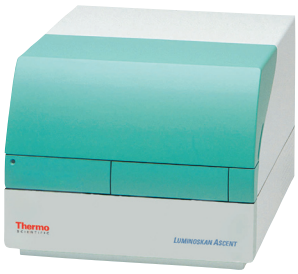
Application Note:
AP-MIB-LUMINO01-1007

Sensitivity of Thermo Scientific Luminoskan Ascent and Fluoroskan Ascent FL in flash type ATP assay

MIB Application laboratory, Thermo Fisher Scientific, Vantaa, Finland

Abstract

Moles ATP / well is a very common way to determine luminometer sensitivity. However, there are several different assays and assay types for measuring ATP. Especially the assay type; flash or glow, moves the system to a very different sensitivity range.
The theoretical sensitivity values for Thermo Scientific Luminoskan Ascent and Fluoroskan Ascent FL instruments were originally given based on a glow ATP assay. Recently, the performance test procedure was changed to utilize flash ATP assay, therefore the new and old sensitivity values differ remarkably.
The purpose of this note is to describe the difference and reason between the old and new luminometric sensitivity specifications of Thermo Scientific Luminoskan Ascent and Fluoroskan Ascent FL luminometers.



Introduction

The theoretical sensitivity values for an instrument can be reported in several different ways. The sensitivity values for Thermo Scientific Luminoskan Ascent and Fluoroskan Ascent FL have traditionally been determined using a glow type ATP assay and being < 1 and 5 fmol/well, respectively. There was a recent change in the performance test system and therefore the performance figures of of the Thremo Scientific luminometers Luminoskan ascent and Fluoroskan Ascent FL have also been changed. This does not mean that there has been a change in the instrument performance. So far the limiting factor has not been the instrument , but the chemistry performance. The diffrence between a flash and glow type ATP assay sensitivity values can be even several decades.
The current test method is a very sensitive ATP flash kit, which enables the detection of ATP on attomolar range.

Principle of the test

All living cells including microorganisms use ATP as their energy source. Therefore ATP is a good indicator for the presence of biomass. ATP is commonly detected by using luminescence with the luciferase reaction, after extracting ATP from the cells.
Checklite HS is a kit for microbial biomass assay based on measuring ATP.
The bioluminescence reagent of the kit contains firefly luciferin and luciferase.
Luciferase specifically reacts with ATP and catalyzes the reaction shown in Figure 1.
The amount of bioluminescence produced in the reaction is directly proportional to the amount of ATP in the sample. With this assay even 100 coliforms/ml (10 cell/assay) can be detected.

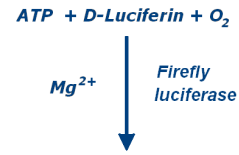


Figure 1. The principle of the assay

Materials and methods

Performance of the instruments was detected using the Checklite HS kit from Kikkoman Corp. (Code 61310). A serial ATP dilution series from 0.1 pM to 1 µM was used to produce the standard curve. The assays were performed on white Thermo Scientific Microlite 1+ 384 square well plates (Product number 8155) according to the kit instructions. In the assay procedure 30 µl of each ATP dilution was manually pipetted to the wells and 30 µl of luciferin-luciferase reagent was added by the dispenser. The luminescence of each well was measured after a lag time of 3 s. The integration time used was 4 s for both instruments.
The instruments used were Thermo Scientific Luminoskan Ascent (Product number 5300170) and Thermo Scientific Fluoroskan Ascent FL (product number 5210460). Both instruments were equipped with a dispenser.
The test was run with three units for both of the instrument types. The sensitivity and dynamic range values were calculated for each unit using the following formulas:

$$Theoretical\ sensitivity = \frac{2SD(blank) \times Conc(dilution\ 3 \times 10^{-13}\ amol/well)}{Signal(dilution\ 3 \times 10^{-13}\ amol/well))}$$

$$Dynamic\ Range = \log\left(\frac{Max}{2SD(blank)}\right)$$

where "Max" is the highest signal in the dilution series.

Results

The instrument performance was evaluated by creating a standard curve from the ATP dilutions and calculating the performance figures for all of the units tested for this note.
A typical standard curve for both of the instruments are presented on figures 2 and 3 and the performance data of all units is collected in tables 1 and 2.

There is a small difference between the two instruments with this test as in the previous glow type test, which was to be expected. The difference is on the same 4 to 5 fold range as in both flash and glow type assay (1 : 5 fmol/well and 9 : 33 amol/well)
The difference between instrument sensitivities can be seen also on the standard curves. The sandard curve of Luminoskan Ascent is linear for the whole teste range, whereas the curve of Fluoroskan Ascent FL starts to flatten on the lower dilutions.

