

# Chemi-luminescent Alkaline Phosphatase Detection in White Clear Bottom Microplates

Monica Kyyhkynen, M.Sc., Thermo Fisher Scientific

## Key Words

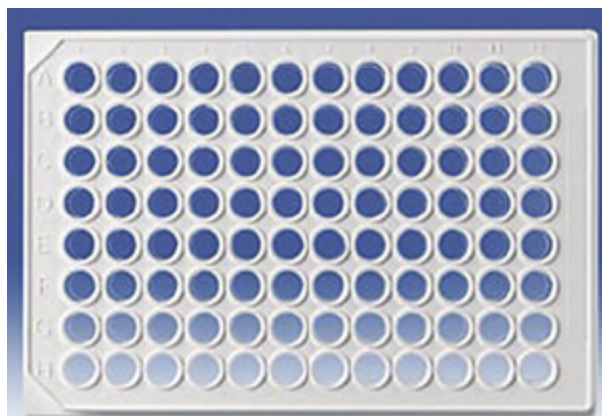
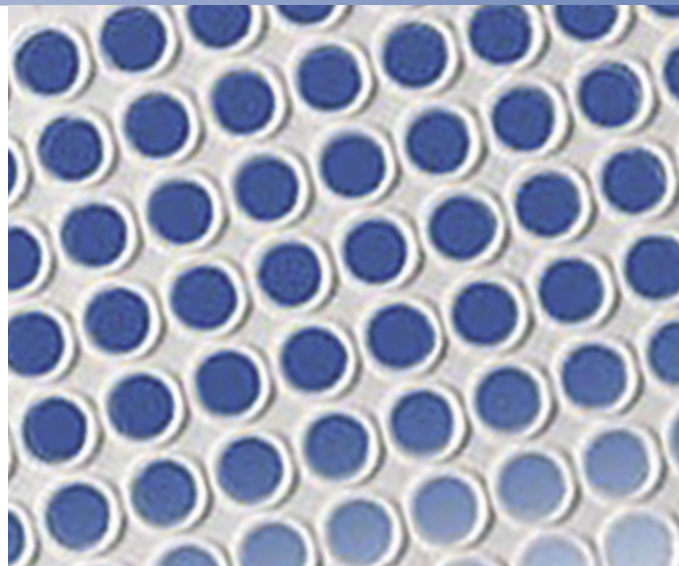
Thermo Scientific™ White Microtiter 96 Well Plate Universal Binding, chemiluminescent detection, alkaline phosphatase enzyme, microscopic viewing

## Goal

The purpose of this application note is to illustrate if the white clear bottom microplate is applicable in chemi-luminescent detection. This is shown using an opaque white plate as reference and comparing this with the white clear bottomed microplate.

## Introduction

BOLD APS™ 540 reagent is a ready-to-use formulation for the chemiluminescent detection of alkaline phosphatase enzyme in a microtiter plate or a cuvette. The reagent produces a luminescent signal that increases in intensity for a period of at least two hours when exposed to alkaline phosphatase enzyme. For luminescent reactions, when performed in microplates, opaque white plates are normally used. We wanted to show whether the white clear bottomed microplates could be applied to the determination.



Thermo White Clear Bottom 96 Plate

## Experimental

The Alkaline Phosphatase preparation used for the assay was Phosphatase, Alkaline from Bovine Calf; Sigma-Aldrich P7923-2KU, containing 23mg of protein and 4391 U/mg of protein. A suitable enzyme dilution ( $5 \times 10^{-5}$  U/well) was prepared and after adding the substrate the kinetic reaction producing chemiluminescent signal was monitored for a period of two hours. The curve achieved is shown in Fig. 1.

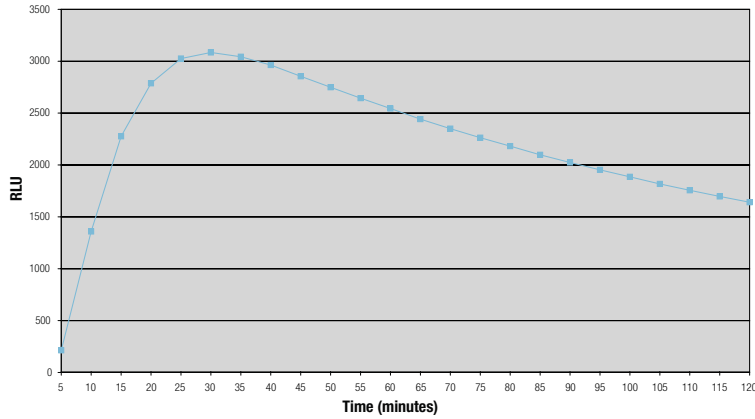


Fig. 1: Kinetics of enzyme reaction monitored in white clear bottom plate,  $5 \times 10^{-5}$  U of ALP per well

