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Sirius L Tube Luminometer FB12/ Sirius Software V2.0

Below amol ATP detection limit with Sirius L and ATP Reagent SS from BioThema

The sensitivity of luminometers is often characterized by the detection limit for ATP. To get ideal results, the measurements on Sirius L with FB12/Sirius Software V 2.0 were performed with the ATP Reagent SS of BioThema.

Materials

Luminometer: Sirius L with 2 injectors
Software: FB12/Sirius Software V2; Single Kinetics Protocol
Reagents: ATP Reagent SS (BioThema Product No 13-101)
ATP Standard 100 nmol/L (BioThema Product No 47-051)
Diluent D (BioThema Product No 24-101).
Brand of tubes: Glass cuvettes from VWR (Product No 212-0301)

Methods

Preparation of ATP free cuvettes

In order to degrade the ATP contamination the cuvettes were heated at 275 °C for 4 hours carefully covered to avoid contamination from the air. The cuvettes were allowed to cool down before they were individually packed in Falcon tubes to avoid re-contamination.

Reagent preparation

ATP Reagent SS (BioThema Product No 13-101) was reconstituted in 10 mL Diluent D (BioThema Product No 24-101). ATP Standard 10 nmol/L was prepared by diluting 1 ml ATP Standard 100 nmol/L (BioThema Product No 47-051) in 9 mL Diluent D (BioThema Product No 24-101).

Instrument preparation

Injectors were carefully washed with water (10 mL), ethanol (10 mL) and water (10 mL) again. The reagent injector was primed with 4x250 µl ATP Reagent SS. The reagent was allowed to incubate in the injector tubings for 3 hours to degrade any remaining background (should degrade the ATP by a factor of $2x10^{-184}$). Before the start of the analysis the reagent injector was primed once more with 3x250 µl ATP Reagent SS, to remove the reagent that has been incubated in the tubings as this reagent may be partially inactivated. The ATP injector was primed with 4x250 µl 10 nmol/L ATP Standard.

Software Setup

Single Kinetics Protocol:

Measurement parameters:
→Delay time: 2.0s
→Total duration: 5min10.0s
→Sampling time: 10.0s
→No. of data points: 31

Injection parameters:
→Injector 1 delay: 182s
→Injector 1 volume: 200 µl
→Injector 2 delay: 242s
→Injector 2 volume: 20µl

Analytical procedure

Instrument blank (IB), cuvette blank (CB) and an ATP Standard curve were measured.
The instrument blank was measured ten times every 10 s during 5 min. Ten empty cuvettes were used to measure cuvette and reagent blanks. Light readings were collected every 10 s during 5 min according to the following procedure:

1. The cuvette is put in the measuring position and the measurement starts automatically. After 60 s delayed fluorescence has faded to zero and the cuvette blank is measured as the average of the readings in the interval 60-170 s.
2. After automatic injection of 200 µL ATP Reagent SS the light is measured in the interval 180-230 s. The second reading (SMP1 at 190 s) and the last reading (SMP2 at 230 s) are used in the calculations.
3. After automatic injection of 20 µL 10 nM ATP Standard (200,000 amol) the light was measured in the interval 240-300 s. The second reading (STD at 250 s) is used in the calculations.

A tenfold dilution series of ATP (five concentrations) was prepared from the 100 nmol/L ATP Standard in ATP free water. Pipette tips and all other disposables were ATP free. Each cuvette was supplied with 10 µL ATP (or 10 µL ATP free water for the assay blank). The measurement was performed in duplicates for each concentration. The measurements were performed as for the empty cuvettes except that the instrument blank was not measured and that no ATP Standard was injected.

Calculations

The instrument blank was 40.8±5.5 counts/s (average±standard deviation). It was expressed in amol (10^{-18} mol) of ATP by using the formula IB/(STD-SMP2)*200,000, where the STD and SMP2 values is the average of these measurements from the ten reagent blanks and where 200,000 is the number of amol from the ATP Standard.

The reagent blank expressed in amol of ATP was calculated as (SMP1-CB)/(STD-SMP2)*200,000, where 200,000 is the number of amol from the ATP Standard.

