FOR RESEARCH USE ONLY

ATPlite 1step

Single Addition
Luminescence ATP Detection Assay System

DOMINIQUE DUTSCHER SAS

PerkinElmer®

For best results, see page 18 for product use recommendations.

Contents	Page
1. Introduction	3
2. Principle	5
3. Advantages of ATPlite™ 1step	9
4. Contents and storage of ATPlite 1step	10
5. Handling	12
6. Stability	12
7. Mixing	12
8. Additional requirements	13
9. ATPlite 1step assay procedure	14
10. ATP standard	16
11. Recommendations for use	18
12. Ordering information	20
13. References	21

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1. Introduction

ATPlite™ 1step is an **A**denosine **T**ri**P**hosphate (ATP) monitoring system based on firefly (*Photinus pyralis*) luciferase. This luminescence assay is an alternative to colorimetric, fluorometric and radioisotopic assays for the quantitative evaluation of proliferation and cytotoxicity of cultured mammalian cells. ATP monitoring can be used to assess the cytocidal, cytostatic and proliferative effects of a wide range of drugs, biological response modifiers and biological compounds [1,2,3,4,5,6].

ATPlite 1step is a true homogeneous high sensitivity ATP monitoring 1-step addition assay kit for the quantification of viable cells. The kit can be used for continuous process systems such as in-line systems in high throughput environments. These in-line systems do not require a long signal half-life since the time between addition of the reagent and reading the resulting luminescence is relatively short (minutes). The decrease of the luminescent light-output is approximately 15% after 30 minutes. This decrease is independent of cell numbers but may differ between cell type and medium. The maximum cell number that can be applied has been determined to be 50,000 cells per well for 96-well and 12,500 cells per well for 384-well microplates. Because the kit needs no stabilization of the luminescence signal, high throughput is preserved.

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The simplicity of the ATPlite 1step assay system in a 96-well microplate is illustrated in Figure 1.

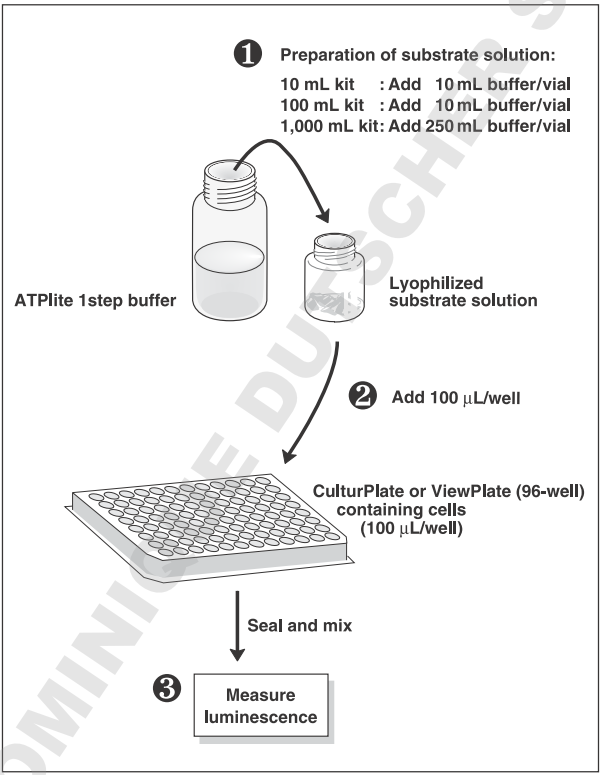


Figure 1: *ATPlite 1 step assay system.*

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2. Principle

ATP is a marker for cell viability because it is present in all metabolically active cells and the concentration declines very rapidly when the cells undergo necrosis or apoptosis. The ATPlite 1step assay system is based on the production of light caused by the reaction of ATP with added luciferase and D-luciferin. This is illustrated in the following reaction scheme:

$$\text{ATP} + \text{D-Luciferin} + O_2 \xrightarrow[Mg^{2+}]{\text{LUCIFERASE}} \text{Oxyluciferin} +$$

$$\text{AMP} + PP_i + CO_2 + \text{Light}$$

The emitted light is proportional to the ATP concentration within certain limits.