The test was repeated in a reference plate, a white opaque microplate (Thermo Scientific White Microtiter 96 Well Plate Universal Binding) to be able to compare the signals obtained in both the clear bottom plate and the opaque one. As expected, the signal obtained in the opaque white plate was somewhat higher than that produced in the white clear bottom plate, but the test seems to be well applicable to the latter one when needed (Fig. 2).

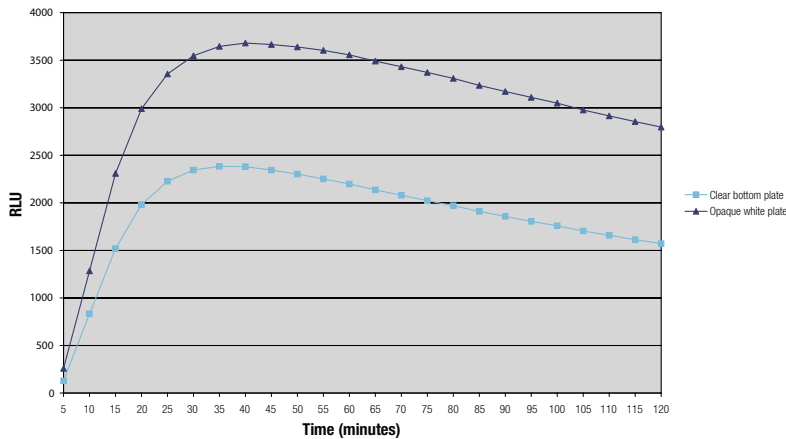


Fig. 2: Enzyme kinetics in white clear bottom plate compared to opaque white plate,  $5 \times 10^{-5}$  U of ALP per well

Signal versus background values for the plates were studied utilizing the BOLD APS 540 application. Three different time points during the kinetic reaction were chosen and the signals detected at those points were compared to the background luminescence level. The corresponding background luminescence for the plates was measured in a luminometer. Thus obtained signal versus background values were calculated and are illustrated in Fig. 3. Different dilutions of the enzyme were prepared and the one giving the best kinetic curve with signal high enough and yet in a measurable range was chosen. The amount of the enzyme preparation used was  $5 \times 10^{-5}$  U per well.

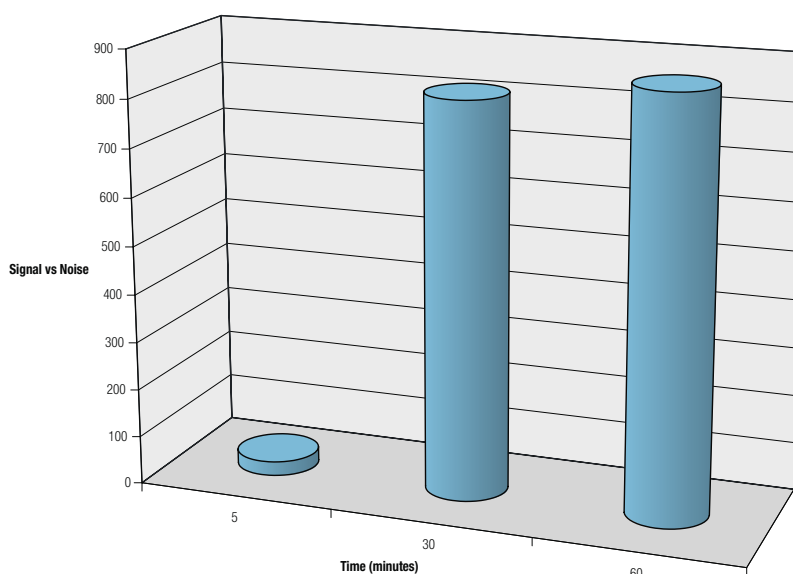


Fig. 3: Signal versus background values detected at different time points during kinetic enzyme reaction

## Discussion

The kinetic Alkaline Phosphatase determination BOLD APS 540 used in the experiment seems to be well applicable to the white clear bottomed microplates. Thus for example for applications where microscopic viewing through the bottoms of the wells needs to be combined with luminometric detection, these microplates offer a great choice.

[thermoscientific.com/diagnosticplates](http://thermoscientific.com/diagnosticplates)

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