

RiboGreen® Assay Linearity, Reproducibility and Sensitivity

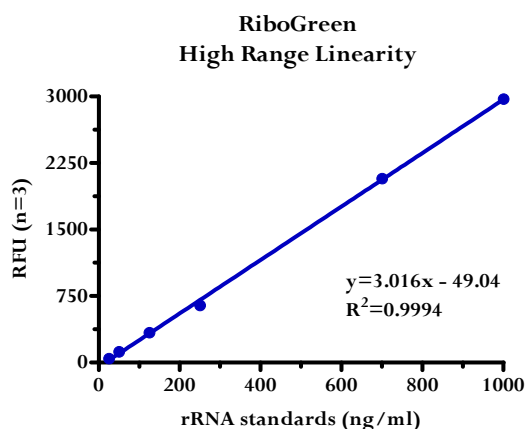
Introduction

The RiboGreen® dye is a fluorescent nucleic acid stain for quantitating intact RNA. Used in conjunction with the micro-volume capability of the Thermo Scientific NanoDrop 3300™ Fluorospectrometer, the RiboGreen® assay provides a highly sensitive means of RNA 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.

Method

Testing was conducted with several prototype microfluorimeters according to the Molecular Probes RiboGreen® Assay protocol. The following data was generated by a single instrument and is representative of the data obtained by all prototypes. The excitation was at 470nm with emission monitored at 525nm. Concentrated RiboGreen® dye stock was diluted in TE either two-hundred (200) fold (used for a High RNA quantitation range (25pg/ul–1000pg/ul) or two thousand fold (2000) (used for a Low RNA quantitation range (5pg/ul – 50pg/ul). Standard curves were generated and sample concentrations determined using 1.5 ul volumes on the NanoDrop 3300

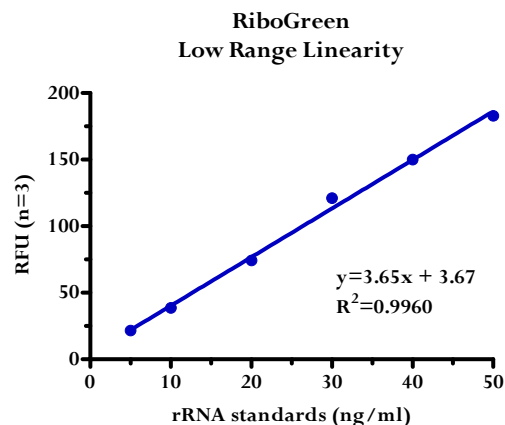
Results



High range

Picograms of rRNA per assay volume	rRNA standards (ng/ml)	Ave RFU (n=3)	Stdev	%CV
37.5	25	43.2	5.1	7.6
75	50	121.6	12.8	8.7
187.5	125	335.6	7.3	2
375	250	644.3	44.6	6.7
1050	700	2074.7	165.9	7.9
1500	1000	2971.6	125.7	4.2

Sensitivity is approximately 37.5 picograms or 25 ng/ml in this data set.



Low range

Picograms of rRNA per assay volume	rRNA standards (ng/ml)	Ave RFU (n=3)	Stdev	%CV
7.5	5	21.5	1.2	3.9
15	10	38.6	2.2	4.7
30	20	74.3	4.1	4.9
45	30	121.1	9.0	7.3
60	40	150.0	15.5	9.7
75	50	182.9	4.1	2.1

Sensitivity is approximately 7.5 picograms or 5 ng/ml in this data set.