When comparing these two instruments, it should be kept in mind that Luminoskan Ascent is a dedicated luminometer, and it has been optimized for the luminometry only. In Fluoroskan Ascent FL as in all multitechnology readers, the performance is always compromice of the technologies implemented
But as seen on the results, both of the instruments are well capable of performing the assay even on the low attomolar range.

Summary

The previously reported sensitivities of the Luminoskan Ascent and Fluoroskan Ascent FL are < 1 fmol/well and < 5 fmol/well, respectively.
The sensitivities of the instrument measured with the flash kit here were 9 amol/well and 16 amol/well. As seen from the results, the effect of the chemistry used to determine the instrument sensitivity may affet the numeric data dramatically. In this case, the difference is from femtomolar to attomolar range.
Comparing luminometric performance of different instruments is a complicated process. There are several ways to report the theoreticl sensitivity of an instrument: moles ATP per well is the most common, but moles luciferase and moles aequorin are also quite commonly used. The fact that there are several completely different types of assaysmakes the comparison even more difficult. Therefore the sensitivity of a luminometer should never be made by given performance spesifications only.

Table 1. Performance charasteristics of Fluoroskan Ascent FL. Mean is the average result of the three instruments

	Instrument 1	Instrument 2	Instrument 3	Mean
Theroretical sensitivity fmol/well (33 amol/well)	0.041	0.027	0.029	0.033
Dynamic range (decades)	5.8	6.0	6.0	5.9

Table 2. Performance charasteristics of Luminoskan Ascent. Mean is the average result of the three instruments

	Instrument 1	Instrument 2	Instrument 3	Mean
Theroretical sensitivity fmol/well (9 amol/well)	0.015	0.0072	0.0059	0.009
Dynamic range (decades)	6.2	6.6	6.6	6.5

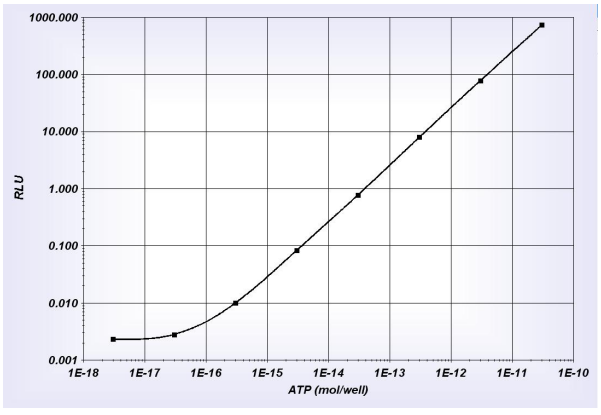


Figure 2. A typical standard curve of the assay with Fluoroskan Ascent FL

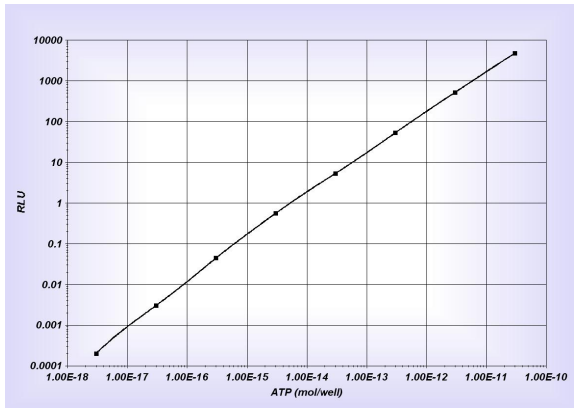


Figure 3. A typical standard curve of the assay with Luminoskan Ascent

North America:
USA/Canada
+1 866 984 3766
Europe: Austria
+43 1 801 40 0,
Belgium
+32 2 482 30 30,
Finland
+358 9 329 100,
France
+33 2 2803 2000,
Germany national toll free 08001-536 376,
Germany international
+49 6184 90 6940,
Italy +39 02 95059 1,
Netherlands
+31 76 571 4440,
Russia/CIS
+7 095 225 11 15,
Spain/Portugal
+34 93 223 3154,
Switzerland
+41 44 454 12 12,
UK/Ireland
+44 870 609 9203
Asia: China
+86 21 6865 4588 or
+86 10 5850 3588,
India
+91 22 5542 9494,
Japan
+81 45 453 9220,
Other Asian countries
+852 2885 4613
Countries not listed:
+49 6184 90 6940 or
+33 2 2803 2000

www.thermo.com