Luminometric Sensitivity of the Thermo Scientific Appliskan™ multimode reader

This application note describes the luminometric performance of Thermo Scientific Appliskan, a filter-based multimode microplate reader. The luminometric sensitivity of the instrument was determined by an ATP (adenosine triphosphate) assay based on flash luminescence.

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Introduction

Luminescence is the emission of light at visible wavelength by a substance. Excitation light is not required because the excitation energy is caused by a chemical or biochemical reaction. Luminescence reactions can be divided into two types according to the duration of the light emission. In flash reactions the light emission decays rapidly lasting only for a couple of seconds. Glow luminescence, on the other hand, can last for hours because light decays very slowly.

ATP is the energy source of all living organisms. ATP assays can therefore be used to detect and quantify microorganisms present in various samples. The flash luminescence reaction of this ATP assay was based on the following reaction catalyzed by firefly luciferase:

$$\text{ATP} + \text{d-luciferin} + O_2 \xrightarrow[Mg^{2+}]{\text{luciferase}} \text{Oxyluciferin} + \text{AMP} + PP_i + CO_2 + \text{light}$$

The intensity of the emitted light is directly proportional to the concentration of ATP when ATP is the limiting component of the reaction.

Material and methods

The luminometric sensitivity of Appliskan was measured with the Kikkoman CheckLite™ HS Set (Kikkoman Corporation, Japan), a kit for highly sensitive microbial biomass assays. A dilution series of ATP (BioThema, Sweden) with a concentration range from 0.1 pM to 1 µM was used. Assays were performed on white Microlite 1+ 384 square well plates and Microlite 1 96-well 12-strip plates

(Thermo Fisher Scientific, Finland). With 384-well plates 30 µl of each ATP dilution was pipetted into the wells and 30 µl of luciferin-luciferase reagent of the kit was added with the instrument's dispenser. With 96-well plates 50 µl of ATP dilution and 50 µl of luciferin-luciferase reagent was used. In blank samples ATP was replaced by the reaction buffer. The flash luminescence of each well was measured instantly after the dispensing step before proceeding to the next well. The measurement time was 2000 ms and the high-sensitive mode of Appliskan was used (no filters, emission wavelength range 300-630 nm).

Results

For this note, the luminometric ATP sensitivity of eight different Appliskan units from two different production lots was compared with both 384 and 96-well plate formats. The theoretical sensitivity of the assay was calculated by the formula:

Theoretical sensitivity = (3 x SD(blank) / I (ref-blank)) x c(ref)

where "ref" was the lowest dilution from the midpart of the linear range with CV-% < 10 and "I" was the signal in relative luminescence units (RLU). The dynamic range was calculated by the formula:

Dynamic range = log (Max / (3 x SDblank))

where "Max" was the highest signal of the dilution series. With 384-well plates the average sensitivity of the eight instruments was 4.6 amol ATP/well and with 96-well plates it was 8.4 amol ATP/well. The sensitivities and dynamic ranges are presented in Table 1 and the linearity of the assay (with 384-well plates) is shown in Figure 1.

According to the technical specifications of Appliskan, the luminometric sensitivity with the high-sensitive measurement mode is < 10 amol ATP/well measured with 384-well plates, and the dynamic range > 5 decades. These specifications are always guaranteed for every Appliskan unit, but the typical sensitivity values are always under this upper limit as the natural variation of different instruments is considered when the instrument specifications are defined.

Table 1. The average of theoretical sensitivity and dynamic range of eight Appliskan units with both 384 and 96-well plates.

	Sensitivity (amol ATP /well), 384-well plate	Dynamic range (decades), 384-well plate	Sensitivity (amol ATP /well), 96-well plate	Dynamic range (decades), 96-well plate
Appliskan average	4.6	> 6.0	8.4	> 5.0

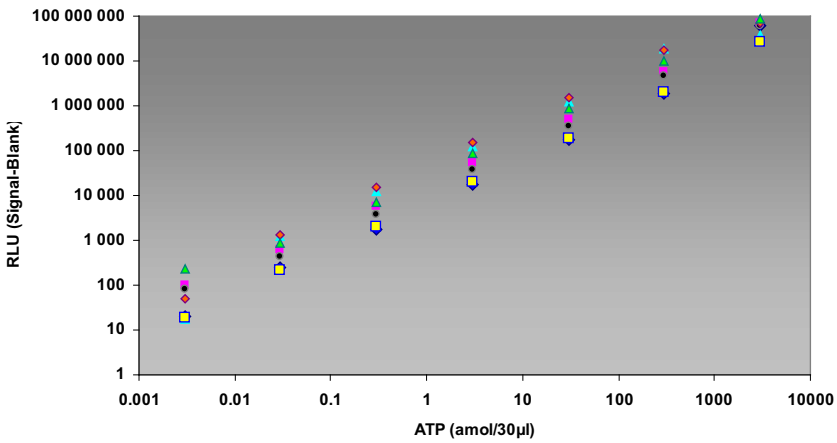

Figure 1. The linearity of the assay (with 384-well plates) of eight Appliskan units. (RLU = Relative Luminescence Unit)

Conclusions

Thermo Scientific Appliskan is well suitable for fast, flash-type luminometric measurements. The rapid detection of flash luminescence requires instrument dispensers for reagent addition. Appliskan can be equipped with up to two on-board dispensers, therefore enabling the best possible performance in flash-type measurements. The highly sensitive luminometry mode of Appliskan uses a dedicated photomultiplier tube with photon counting and specially designed optics to ensure excellent sensitivity and a wide dynamic range in all luminometric applications.

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