Thermo SCIENTIFIC

PERFORMANCE DATA NanoDrop 3300

PicoGreen® Assay
Linearity, Reproducibility and Sensitivity

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 the Thermo Scientific NanoDrop™ 3300 Fluorospectrometer, the PicoGreen® assay provides a highly sensitive means of dsDNA quantitation with minimal consumption of sample. 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 pg/ul – 1000 pg/ul, and has proven to be 75 times more sensitive then using the Hoechst 33258 dye with this system. Readings taken at the lowest detection limit consume only 2 picograms of dsDNA per measurement.

Method

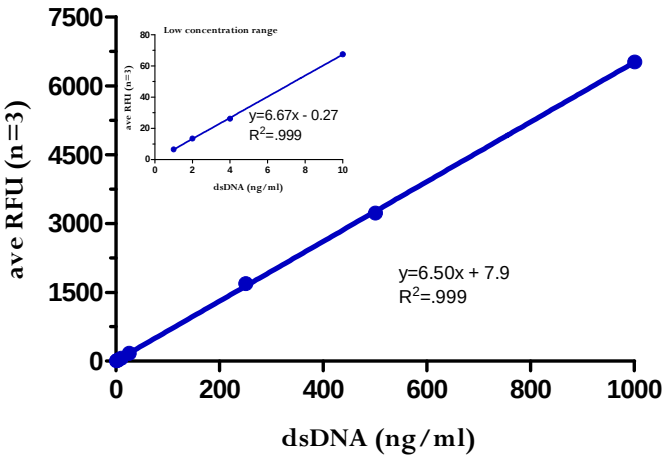
Testing was conducted with several prototype microfluorometers according to the Molecular Probes PicoGreen® Assay protocol. The following data was generated by a single instrument and is representative of the data obtained by all prototypes. Excitation was at 470nm with emission monitored at 525nm.

1. Concentrated PicoGreen® dye stock was diluted two-hundred (200) fold with 1X TE.
2. Serially diluted dsDNA standards were prepared at 2x concentrations in nuclease free vials.
3. A negative control (0 pg/ul) or Reference solution was prepared using equal volumes of 1X TE and PicoGreen® working solution.
4. Diluted dsDNA standards and diluted unknown dsDNA samples were transferred into clean amber tubes and mixed with an equal volume of the PicoGreen® working solution.
5. The solutions were mixed thoroughly and allowed to equilibrate for 5 minutes at room temperature.
6. Standard curves were generated and sample concentrations determined using 1.5 ul volumes on the NanoDrop 3300.

Results

Picograms of dsDNA per assay	dsDNA (ng/ml)	Ave RFU (n=3)	Stdev	%CV
2	1	6.6	1.6	13.5
4	2	13.5	0.9	4.8
8	4	26.3	1.0	3.0
20	10	67.5	3.1	4.3
50	25	173.7	4.6	2.6
500	250	1693.7	95.3	5.6
1000	500	3225.0	64.1	2.0
2000	1000	6520.1	332.5	5.2

PicoGreen Linearity on the NanoDrop 3300



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