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

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Abstract

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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

Before start

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.

Materials

Quant-it™ PicoGreen® dsDNA Assay Kit P7589 by Life Technologies

Protocol

Pico Green dsDNA Assay

Step 1.

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

REAGENTS

Quant-iT PicoGreen dsDNA kit P7589 by Thermo Scientific

NOTES

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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.

Pico Green dsDNA Assay

Step 2.

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

NOTES

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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.

Pico Green dsDNA Assay

Step 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).

NOTES

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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.

Pico Green dsDNA Assay

Step 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.

NOTES

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

Pico Green dsDNA Assay

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

NOTES

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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.

Pico Green dsDNA Assay

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

Pico Green dsDNA Assay

Step 7.
Tap plate to mix.

Pico Green dsDNA Assay

Step 8.
Incubate 5 minutes at room temperature keeping plate in the dark.

DURATION

00:05:00

Pico Green dsDNA Assay

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

Pico Green dsDNA Assay

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