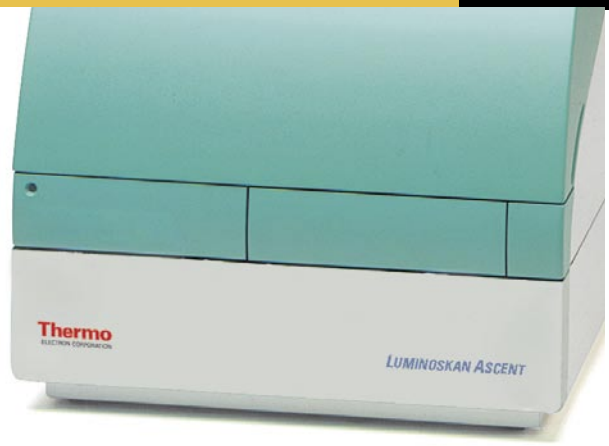

Ultrasensitive Luminometric ATP Assay with Luminoskan Ascent® and Flash Type ATP Chemistry

This note describes how a highly sensitive luminometric ATP assay is performed with the Thermo Electron Luminoskan Ascent microplate luminometer. The assay uses modern flash type chemistry, offering greater sensitivity than the common glow type ATP assay chemistry. Using this flash type ATP chemistry and an automatic dispenser, it is possible to measure ATP concentrations below 5 amol/well with the Luminoskan Ascent.

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Introduction

The luminometric ATP assay is the most common method for conducting quantitative ATP measurements. The assay is based on the following reaction:

ATP + D-Luciferin + O_2
luciferase
---------->
Oxyluciferin + AMP +
PP_i + CO_2 + LIGHT

When the ATP concentration is measured, D-luciferin and luciferase are added in saturated concentration from the assay reagent, and ATP is derived from the unknown sample. With this setting the amount of produced light is directly proportional to the ATP concentration. The assay is normally calibrated with known ATP concentrations, and the concentration of unknown samples is revealed based on the calibration curve using linear regression.

The main benefits of the luminometric ATP assay are a very wide dynamic range and high sensitivity. At present there are two common types of ATP assay kits on the market.

One type is based on stable, glow type light production where the reaction produces a constant light production signal over a long period of up to several hours. This is convenient when all reagents are added manually because the signal level can be measured at any time during this stable light production period. This stable reaction is normally achieved by adding inorganic pyrophosphate (PP_i) and L-luciferin to the assay reagent. Both compounds are inhibitors for the D-luciferin/luciferase reaction and this inhibition converts the light reaction kinetics to a stable, constant format. An important disadvantage of this glow type reaction is that reaction inhibition reduces the sensitivity of the assay.

An alternative assay type uses the ATP flash reaction where there are no inhibitors in the assay reagent. This natural form of D-luciferin/luciferase reaction produces instable flash kinetics where the signal maximum is reached in a few seconds after the reaction start, typically 0 to 1 s, and the signal level decreases at high speed. This assay type has much higher sensitivity than the glow type assay, but performing the assay is only possible with instrumentation that has automatic dispensers for reagent addition.

Material and Methods

Performance of the Luminoskan Ascent microtiter plate luminometer with the luminometric ATP assay using flash chemistry was detected using the ATP Biomass HS kit (product number 266-311) obtained from the Swedish company BioThema AB (http://www.biothema.com). An ATP standard (9.7 pmol/ml) was serially diluted 1:10 with ATP-free distilled water to produce an ATP standard curve over the concentration area of 1 to 100 000 amol/well with a 96-well microtiter plate and 0.3 to 30 000 amol/well with a 384-well microtiter plate.

With the 96-well microtiter plate, a 100 µl of different ATP concentrations together with distilled water blanks were added as replicates into the white Thermo Microtiter® 96-well strip plate (product number 95029510). Then the plate was placed into the Luminoskan Ascent luminometer with an automatic dispenser (product number 5300170). The Luminoskan Ascent was programmed to dispense 200 µl of D-luciferin/luciferase reagent and immediately integrate the luminescence signal over 7 seconds.

With the 384-well microtiter plate, 30 µl of ATP standards and blank were added into the white Thermo Microlite 1 plate (product number 8155) and 60 µl of D-luciferin/luciferase reagent was added with the automatic dispenser. The same 7-second integral measurement was also used with the 384-well plates.

When the assay was finished, the raw data results were transferred to Microsoft® Excel and all further calculations and graphics were made with Excel. Assay sensitivity was calculated from the ATP calibration curve by dissolving the point where the calibration curve crosses the signal level of the Blank well background signal + 3 * Standard deviation of the blank background.

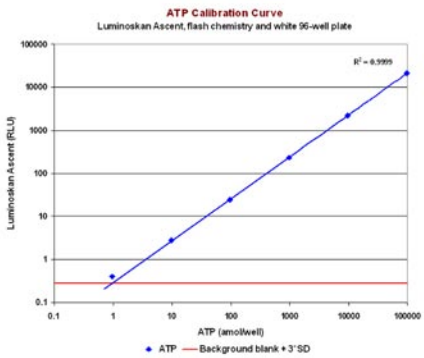
Results

The results of the 96-well microtiter plate assay are collected in Table I, and the ATP calibration curve is shown in Figure 1. The result values are signal integrals over the whole 7-second measurement period.

Table I

96-well microtiter plate Assay		
Concentration (amol/well)	Integrated signal (average, RLU)	Signal CV%
0	0.2277	7.2
0.97	0.3951	2.4
9.7	2.752	1.3
97	24.33	1.8
970	231.1	3.7
9700	2215	2.2
97000	21596	3.8
Assay sensitivity: 1.0 amol/well		

Figure 1.

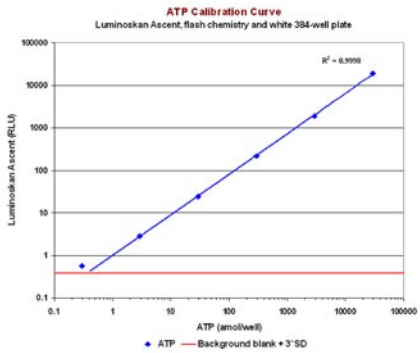


The results of the 384-well microtiter plate assay are collected in Table II, and the ATP calibration curve is shown in Figure 2. The result values are signal integrals over the whole 7-second measurement period.

Table II

384-well microtiter plate Assay		
Concentration (amol/well)	Integrated signal (average, RLU)	Signal CV%
0	0.3137	7.2
0.29	0.5709	7.4
2.9	2.857	4.3
29.1	23.88	5.5
291	218.2	3.8
2910	1899	3.2
29100	18886	2.9
Assay sensitivity: 0.4 amol/well		

Figure 2.



Conclusions

The sensitivity of the Luminoskan Ascent with ATP stable glow chemistry is <1 fmol/well. As seen from the results, much greater sensitivity is achieved when using ATP flash chemistry. With 96-well microtiter plates, assay sensitivity is clearly under 5 amol/well and even 1 amol/well concentrations can be measured. Even higher sensitivity is achieved with 384-well microtiter plates where the sensitivity of the ATP flash assay can be as low as 0.5 amol/well.

Based on these results, it can be concluded that it is distinctly possible to measure ATP concentrations reliably below 5 amol/well using the Luminoskan Ascent and the ATP assay kit based on flash chemistry.

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