The kit has been formulated in such a way that within a measuring window of 0 to 30 minutes, a very bright signal will be obtained, when using up to the maximum cell number per well. Within this measuring window the decrease of the luminescent signal in time is independent of the cell number per well.
To show that there is a linear correlation between the luminescence signal and the cell number the following experiment was performed. Serial 2-fold dilutions of U 937 cells in RPMI-1640 supplemented with 10% FBS were made in a 96-well black CulturPlate™, 100 µL cell suspension per well. The plate was incubated for

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2 hours at 37 °C / 5% CO_2. The plate was taken from the incubator and equilibrated at room temperature for 30 minutes. Next, 100 µL of ATPlite 1step reagent was added to the wells and the plate was shaken for 2 minutes at 700 rpm using an IKA® MTS4 plate shaker. The resulting luminescence was monitored at 5 minutes intervals in a temperature controlled (22 °C) TopCount® NXT and expressed as counts per second (CPS). The results

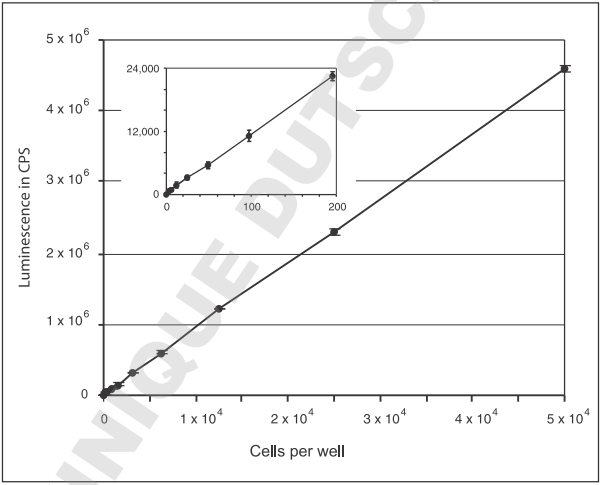


Figure 2: *Serial dilution (2-fold) U 937 cells in RPMI-1640 10% FBS. Luminescence measured after 10 minutes incubation. Inset shows linearity at low cell numbers per well.*

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presented in Figure 2 shows that there is a linear correlation between cells per well and luminescence. Monitoring luminescence over 40 minutes (Figure 3) shows that the decrease in luminescence is independent of the cell numbers per well, in this time frame.

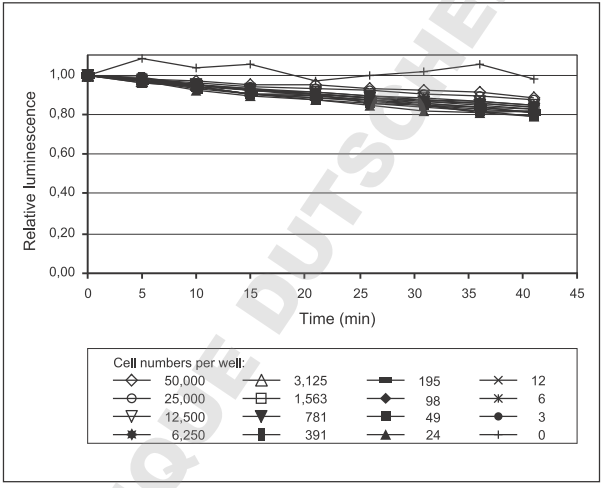


Figure 3: *Relative luminescence versus time for different cell numbers per well. Serial dilution (2-fold) U 937 cells in RPMI-1640 10% FBS.*

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Organic solvents are often used to introduce screening compounds, resulting in the presence of a small percentage of organic solvent in the culture medium. The effect of organic solvents on the light-output of ATPlite 1step system was investigated in the following experiment. 50 µL per well of a BHK-21 cell suspension in MEM supplemented with 1mM Sodium Pyruvate, 1% MEM Non-Essential Amino Acids (NEAA) and 10% FBS was added to a black 96-well CulturPlate. The plate was

