vol. µl 1x TE	Vol µl PicoGreen (1:200)	Final DNA Standard ng/mL
Low DNA Standard	0	100	100	0
(1:1000 of 100 µg/ml)	1	99	100	0.5
	5	95	100	2.5
	10	90	100	5.0
	20	80	100	10
	50	50	100	25
	80	20	100	40
	100	0	100	50
High DNA Standard	0	100	100	0
(1:50 of 100 µg/ml)	1	99	100	10
	5	95	100	50
	10	90	100	100
	20	80	100	200
	50	50	100	500
	80	20	100	800
	100	0	100	1000

MATERIALS

NAME	CATALOG #	VENDOR
Quant-it™ PicoGreen® dsDNA Assay Kit	P7589	Life Technologies

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Quant-iT PicoGreen dsDNA kit	P7589	Thermo Scientific

BEFORE STARTING

Determine number of samples **and** standards to test in 96 well plate format. Multiply by 2 if running everything in duplicate for total number of wells.

Use a black-walled plate with black bottoms if possible. Black-sided wells with clear bottoms or white-sided wells will also work, but background will be higher due to reflected fluorescence in the wells. Do not use clear microtiter plates for fluorescence readings.

You will need 100 µl diluted PicoGreen reagent per well.

Total amount of 1X TE per assay will be 200 µl per well which includes the amount of TE used to dilute the PicoGreen reagent.

Pico Green dsDNA Assay

1 Warm Quant-iT PicoGreen reagent to room temp in the dark.

PicoGreen reagent is diluted in dimethylsulfoxide (DMSO) which solidifies at refrigerator temperatures. It must be completely liquified before use by allowing it to come to room temperature. Vortex solution briefly to mix well and centrifuge for 5 sec to bring liquid to bottom of tube; then dispense for use in the assay. PicoGreen reagent is also light-sensitive, so reagent should be protected from light.

Quant-iT PicoGreen dsDNA kit
by Thermo Scientific
Catalog #: P7589

2 Prepare 1XTE buffer from 20X stock solution using nuclease-free water: will need 200 µl/well (for diluting standards, samples and PicoGreen).

Prepare 1X TE by pipetting 2.5 mL of 20X stock TE into a sterile 50 mL centrifuge tube and filling to 50 mL mark with molecular biology grade water. Invert tube to mix.

3 Dilute DNA standard to either “High” 2 µg/mL (1:50 of λ DNA stock) or “Low” 50 ng/mL (1:1000 of λ DNA stock).

It is best to run standards in duplicate, and if amount of DNA in samples is unknown or varys widely, it is also best to run both the high and low DNA standards.

4 Determine amount of sample to assay (eg, 2µl sample in total of 100µl TE buffer). Add correct amount of TE buffer to all wells. Add standards to wells. Then add samples to wells.

See Guidelines for amount of DNA standards to add to standard wells.

5 Dilute PicoGreen 1:200 in TE buffer and protect from light until ready to add to plate.

A 1:200 dilution of PicoGreen reagent is prepared by adding 10 µl of PicoGreen per 2 mL of 1X TE buffer. You will need 100 ul diluted PicoGreen per well containing 100 ul sample.

6 Add equivalent volume (100 µl) of diluted PicoGreen to every well (keeping plate in the dark as much as possible).

7 Tap plate to mix.

8 Incubate 5 minutes at room temperature keeping plate in the dark.
00:05:00

9 Take fluorescent readings using 485nm excitation and 535nm emission filters.

10 Determine standard curve and calculate concentration of DNA in samples (see table in the guidelines).

COMMENTS

Debashish Banerjee Aug 23, 2016 06:59 PM

I am using the Picogreen Assay kit http://www.nature.com/protocolexchange/system/uploads/3551/original/Quant-iT_Picogreen_dsDNA.pdf?1423512049 I normally use a total reaction volume of 100 ml out of which 50 ml is 1:200 fold diluted Picogreen (as per manufacturer recommendation) and 50 ml is standard DNA or my sample.For the standard, the final concentration expressed in ng/ml is as per the manufacturer after diluting with TE buffer. My query is regarding the amount of DNA in my sample.I use a 5 ml of a sample (which has been pre-diluted already 10 times before being used) and obtain a DNA concentration ofe.g 800 ng/ml, the actual concentration of my sample would be= (800 ng/1000 ml)*20*10 = 160 ng/ ml (5 ml of sample in 100 ml of reaction volume and 10 fold

Bonnie Poulos Aug 23, 2016 08:17 PM
Matthew Sullivan Lab, University…

Debashish,

I am having trouble figuring out if your calculations are correct. You seem to be using milliliters (ml) when you mean microliters (ul). However, in the example you propose, 160 ng/ml would actually be 0.16 ng/ul (or 160 pg/ul) which means you would have to load 31.25 ul of your undiluted DNA in a gel lane to see a 50 ng band - is that what you did? I suggest two options to figure out whether your calculations are correct: one is to load the PicoGreen standard DNA in a gel using a range from 10 ng to 200 ng and then compare those bands to your DNA sample that you think should be at a certain amount. The other suggestion is to call the company that makes