

Fluorometric protein quantitation in black microplates

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This application note describes the suitability of Thermo Scientific black and black clear bottom microplates for fluorometric protein quantitation. The measurements were performed with Thermo Scientific Fluoroskan Ascent and reagents from a commercial available kit.

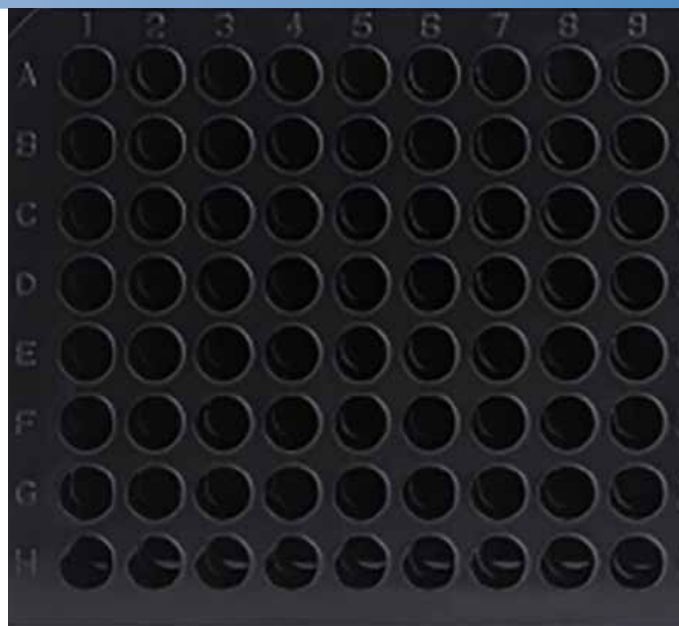
Introduction

Quantitation of proteins is a common analysis in a wide variety of biological applications. There are several commercial kits available for this purpose. In this application note we used Quant-iT Protein Assay Kit (Molecular Probes). The Quant-iT protein reagent is fluorescent when bound to a protein. To determine the concentration of a protein the fluorescence can be compared to a standard curve.

We tested the suitability of Thermo Scientific universal binding black 96-well plate and black clear bottom 96-well plate (tissue culture treated) for use with this kit. Also competitor's black universal binding plates were included in the testing.

Experimental

The assay was performed according to the manufacturer's instructions for use with the bovine serum albumin (BSA) standards that were included in the kit. The concentrations ranged from 0 to 500 ng μl^{-1} , which represented 0 to 5 $\mu\text{g well}^{-1}$. The fluorescence was measured using Thermo Scientific Fluoroskan Ascent with filter pair excitation 485 nm and emission 584 nm. The background fluorescence of the plates was measured in a similar way.



Results and discussion

The measured fluorescence intensity was plotted against the BSA mass (μg) (Fig. 1). The obtained standard curve had a sigmoidal shape. The standard curve was similar for all used microtiter plates. This indicates that all plates were suitable for use with Quant-iT Protein Assay Kit.

The signal to noise ratio of the measured fluorescence was calculated for Thermo Scientific black plates and black clear bottom plates. The results are shown for 1, 3 and 5 μg BSA (Fig. 2). The ratio varied from 4 to 32 for Thermo Scientific black clear bottom plates and from 8 to 69 for Thermo Scientific black plates. This illustrates that the black plate gave higher signals than the clear bottom plates. The clear bottom plates however are a good choice when combining protein quantitation with direct microscopic observation.

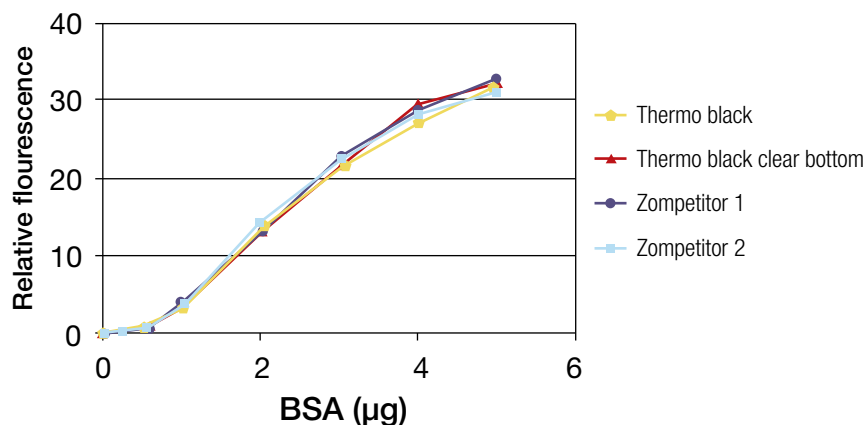


Figure 1. BSA standard curve with Quant-iT protein assay kit.

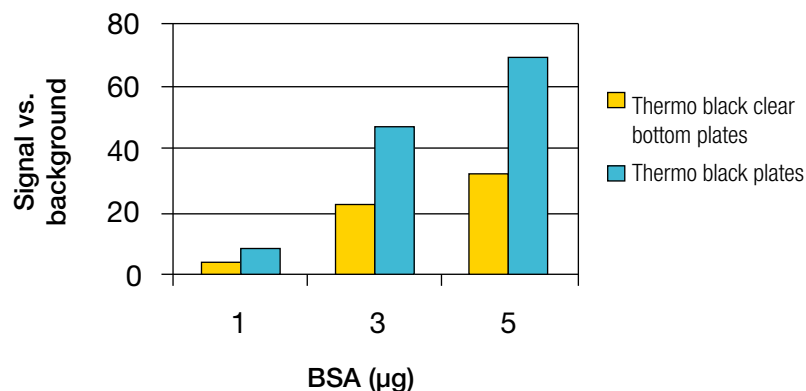


Figure 2. Signal vs. background values for Thermo Scientific black and black clear bottom plates for three different BSA quantities.

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