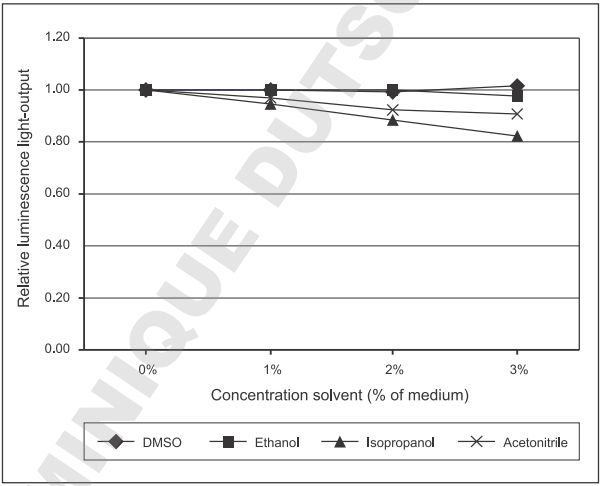


Figure 4: *Effect organic solvent concentration on luminescence of ATPlite 1step using BHK-21 cells in MEM 10% FBS without Phenol Red.*

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incubated at 37 °C / 5% CO_2 to let the cells adhere. The plate was taken from the incubator and equilibrated at room temperature. Next, 50 µL of medium with various concentrations of organic solvents was added to the wells and mixed gently. Hereafter 100 µL of ATPlite 1step was added to the wells and the plate shaken for 2 minutes at 700 rpm using an IKA MTS4 orbital plate shaker. The resulting luminescence was measured on a TopCount NXT. The results presented in Figure 4 shows that DMSO and Ethanol have a minimal effect. With Isopropanol and Acetonitrile the luminescence decreases gradually with increasing solvent concentrations.

3. Advantages of ATPlite 1step

- *Simple and reproducible*
  - only one reagent addition step, no separation steps

- *Suitable for both 96- and 384-well microplates*

- *Linear correlation between cell number and luminescent signal*
  - up to 50,000 cells per well for 96-well microplates and 12,500 cells per well for 384-well microplates

- *Designed for continuous process systems*
  - luminescence should be measured between 0 and 30 minutes after reagent addition and plate shaking

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- *Homogeneous*
  - no cell harvesting or centrifugation required

- *Highly sensitive*

- *Reduced Phenol Red dependency*

- *High light-output*
  - kit can also be used with less sensitive luminescence readers like multi-label readers

- *Fast*
  - no luminescence signal stabilization time required

4. Contents and storage of ATPlite 1 step kit

6016736 - ATPlite 1step 10 mL kit
Each assay kit contains the following components:

1. 1 x 10 mL of substrate buffer solution
2. 1 vial of substrate solution (lyophilized)
3. 1 vial of ATP standard (lyophilized)
4. Instruction booklet

Using the recommended assay volumes of 100 µL for 96-well microplates and 25 µL for 384-well microplates this kit is sufficient for 100 and 400 assays respectively.

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6016731 - ATPlite 1step 100 mL kit
Each assay kit contains the following components:

1. 1 x 100 mL substrate buffer solution
2. 10 vials of substrate solution (lyophilized)
3. 2 vials of ATP standard (lyophilized)
4. Instruction booklet

Using the recommended assay volumes of 100 µL for 96-well microplates and 25 µL for 384-well microplates this kit is sufficient for 1,000 and 4,000 assays respectively.

6016739 - ATPlite 1step 1,000 mL kit
Each assay kit contains the following components:

1. 2 x 500 mL substrate buffer solution
2. 4 vials of substrate solution (lyophilized)
3. 4 vials of ATP standard (lyophilized)
4. Instruction booklet

Using the recommended assay volumes of 100 µL for 96-well microplates and 25 µL for 384-well microplates this kit is sufficient for 10,000 and 40,000 assays respectively.

