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:

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

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.