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We use this protocol and it's working.

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DNA quantification with PicoGreen

Anna Székely[1]

[1]Uppsala Universitet

SEEC

Anna Székely

ABSTRACT

We use the Quant-iT™ PicoGreen® dsDNA kit for quantification of both extracted and amplified DNA.
The Quant-iT™ PicoGreen® dsDNA reagent is a proprietary, asymmetrical cyanine dye. Free dye does not fluoresce, but upon binding to dsDNA it exhibits a >1000-fold fluorescence enhancement. PicoGreen is 10,000-fold more sensitive than UV absorbance methods, and highly selective for dsDNA over ssDNA and RNA.
For moreinformation see:
https://www.thermofisher.com/order/catalog/product/P7589

GUIDELINES

IMPORTANT:

When quantifying non-amplified DNA (for example DNA extracted from filters), work in the prePCR area of the lab using the kit designated for prePCR.
When measuring amplified DNA (for example PCR products), work in the postPCR usinf the kit located there.

QUALITY ASSURANCE:

1. **Standards:** always measure your samples with appropiate standards. The concentration of all of your sample has to fall within the standard range.
2. **Replicates:** always measure both for your samples and for the standards at least 2 technical replicates.
3. **Kit properties:** Always write down the LOT number of the kit you use.

MATERIALS

MATERIALS

Quant-iT™ PicoGreen® dsDNA Assay Kit **Life Technologies Catalog #P7589**

BEFORE START INSTRUCTIONS

Take out PicoGreen (supplied in the PicoGreen kit) from fridge to thaw, before starting.
Protect it from light.

PicoGreen is solid at 4°C and thaws at room temperature. The reagent is light sensitive and should be kept wrapped in foil both while thawing and in the diluted stage.

1 Prepare TE

Calculate the total volume of 1xTE needed:

- 200 µl per each technical replicate of your samples
- 100 µl per standard
- Total of 3-400 µl for the DNA standard dilution (depending on the assay you use, see step 2)
- Make enough to count for pipetting losses!!!
- E. g. for high range: 20 samples + 2* 4 standards + DNA standard the amount is 20*200 + 8*100 + 300 = 5100 µl -> make 5500 µl

Dilute from 20xTE stock (supplied in the PicoGreen kit).

- Volume of 20xTE = final volume/20;
- Volume of water = final volume – volume of 20*TE
- E.g. To make 5 ml: mix 250 µl of 20*TE with 4750 µl of water.

2 Prepare DNA standard

Dilute 100 µg/mL **DNA standard** (supplied in the PicoGreen kit) to working dilution.

- For high range prepare 2 ng/µl: mix 6 µl of DNA stock with 294 µl of 1*TE
- For middle range prepare 0.5 ng/µl: mix 2 µl of DNA stock with 398 µl of 1*TE
- For high sensitivity prepare 0.05 ng/µl: mix 0.2 µl* of DNA stock with 399.8 µl of 1*TE

*As pipetting of 0.2 µl is not accurate with our pipettes, it is recommended to prepare first a 10-times dilution of the DNA standard and use 2 µl of that into 398 µl of 1*TE for the high sensitivity DNA standard dilution.

3 Prepare PicoGreen

Calculate the total volume of 1:200 diluted PicoGreen reagent needed.

- For each standard and each unknown sample, a volume of 100 µL will be needed = total number of samples (standard + unknowns) x100 µl + some for pipetting losses.
- e.g. For standard series of 8 and 20 samples (=28 total) prepare 28*100 µl =2800 µl: -> make 3000 µl

Dilute PicoGreen reagent with 1*TE.

- Volume of PicoGreen = final volume/200;
- Volume of TE = final volume – volume of PicoGreen
- e.g. Mix 15 µl of PicoGreen with 2985 µl of 1*TE

KEEP diluted PicoGreen IN THE DARK UNTIL USE!!!

4 Prepare plate - add standards

- Use **NUNC 96-well flat bottom black** plates. Cover with aluminium foil inside the lid.
- Mark samples needed to be measured on the lid and also diagram your plate layout in the table as follow (When possible, measure samples at least in duplicates).
- Plan the standard curve in the microtiter plate.

Table 1 High range standard curve: for 2ul samples, test limit is **0 to 100** ng/ul - for higher concentration dilute your samples.

	Plate Well	volume of TE (uL)	volume (uL) of **2ug/mL** stock DNA	volume of diluted picog dye (uL)	final DNA concentration in assay (ng/mL)	total DNA in well (ng)
	A1 & A2	0.00	100.00	100	1000	200
	B1 & B2	90.00	10.00	100	100	20
	C1 & C2	99.00	1.00	100	10	2
	D1 & D2	100.00	0	100	0	0

Table 2 Low range standard curve: for 2ul samples, test limit is **0 to 25** ng/ul

	Plate Well	volume of TE (uL)	volume (uL) of **0.5ug/mL** stock DNA	volume of diluted pg reagent (uL)	final DNA concentration in assay (ng/mL)	total DNA in well (ng)
	A1 & A2	0.00	100.00	100	250	50
	B1 & B2	90.00	10.00	100	25	5
	C1 & C2	99.00	1.00	100	2.5	0.5
	D1 & D2	100.00	0	100	0	0

Table 3 Low range standard curve: for 2ul samples, test limit is **0 to 2.5** ng/ul

	Plate Well	volume of TE (uL)	volume (uL) of **0.05ug/mL** stock DNA	volume of diluted pg reagent (uL)	final DNA concentration in assay (ng/mL)	total DNA in well (ng)
	A1 & A2	0.00	100.00	100	25	5

	B1 & B2	90.00	10.00	100	2.5	0.5
	C1 & C2	99.00	1.00	100	0.25	0.05
	D1 & D2	100.00	0	100	0	0

5 **Prepare plate - add TE**

- Add 1*TE to each standard well according to table 1-3.
- Add 98 µl of 1*TE to each sample well. Prepare 2 wells for each sample (duplicates).

6 **Prepare plate - add DNA samples**

- Add 2-5 µl of sample to each sample well.

7 **Prepare plate - add DNA standard**

- Add diluted DNA standard according to Table 1-3.

8 **Prepare plate - add PicoGreen**

- Add 100 µl of diluted PicoGreen to each of the wells (both sample and standard wells) and mix by pipetting 5-10 times.

9 Incubate in room temperature in dark for at least 5 minutes and measure with the plate reader (ask for help using the plate reader if using it for the first time).

00:05:00

10 To calculate your DNA concentration:

1. Create a calibration curve in excel or similar from the standards (x axis: measured fluorescens, y axis: total DNA in standard wells in ng from corresponding tables in step 4).
2. Use the equation of the fitted trendline (excel) to calculate the amount of DNA (ng) in your samples.
3. To calculate the DNA concentration of your samples (ng/µl), divide the calculated amount of DNA (ng) by the volume of DNA (µl) that you added in step 6 (2-5 µl).