

Promega ENLITEN[®] Kit Performed on the FLUOstar and LUMIstar



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Application Note 110

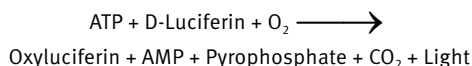
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- ENLITEN[®] kit performs as expected on both readers
- FLUOstar LOD = 0.81 fmol/well
- FLUOstar is linear through-out kit range, $R^2 = 0.99595$

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 microorganisms 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) of the FLUOstar in Luminescence mode. The LUMIstar is also used in this application note to show its performance with the kit. However, at the concentrations presented in this application note its sensitivity will not be challenged as the LUMIstar can detect ATP below the published linearity for this kit. Promega indicates that the kit will detect ATP down to 10^{-16} mol or 0.1 fmol. More information about Promegas' ENLITEN[®] kit is available on their website at www.promega.com. Refer to Technical Reference no. TMF005¹ for the protocol for the use of this product.

Materials

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

- Promega ENLITEN[®] kit, Promega Catalog no. FF2000
- Distilled water
- FLUOstar Galaxy, Serial no. 403-0760, BMG LABTECH, Offenburg Germany
- LUMIstar Galaxy, Serial no. 502-0027, BMG LABTECH, Offenburg Germany
- 3 mm Luminescence Optic for FLUOstar and LUMIstar, BMG LABTECH, Offenburg, Germany
- Microplates, White 96 well, Costar

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

Experimental

A complete description of this preparation for microplate assays can be found in the Promega technical reference no. TMF005. All reagents were prepared and the assay run according to this document.

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 μ L) was injected into each well prior to measurement using the microplate readers 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:

FLUOstar parameters:

- Emission filter: Empty
- Read Mode: Well
- Gain: HV-Model 1 (0-127): 110
HV-Model 2 (0-255): 202
- Optics: 3 mm Lumi Optic
- No. of Intervals: 20
- Integration Time: 0.5 s
- Position delay: 1 s

LUMIstar parameters:

- Read Mode: Well
- Gain: HV-Model 1 (0-127): 80
HV-Model 2 (0-255): 126
- Optics: 3mm Lumi Optic
- No. of Intervals: 20
- Integration Time: 0.5 s
- Position delay: 1 s

The data from the measurement was evaluated using the BMG LABTECH FLUOstar Excel[™] evaluation package. 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 and Discussion

The performance of the ATP kit was linear over a range of 100 fmol/well to 0.195 fmol/well for the LUMIstar and down to 3.125 fmol/well for the FLUOstar. A linear regression was performed on the data, which yielded an R^2 value of 0.99859 for the LUMIstar and 0.99595 for the FLUOstar. Blanks were repeatedly measured ($n=20$) to determine the standard deviation of a well measurement. The STDEV of the blanks were 138 RLUs for the LUMIstar and 405 RLUs for the FLUOstar. Using these parameters the LOD, LOQ and R^2 were calculated and are summarized in Table 1.

Table 1: FLUOstar and LUMIstar 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 FLUOstar and the LUMIstar perform within the stated range. However, the LUMIstar can read to much lower limits of detection, beyond the linearity of the Promega ENLITEN® ATP kit. The FLUOstar was used near its full potential and the results generated should adequately reflect the kits performance on both the FLUOstar and the POLARstar. 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 FLUOstar Microplate reader or LUMIstar Microplate reader as a whole but should give the user a reasonable expectation of performance. Results may or may not vary from instrument to instrument.

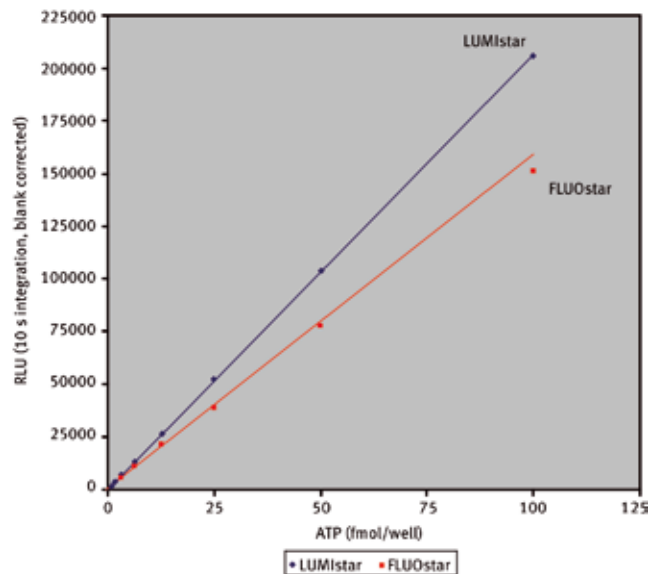


Fig. 1: FLUOstar and LUMIstar linearity data from 100 fmol/well to 0.195 fmol/well

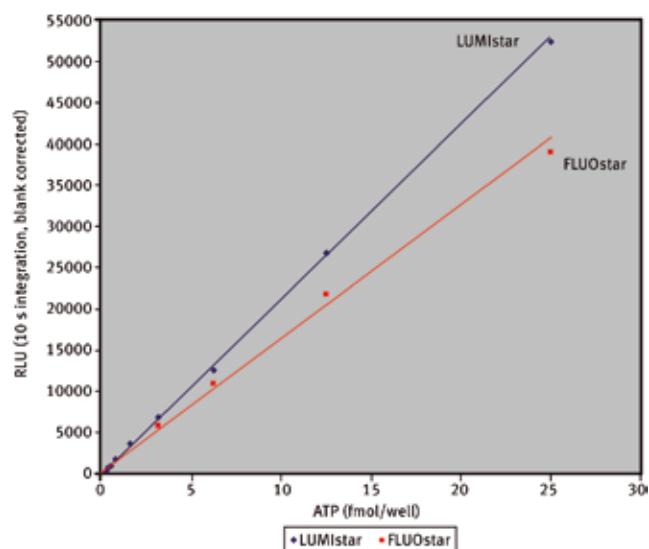


Fig. 2: Expanded range from Figure 1, 0-30 fmol/well

References

- Promega Technical Resource no. TMF005, ENLITEN® Luciferase/ Luciferin Reagent Bioluminescence Detection Reagent for ATP Measurement Technical Manual.

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