Storage conditions:
Upon arrival, store kit at 2 - 8 °C.

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5. Handling

Care should be taken during handling of the kit components, such as opening vials and bottles, to ensure that the contents of these are not contaminated with ATP. Such contamination will cause high background levels. In handling the kit, the skin of the fingers is a very potent source of ATP-contamination, therefore the use of clean gloves is strongly recommended. Use also ATP-free dispensing materials.

6. Stability

Reconstituted ATPlite 1step is stable (approximately 10% loss of activity) for 8 hours at 20 °C. At 2 °C storage the loss in activity is less than 10% after 48 hours. Freshly prepared reagents can be aliquoted and stored at -80 °C for one month.

7. Mixing

Mixing of the added ATPlite 1step reagent with the contents of the wells is very important. Improper mixing results in lower signals and deviations in the correlation between high and low cell numbers per well. It was found that using the IKA MTS 4 laboratory orbital

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microplate shaker (diameter of the orbit: 3 mm) set at 700 rpm for 2 minutes gave consistent results using 96-well plates. It was also found that with these settings the contents of 384-well microplates could not be mixed properly, even with prolonged shaking times. Increasing to 1,100 rpm for 2 minutes resolved this.

8. Additional requirements

1. Detection instrument such as the PerkinElmer TopCount, MicroBeta®, LumiCount®, VICTOR^{3TM} Multi Label Reader, VICTOR Light, EnVision™ or EnSpire®. CCD camera systems, such as PerkinElmer ViewLux™ can be used for high throughput applications.

2. Sterile, tissue culture treated, white or black 96- or 384-well microplates such as the PerkinElmer CulturPlate and ViewPlate®.

3. Pipette (multichannel) or automated pipetting device.

4. Microplate shaker suitable for efficient mixing of the plate used.

5. ATP-free dispensing material.

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9. ATPlite 1step assay procedure

General procedure for 96-well microplate (for 384-well microplates the numbers are shown in brackets).

1. Equilibrate the substrate vial and the buffer solution at room temperature before reconstitution. A water bath set at 20 - 22 °C can be used for this.

2. Reconstitute the lyophilized substrate solution by adding the appropriate volume of buffer to the substrate vial. Mix the contents of the vial by inversion and leave the solution to stand for 5 minutes. This should result in a clear homogeneous solution.

3. For 96 (384)-well microplates add 100 µL (25 µL) of the reconstituted reagent to each well containing cells, growth factors or cytotoxic agents to a final volume of 100 µL (25 µL). Ensure that the microplate is equilibrated at room temperature (20 - 22 °C) before adding the reagent.

4. Shake the 96 (384)-well microplate for 2 minutes at 700 (1,100) rpm using an orbital microplate shaker with an orbit diameter of 3 mm.

5. Measure luminescence.

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If needed, dark adapt white plates for 10 minutes to reduce plate phosphorescence. Black plates show minimal plate phosphorescence and therefore there is no need for dark adaptation. Because there is no need to stabilize the luminescent signal, the luminescence can be measured directly after shaking the plate.

Note: *Please realize that ATP is everywhere. ATP is the universal energy carrier in nature; both eukaryotes and prokaryotes utilize the molecule for energy storage and transfer. As a result, ATP is abundantly present both in microbial, animal or plant cells and also as free ATP. ATP is fairly heat-stable so mere autoclaving is not always sufficient for complete reduction. Therefore, it is important that direct contact of reagents by hand or fingertips is avoided. Open vials carefully and do not touch the mouth of the bottle. Be careful removing the rubber stopper from the vials. Use ATP-free pipette tips. Handle microplates carefully and use lids to avoid contamination.*

The ATPlite 1step general assay procedure for 96-well format is outlined in Figure 4.

