Labrox Application Note #010

Labrox® multimode plate reader:

Luminescent determination of ATP concentration with the ENLITEN® ATP Assay System using the Labrox reader

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Introduction

Adenosine triphosphate (ATP) is fundamental in cellular energetics, metabolic regulation and cellular signaling. Therefore, many different means to measure this important compound have been developed over time (1). The ENLITEN® ATP Assay Kit, which we used in these experiments, is developed for quantitative measurement of ATP levels. This assay uses recombinant luciferase (rL) to catalyze the reaction:

$$ATP + D\text{-}Luciferin + O2 \rightarrow Oxyluciferin + AMP + PPi + CO2 + Light\,(560\,nm)$$

The emitted light is proportional to the ATP concentration (2).

In this application note we demonstrate, by measuring a standard ATP standard curve with this kit, that the Labrox reader can accurately measure ATP concentration by luminescence.

Figure 1. Labrox plate reader with a dispenser.

Materials and methods

- ENLITEN® ATP Assay Kit (Promega)
- PerkinElmer OptiPlates (white)
- Micropipettes and sterile tips
- Tubes (ATP free)
- vortex
- Lonza AccuGENE™ Molecular Biology Water (cat. BE51200)
- Labrox Multimode Reader

Methods

All the reagents were prepared following the kit´s instructions. NOTE. Care was taken in order to prevent contamination by external ATP: gloves were used and all material (pipette tips, microfuge tubes, microplates) was sterile and ATP free. The dispenser was carefully disinfected by a series of washes with pure water and ethanol.

A dilution series of ATP was prepared (10^{-7} to 10^{-13} M). After preparation of the standard curve dilutions, 3 replicates of 10µl each were placed into the microplate and the plate inserted into the reader. Then 100 µl of rL/L reagent were added using the reader´s dispenser. After a 2 seconds delay luminescence was measured (10 seconds/well).

Measurement parameters:

1. Dispenser: 100 µl rL/L reagent
2. 2 seconds delay
3. LUM EndPoint top measurement:

    Z-Focus: 8,5
    No mirror
    Ems filter: Unfiltered LUM
    Ems spot size: 4
    Measurement time/ms: 10000

Sensitivity (LoD) calculation:

The LoD was calculated according to IUPAC standards:
LoD = 3* SD blank / slope standard curve.

Results

The results are shown in Table 1 and Figures 2 and 3 below.

Table 1. ATP concentrations, average of the 3 replicates luminescence readings, standard deviation (SD) and SD % are presented.

[ATP] M	Average	SD	SD %
10^{-13}	33039	155	0
10^{-12}	34712	1251	4
10^{-11}	38488	1039	3
10^{-10}	44935	2915	6
10^{-9}	108154	16695	15
10^{-8}	599435	72069	12
10^{-7}	5106251	127331	2

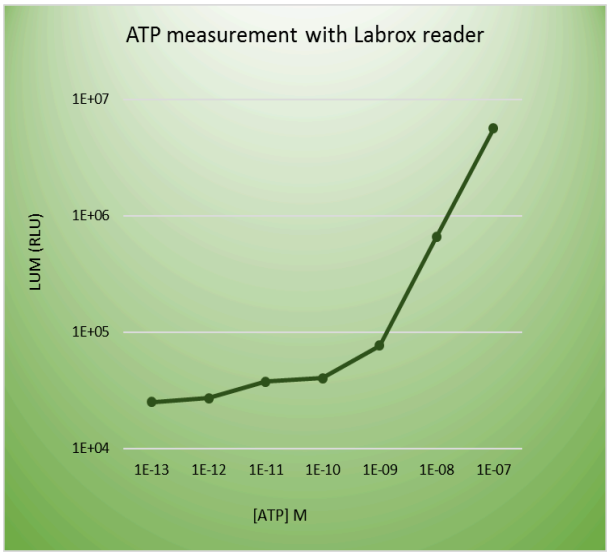
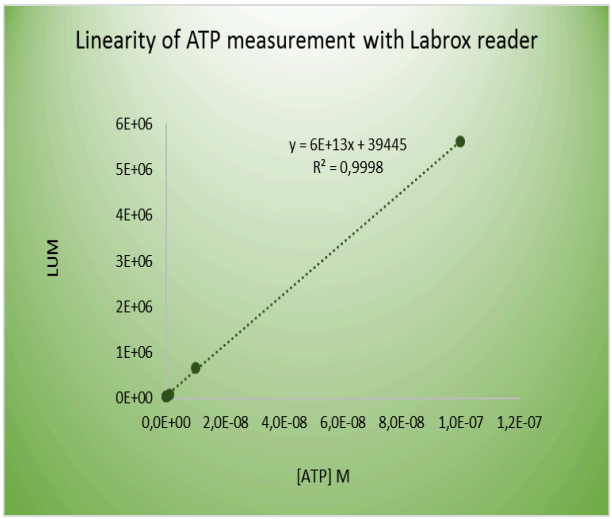


Figure 2. ATP standard curve.

Figure 3. Linearity of Luminescent response to ATP



Discussion

The measurements showed a high linearity relation between ATP concentration and luminescence output over a broad concentration range: 10^{-13} to 10^{-7} M (Figs. 1 and 2). Unknown samples can be interpolated with a high degree of confidence as the coefficient of correlation (R2) of a linear regression is 0,9998. The limit of detection was determined to be ≤ 1 pM.

Conclusions

The results presented in this application note clearly demonstrate that the Labrox reader is an ideal instrument to measure ATP levels using luminescence. The obtained standard curve presented a high linearity over several orders of magnitude of ATP concentration and with a detection limit of 1 pM the reader is able to detect very small amounts of ATP.

References

1. Kerr, SE and L. Daoud (1935). “A study of the Organic Acid-soluble Phosphorus of the Erythrocytes of Various Vertebrates”, J. Biol. Chem. 109:301.

2. Promega ENLITEN ATP Assay Manual

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AN#010 Ver. 01
11. 2016