

Fluorescence based Protein Assays on the NanoDrop 3300 Fluorospectrometer

Fluorescent assays may be used for cell lysates and uncharacterized protein solutions where interfering substances prohibit UV spectroscopy or colorimetric methods for protein concentration determination. The table below is a quick and useful guide to the Thermo Scientific NanoDrop™ 3300 Fluorospectrometer software protein assay modules:

Method	Detection Range	Advantages of Method	Disadvantages of Method	Mode of Action	LED source/ EM wave-length
Fluorescamine	10-250 ug/ml	Smaller proteins and peptides Brief incubation time.	Buffers with primary amines like Tris or glycine should be avoided. Acylated proteins will not react. Protein-to-protein and peptide-to-peptide variation	Reacts with primary amines	UV/470
FluoroProfile (Eppicoccinone)	3-100 ug/ml	Reversible reaction for downstream processes like mass spec Low protein-to-protein variation	Heme containing proteins interfere. Buffers with primary amines like Tris or glycine should be avoided.	Covalent binding of protein	Blue/614
OPA, Flouraldahyde (O-phthaladahyde)	Low Range: 5-500 ug/ml High Range: 50-2000 ug/ml	Smaller proteins and peptides. Brief incubation time. Reducing agents, most detergents and metal chelators do not interfere. Broad linear range	Buffers with primary amines like Tris or glycine should be avoided. Acylated proteins will not react. Protein-to-protein and peptide-to-peptide variation	Reacts with primary amines	UV/455
Quant-iT Protein	Low Range: 5– 100 ug/ml High Range: 25-500 ug/ml	Low protein-to-protein variation	Buffers with detergents should be avoided	Binds detergent bound protein	Blue/600

Pre-formulated reagents, utilized in the above assays, are available in kit form from specific manufacturers. Please see the manufacturer's recommendations for the particular assay of interest. Protein standards, pre-diluted standards and protein purification products are available from Thermo Fisher Scientific at the following website: <http://www.piercenet.com>.