

Quant-iT™ Protein Assay Linearity, Reproducibility and Sensitivity

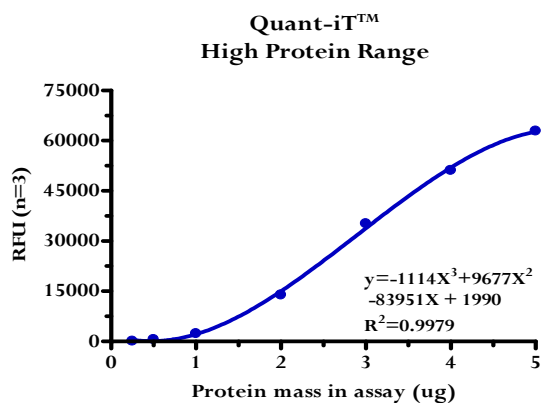
Introduction

The Quant-iT™ protein dye is a fluorescent stain for quantitating minute amounts of protein in solution. Used in conjunction with the micro-volume capability of the Thermo Scientific NanoDrop™ 3300 Fluorospectrometer, the Quant-iT™ protein dye provides a highly sensitive means of protein quantitation with minimal consumption of sample. The ability of the NanoDrop 3300 to measure as little as 2 ul of protein samples allows significantly scaled-down reaction volumes, thereby using only a fraction of sample commonly needed for conventional cuvette-based fluorometers.

Method

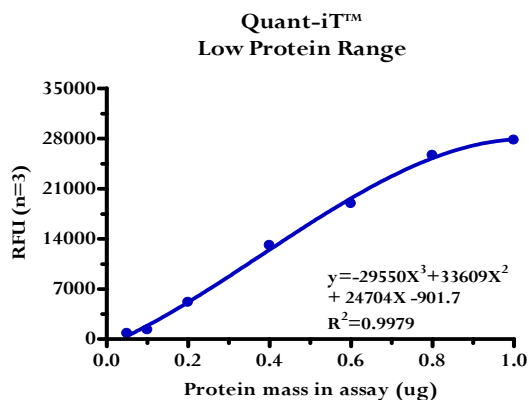
Testing was conducted with several prototype microfluorometers according to the Molecular Probes Quant-iT™ protocol. The following data was generated by a single instrument and is representative of the data obtained by all prototypes. The excitation was at 470nm with emission monitored at 590-610nm. The largest dynamic range for the assay can be obtained by utilizing two distinct working reagent concentrations. The high 0.25ug to 5ug range (total mass per measured 2ul volume) is covered by mixing 10 ul of protein sample with 20 ul of a twenty fold (20X) diluted working reagent. The 0.05ug - 1ug range (total mass per measured 2ul volume) is covered by mixing 2 ul of protein sample, 8 ul buffer and 20ul of an eighty fold (80X) diluted working reagent.

Results



High range

Micrograms of BSA per assay	Ave RFU (n=3)	Stdev	%CV
0.25	112.58	52.4	8.7
0.5	565.2	60.8	5.7
1	2372.4	104.0	3.6
2	13897.5	245.6	1.7
3	35218.2	410.9	1.2
4	51128.0	761.7	1.5
5	62897.8	1535.9	2.4



Low range

Micrograms of BSA per assay	Ave RFU (n=3)	Stdev	%CV
0.05	808.7	252.5	14.0
0.1	1290.2	332.9	14.6
0.2	5150.8	171.2	2.8
0.4	13068.4	392.8	2.8
0.6	18902.0	702.2	3.5
0.8	25652.3	663.9	2.5
1	27774.4	733.7	2.5