The light readings for the standard curve were corrected for the assay blank (10 µL ATP free water). Thereafter the logarithms of the light readings were plotted versus the logarithms of the ATP amount in Fig. 2.

Results

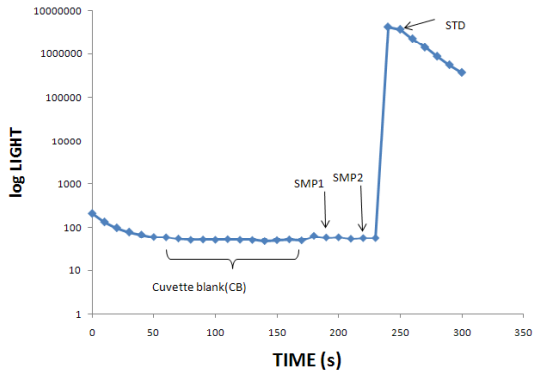

Fig. 1: Light intensity during measurement of an empty cuvette

Figure 1 shows that the delayed fluorescence from the cuvettes fade away in about 60 s. It also shows that the reagent blank is visible but very low. The first value after adding the ATP Standard is somewhat too low, most likely an effect of poor mixing (20 µL injected into 200 µL). Please note that thereafter the light decays as a first order reaction (a straight line is obtained in the semi-log plot).

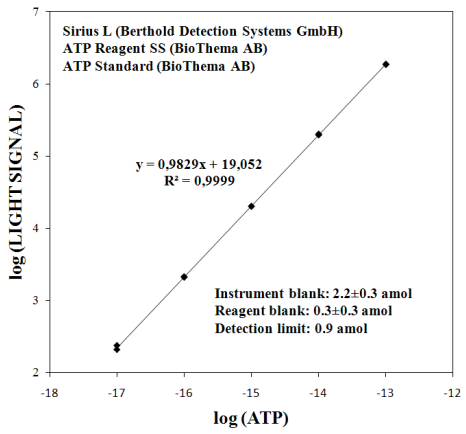

Fig.2: ATP Standard curve

Results in Figure 2 show that only with the lowest ATP level one can see a difference between duplicates. The figure also shows that the slope of the linear regression line is close to 1, which is required for a linear relationship in a log-log plot. The instrument blank is 2.2±0.3 amol (average±standard deviation) and the reagent blank is 0.3±0.3 amol. The constant portion of the instrument blank (2.2 amol) is not relevant from detection point of view as it is subtracted in the calculation of the reagent blank. Furthermore in many luminometers it can be electronically adjusted to zero. The detection limit of the instrument with ATP Reagent SS is therefore 3 times the standard deviation, i.e. 0.9 amol. The fact that we detect a reagent blank is most likely an artefact. ATP Reagent SS consumes its own background by one order of magnitude every minute.

Discussion

The detection of 1 amol ATP requires:

1. ATP free cuvettes
2. A sensitive ATP reagent that rapidly degrades ATP producing a peak of light
3. A sensitive luminometer with reagent injectors

The present study shows that all three requirements were achieved. ATP free glass cuvettes can be achieved by heating. ATP Reagent SS has a high luciferase activity resulting in a high light emission. Ninety percent of the ATP is consumed in 1 min, which also means that the reagent can be used to degrade contaminating ATP in the reagent injector. Sirius L is a very sensitive luminometer and it has the reagent injectors required for flash light ATP reagents.
The lack of mixing facilities must be remembered when deciding on volumes of sample, reagent and ATP standard.
The slight ATP contamination that we found with empty cuvettes (0.3±0.3 amol) may come from the glass cuvettes in spite of the heat treatment. Another possibility is that the reagent injector has a “hidden” source of ATP slowly leaking out its contents. Such a hidden source may be the sides of the plunger of the syringe or the valves in the injector. The fact that we had to incubate the reagent in the injector for 3 hours to degrade the ATP indicates that there are hidden sources of ATP in the injector.

Acknowledgement:

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Figures and text by courtesy of BioThema AB, Sweden

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