

Hoechst Assay Linearity, Reproducibility and Sensitivity

Introduction

Hoechst 33258 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 Hoechst 33258 dye provides dsDNA quantitation with minimal consumption of sample. Hoechst 33258 dye circumvents contributions from interfering substances by exhibiting an emission maximum at 450nm when bound specifically to dsDNA (unbound Hoechst 33258 dye 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 Hoechst 33258 dye of 75pg/uL– 1500pg/uL.

Method

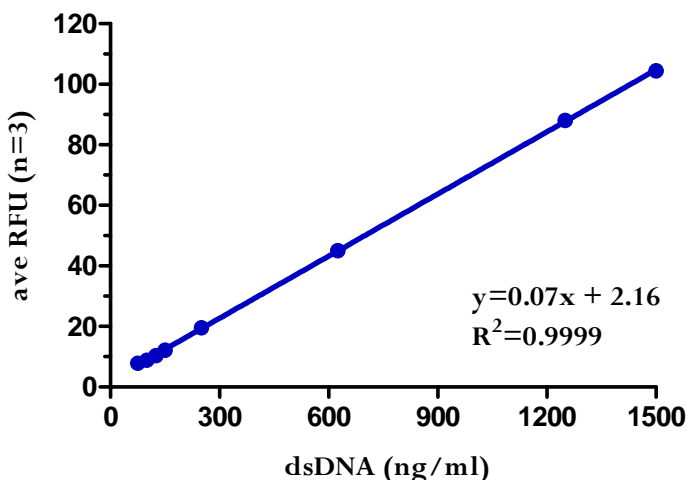
Serially diluted lambda DNA was measured using the Hoechst dye 33258 with the UV LED excitation source monitoring the emission wavelengths at 450 nm.

1. The Hoechst Dye stock (10 mg/mL) was diluted fifty-thousand (50,000) fold to obtain a 0.2 ug/mL solution in 1X TNE buffer.
2. Serially diluted dsDNA standards were prepared at 2x concentrations in nuclease free vials.
3. A negative control (0pg/ul) or Reference solution was prepared using equal volumes of 1X TNE and the 2X working Hoechst 33258 dye 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 0.2ug/ml Hoechst working solution (final concentration of the Hoechst dye will be 100ng/ml).
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.

Picograms of dsDNA per assay	dsDNA (ng/ml)	Ave RFU (n=5)	Stdev	%CV
150	75	7.8	0.46	5.89
200	100	8.8	0.46	5.22
250	125	10.3	0.38	3.66
300	150	12.1	0.25	2.05
500	250	19.5	0.3	1.57
1250	635	45.1	0.96	2.12
2500	1250	88.1	1.91	2.17
3000	1500	104.4	1.45	1.39

Results

Hoechst Dye 33258 Linearity on the NanoDrop 3300



Sensitivity is approximately 150 picograms in