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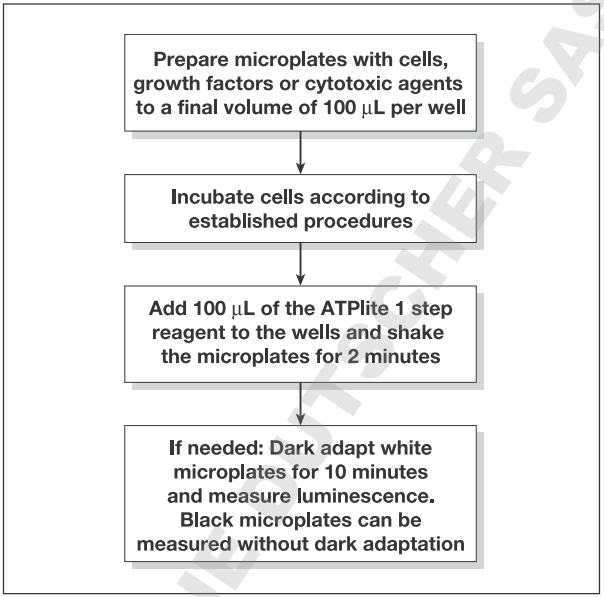


Figure 4: The ATPlite 1 step assay flow chart (96-well).

10. ATP Standard

In cases where it is necessary to quantify the ATP released from the cells, perform the following procedure:

1. Reconstitute a vial of lyophilized ATP standard solution with water so that a **10 mM** stock solution is obtained. E.g., add 1,170 µL of water if the ATP

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amount printed on the label is 11.7 µmole or add 960 µL of water if the amount is 9.6 µmole. After addition of the water, allow the ATP to dissolve completely by swirling the vial for one minute.

2. Set up a standard curve in the same microplate that will be used for the experimental samples:

   a. Take an aliquot of the ATP standard solution and prepare a 1 µM ATP in culture medium.
   b. Prepare a 10-fold serial dilution series of ATP in culture medium (1 µM to 1 pM)
   c. Pipette this dilution series in the wells of a microplate (100 µL for a 96-well and 25 µL for a 384-well microplate).

3. Add ATPlite 1step reagent to these wells, 100 µL for a 96-well and 25 µL for a 384-well microplate.

4. Mix contents of the wells for 2 minutes using a plate shaker.

5. Measure luminescence.

Note: *Endogenous ATPase's may be present in sera resulting in a reduction of the ATP concentration of the ATP dilution series. Therefore the ATP dilution series should be made just before the addition of the ATPlite 1step reagent.*

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11. Recommendations for use

Phenol red, as well as other colored compounds, will not interfere with the luciferin/luciferase reaction, but will physically absorb some of the emitted light, resulting in lower assay signals. For the highest light-output, the culture medium can be substituted with medium without phenol red or (Dulbecco's) PBS prior to the addition of the ATPlite 1step reagent.

Whether to choose white or black microplates depends very much on the luminescence reader used. With some readers the detectors of the instrument may saturate when using white plates and high ATP concentrations due to the very high light-output of the ATPlite 1step reaction. This can occur especially with 96-well white plates. In this case black plates are recommended.

Optimize liquid handling procedures to attain optimal reagent/medium mixing.

When handling the plates prior to measurement, work under subdued light conditions and avoid direct sunlight or bright fluorescent light. Bright light may cause plate phosphorescence resulting in higher background levels. Phosphorescence has a half-life of several minutes.

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If more than one vial of substrate is reconstituted for the assay, we recommend these solutions be combined before addition to the plates.

Optimal room and instrument temperature is 22 °C. Allow plates to adapt at room temperature upon removal from the incubator and prior to the addition of the reagent. An adaptation time of 30 minutes is usually sufficient.

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12. Ordering information

ATPlite 1 step	Reorder No.
10 mL ATPlite 1step assay kit	**6016736**
100 mL ATPlite 1step assay kit	**6016731**
1,000 mL ATPlite 1step assay kit	**6016739**

For further information on luminescence readers, microplates, seals and luminescence applications please contact your local PerkinElmer representative or visit our website: **http://www.perkinelmer.com**

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13. References

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