**PicoGreen ds DNA Assay**

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Specifically quantitates low quantity ds DNA, as little as 50 pg DNA in 200 ul assay volume.

Complete protocol: <https://tools.thermofisher.com/content/sfs/manuals/mp07581.pdf>

Reagents

1. Quant-IT PicoGreen Assay from Molecular Probes, item #P7589 – includes 20X TE, PicoGreen dye, and Lambda DNA standard
2. 96 well assay plate, Costar item #3915 – black, flat bottom, polystyrene
3. BioTek FLx800 plate reader in Henikoff lab

Protocol

1. Turn on plate reader before setting up assay to allow the machine to warm up.
2. Determine how many samples will be read, including standards. 4 standards + blank + unknowns, all in duplicate. Reaction volumes will be 200ul per sample/well.
3. Prepare 1X TE from 20X stock using di DNase-free water. Will need 200ul 1X TE per sample, plus extra for diluting standard DNA.
4. Prepare a working stock of PicoGreen reagent. Allow PicoGreen stock to come to room temp before opening tube (stock is in DMSO). Dilute 200-fold into prepared 1X TE in plastic tubes (no glass). Will need 100 ul working stock of PicoGreen per sample. Cover with foil until needed, working solution should be used within a few hours of preparation.
5. Prepare working solution of standard DNA. Stock Lambda DNA provided is 100 ug/ml. Dilute to 2 ug/ml in 1X TE (6 ul 100 ug/ml DNA + 294 ul TE). Low range standards will need an additional dilution to 50 ng/ml (8 ul 2 ug/ml DNA + 312 ul TE).
6. Dispense appropriate volume of 1X TE to each well, then add DNA standards and unknown DNAs so that final volume in each well is 100 ul. **\*\*Important!\*\*** The Henikoff template on the plate reader is set up to use well A1 as the “PMT sensitivity” reference well, so this well must have the most DNA in it. Plan on using A1 for the highest DNA standard (S1).
7. Dispense 100 ul working solution of PicoGreen to each well, pipetting up and down several times.
8. Cover plate with foil and incubate at room temperature for 5 minutes. Read plate. (Plate can be read up to an hour after adding the PicoGreen, and possibly longer)

**High-Range Standard Curve**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Volume of TE (ul) | Volume of 2 ug/ml DNA | Total DNA in well (ng) |
| S1 | 0 | 100 | 200 |
| S2 | 90 | 10 | 20 |
| S3 | 99 | 1 | 2 |
| S4 | 99.9 | 0.1 | 0.2 |
| B | 100 | 0 | 0 |

**Low-Range Standard Curve**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Volume of TE (ul) | Volume of 50 ng/ml DNA | Total DNA in well (ng) |
| S1 | 0 | 100 | 5 |
| S2 | 90 | 10 | 0.5 |
| S3 | 99 | 1 | 0.05 |
| S4 | 99.9 | 0.1 | 0.005 |
| B | 100 | 0 | 0 |

Read Plate

1. Open software “Gen5 1.11” on computer to the left of the plate reader.
2. Click “Expt”, then “P.G. Auto ‘Henikoff\_Picogreen\_Auto’” template.
3. Click the left arrow icon at the top, which is the “read plate” button.
4. Click “READ”. Next, insert your plate into the plate reader when it tells you to do so (there is a metal clip on the bottom right that the plate inserts underneath). Then click “OK”.
5. Plate will be read and readings will be displayed in a 96 well format. These values can be exported as an Excel file.

Notes

Henikoff lab says that they have tried washing and reusing the plates, but are not able to wash them out thoroughly enough. The same plate can be used in a different assay, but new wells must be used. (Can wash out well A1 and reload a duplicate high DNA sample in this well since it will still be used as a reference well)