

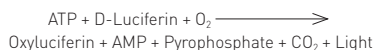
Promega ENLITEN® kit performed on a BMG LABTECH microplate reader

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- ENLITEN® kit performs as expected
- LOD = 0.81 fmol/well
- BMG LABTECH microplate reader is linear through-out kit range, $R^2 = 0.99595$

Introduction

The Promega ENLITEN® kit is used for the detection of ATP (adenosine 5'-triphosphate). The measurement of ATP is widely used to determine the amount of micro-organisms in food and it can be used in the quantitation of cells in tissues and serum. The ENLITEN® kit uses L/L (Luciferase/Luciferin) to rapidly quantitate ATP. The L/L catalyzes the 560 nm light-generating reaction shown below with ATP being the limiting reagent.



The purpose of this experiment is to determine the analytical limit of detection (LOD). Promega indicates that the kit will detect ATP down to 10^{-16} mol or 0.1 fmol. More information about Promega's ENLITEN® kit is available on their website at www.promega.com.

Materials & Methods

All materials were obtained through normal distribution channels from the manufacturer stated.

- Promega ENLITEN® kit
- Distilled water
- BMG LABTECH microplate reader with injectors
- Microplates, White 96 well, Costar

In addition, consumables such as pipette tips and micro-centrifuge tubes were used as needed from various manufacturers.

Experimental

All reagents were prepared and the assay run according to Promega's protocol. The most important aspect of this procedure is to make absolutely certain that there is no contamination of the samples. ATP is a common compound found on almost everything and great care must be taken when handling the kit. The ATP solution was pipetted into the plate using a serial dilution with concentrations ranging from 1000 fmol/well to 0.1 amol/well. L/L solution (100 µL) was injected into each well prior to measurement using the microplate reader built-in reagent injector. The injector was cleaned with a solution of 10% bleach that was allowed to sit in the injector and tubing for 30 minutes prior to use. The tubing was then rinsed with distilled water to remove the bleach solution.

The plates were inserted into the instruments and then read in luminescence mode using the following parameters:

BMG LABTECH reader parameters:

- Emission filter: Empty (depending on reader)
- Read Mode: Well
- Gain: 3300-3500 (depending on model)
- No. of Intervals: 20
- Integration Time: 0.5 s
- Position delay: 1 s

The average value of the blank measurement was subtracted from the duplicate measurements made at each concentration and the results plotted. A linear regression was performed on the standard curve to provide the calculated values that appear in Figure 1 and Figure 2.

Results & Discussion

The performance of the ATP kit was linear over a range of 100 fmol/well to 0.195 fmol/well. A linear regression was performed on the data, which yielded an R^2 value of 0.99859. Blanks were repeatedly measured ($n=20$) to determine the standard deviation of a well measurement. The standard deviation of the blanks were 138 RLU. Using these parameters the LOD, LOQ and R2 were calculated and are summarized in Table 1.

Table 1: Parameters.

Parameter	FLUOstar®	LUMIstar®
R^2	0.99589	0.99895
LOD	0.81 fmol/well	0.20 fmol/well
LOQ	2.70 fmol/well	0.67 fmol/well

The Technical Resource Manual that follows the kit states that this kit is designed to measure ATP from 10^{-11} to 10^{-16} mol. As shown by the data calculated the BMG LABTECH microplate reader performs within the stated range. However, BMG LABTECH microplate readers can read to much lower limits of detection, beyond the linearity of the Promega ENLITEN® ATP kit. The LOD for ATP using this ENLITEN® ATP kit is approximately 1 fmol per well for the FLUOstar/POLARstar series of instruments, entirely reasonable for a multifunctional microplate reader and certainly within the realm of general purpose laboratory use. These results were taken from one representative instrument and should be taken as single instrument



data only. They do not accurately reflect the reader as a whole but should give the user a reasonable expectation of performance. Results may or may not vary from instrument to instrument.

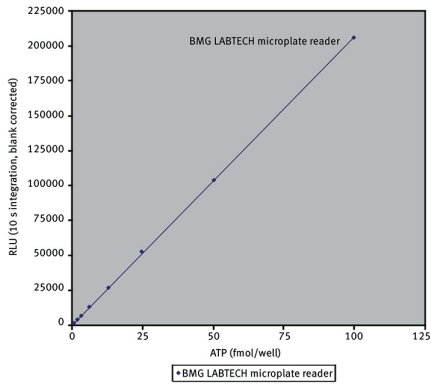


Fig. 1: Linearity data from 100 fmol/well to 0.195 fmol/well.

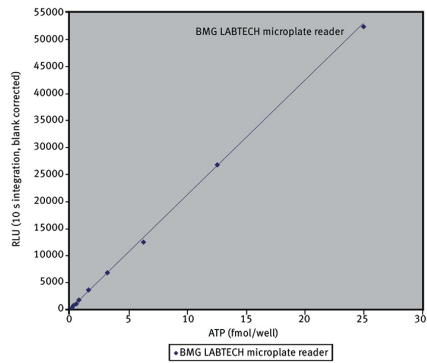


Fig. 2: Expanded range from Figure 1, 0-30 fmol/well.



PHERAstar® FSX

*The PHERAstar FSX is the newest PHERAstar reader.



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