Thermo SCIENTIFIC

PROTOCOL ND-3300

# PicoGreen® Assay for dsDNA

## Introduction

The PicoGreen® dye is a fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA). Used in conjunction with the micro-volume capability of the Thermo Scientific NanoDrop 3300 Fluorospectrometer, the PicoGreen® assay provides a highly sensitive means of dsDNA quantitation with minimal consumption of sample. The main disadvantage of general UV spectroscopy for dsDNA quantitation is the contribution of signal from single-stranded DNA (ssDNA) and other contaminants, such as protein and extraction buffers. PicoGreen® reagent circumvents such contributions from interfering substances by exhibiting an emission maximum at 530nm when bound specifically to dsDNA (unbound PicoGreen® reagent exhibits minimal fluorescence in solution). The ability of the NanoDrop 3300 to measure as little as 1 ul of sample, allows significantly scaled-down reaction volumes, thereby using only a fraction of sample commonly needed for conventional cuvette-based fluorometers. The NanoDrop 3300 has demonstrated a detection range for dsDNA bound with PicoGreen® reagent of 1 ng/ml – 1000 ng/ml, and has proven to be 75 times more sensitive than using the Hoechst 33258 dye with this system. Readings taken at the lowest detection limit consume only 2 picograms of dsDNA per measurement. The following protocol is an adaptation of the Molecular Probes™ dsDNA Quantitation Reagent and Kits product information sheet.

## PicoGreen® Assay Supplies

Equipment:

- NanoDrop 3300 Fluorospectrometer
- 2uL pipettor (low retention nuclease free pipette tips)

Materials:

- Low lint laboratory wipes
- Nuclease free amber or foil covered 1.5 ml polypropylene tubes

Reagents:

- PicoGreen® dsDNA reagent kit (Molecular Probes catalog # P-11496)
- Nuclease free $dH_2O$

## PicoGreen® Assay Protocol

This protocol is written to accommodate small volume reactions (10 ul total volume per tube.) ranging from 10 ul and 200 ul total reaction volumes and have been shown to yield comparable results.

1. Prepare 1X TE with nuclease free $dH_2O$. The volume needed depends on total number of samples to be measured and the volume of PicoGreen® working solution needed.

2. Dilute the concentrated PicoGreen® Dye stock two-hundred (200) fold.

   *Example*: For the PicoGreen® working solution, dilute the PicoGreen® stock (200X concentrate) by mixing the dye thoroughly and transferring 995 ul of 1X TE and 5ul of the dye stock to a 1.5 ml amber snap cap tube. Mix well and protect from light. *(Prepare fresh as the diluted PicoGreen® dye is stable for only a few hours).*

3. Thaw the standard and unknown dsDNA samples. Once thawed, mix each individual solution gently but thoroughly.

4. Prepare serially diluted dsDNA standards at 2X the final concentrations in nuclease free vials or tubes (please refer to the example standard curve dilution series on page 2).

5. Transfer one volume of the diluted dsDNA standards into labeled nuclease free amber or foil covered tubes.

6. Aliquot an equal volume of diluted unknown dsDNA samples into appropriately labeled nuclease free amber tubes.

   Note: It is recommended that dilutions of unknown dsDNA samples be performed in 1X TE at an estimated concentration that will likely fall within the standard curve.

7. Transfer an equal volume of the PicoGreen® working solution to each amber tube containing either the 2X standard dsDNA solution or unknown sample.

8. Prepare the or Reference solution (negative control) by adding equal volumes of 1X TE and PicoGreen® working solution.

9. Mix each standard dilution and unknown sample thoroughly and allow to equilibrate at room temperature for five minutes.

10. Proceed to the NanoDrop 3300 standard curve protocol.

## Standard Curve Protocol

1. Clean both sampling pedestals with 2 uL of nuclease free deionized water.

2. Open upper arm and firmly blot the two pedestals with a dry lab wipe. Make sure there are no traces of lint on the pedestals before continuing.

3. Open the operating software. Click on the Nucleic Acid Quantitation button and select the PicoGreen method.

4. Add 2 uL of assay buffer (no dye, no sample) to the lower pedestal. Lower the arm and click F3 or the Blank button. When the measurement is complete, lift the arm and use a dry laboratory wipe to blot the buffer from both the bottom and upper measurement surfaces. Use a fresh aliquot of buffer to verify a proper baseline.

5. Under Measurement type, click on the Standards tab. Highlight the Reference standard.

6. Mix the reference solution (assay buffer and dye, no sample) briefly and transfer 2 uL of the solution onto the lower pedestal. Lower the arm and click F1 or the Measure button. A pop up window will ask for confirmation of the units. (Recommended ng/mL or pg/uL)

7. Measure up to 5 replicates of the reference solution using a fresh 2 uL aliquot for each measurement.

8. Select Standard 1 to enter a value. Enter values for up to 7 standards.

9. Mix the standard solution briefly and transfer 2 uL onto the lower pedestal. Lower the arm and click F1 or the Measure button. Measure up to 5 replicates of each standard using a fresh 2 uL aliquot for each measurement.

10. Once the standard curve is completed, select the Standard Curve Type (Interpolation, Linear, 2° polynomial, 3° polynomial) that best fits the standards data set.

11. Click on the Sample tab under Measurement Type, and enter the unknown samples' respective ID information. If a dilution of the unknown sample was made, enter the dilution factor in the box below the sample ID window.

12. Add 2 ul of the sample and use the F1 key or click the Measure button to initiate the measurement cycle. Use a fresh aliquot of sample for each measurement.

Example of standard curve dilution series.

| Stock dsDNA (ng/ml | 2X dsDNA volume (ul) | 1X TE buffer volume (ul) | 2X dsDNA standard (ng/ml) | Final dsDNA (ng/ml) |
|---|---|---|---|---|
| 2000 | 10* | 0 | 2000 | **1000** |
| 2000 | 10** | 10 | 1000 | **500** |
| 1000 | 4 | 16 | 200 | **100** |
| 200 | 5 | 15 | 50 | **25** |
| 50 | 8 | 12 | 20 | **10** |
| 20 | 10 | 10 | 10 | **5** |
| 10 | 4 | 16 | 2 | **1** |
| 0 | 0 | 10 | 0 | **0** |

**Initial stock undiluted* *** Initial stock diluted 1:1*

- The final concentration of the 1:1 dilution of PicoGreen® working solution is shown in the last column the table 1.
- The software will generate standard curves using between 1 to 7 standard concentrations along with the reference solution.
- When determining dsDNA concentrations near the lower detection limit, use a higher density of standards at the lower end.
- Excess volume of the 2x standards are prepared to facilitate the easy transfer of 5 ul into amber tubes during step 7 on page 1.

Example spectrum of a PicoGreen-dsDNA sample