FOR RESEARCH USE ONLY

ATPlite

Luminescence ATP Detection Assay System

PerkinElmer®

For best results, see page 15 for product use recommendations.

1. Introduction

ATPlite™ is an Adenosine TriPhosphate (ATP) monitoring system based on firefly (*Photinus pyralis*) luciferase. This luminescence assay is *the* 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].

The major advantages of this system are high sensitivity, excellent linearity, simplicity, fast results and the lack of cell harvesting or separation steps. Furthermore, the PerkinElmer ATPlite assay system produces a long lived "glow" type signal with a half-life of greater than five hours, therefore a special luminometer with injectors is not required. The kit is ideal for use with the PerkinElmer luminescence detection instruments in both 96- and 384-well microplates. The simplicity of the ATPlite assay system in a 96-well microplate is illustrated in Figure 1.

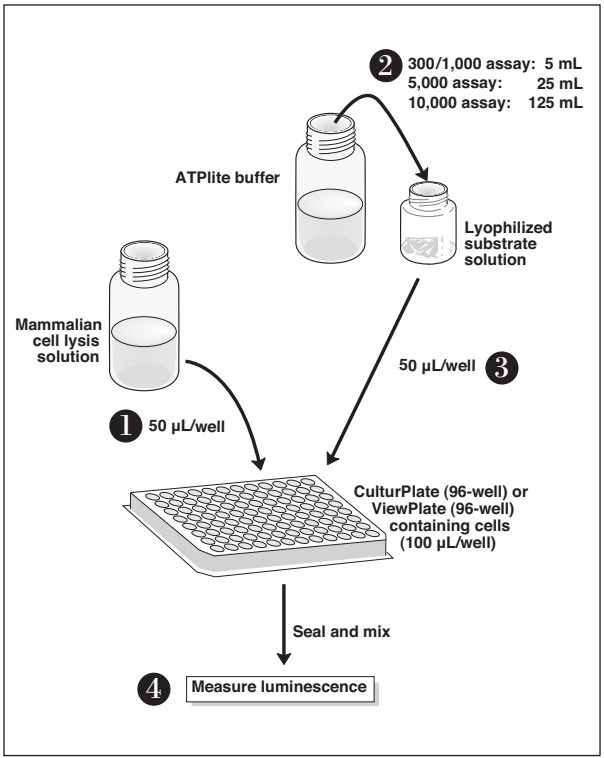

Figure 1: ATPlite assay system.

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 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.

A limitation associated with common luciferase assay technology is the short half-life of the light emission. This flash-type signal requires luminometers with reagent injectors to measure the quick reaction. ATPlite is a mixture of several substances that extends the signal half-life to over 5 hours.

A problem with some ATP assay kits that are currently on the market is that the lysing solutions that release the ATP do not irreversibly inactivate endogenous ATP degrading enzymes (ATPases). Also, some lysing solutions contain chaotropic agents like TCA, which

have a negative effect on the luciferase activity. The ATPlite kit overcomes these problems by raising the pH of the cell culture medium through the addition of the mammalian cell lysis solution. The lysis solution inactivates the endogenous ATPases. The subsequent addition of the substrate solution (Luciferase/Luciferin) lowers the pH to a suitable level so that the reaction can occur.

Figure 2 illustrates the performance of the ATPlite

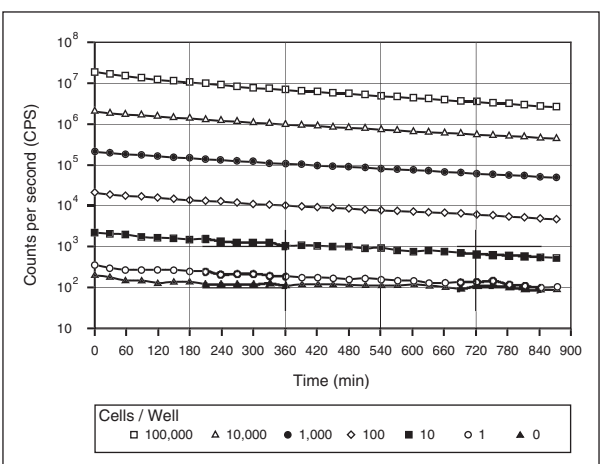

Figure 2: Signal stability, dynamic range and linearity of ATPlite with CHO cells cultured and measured in a white 96-well CulturPlate™ from PerkinElmer.

kit used with a serial dilution of Chinese Hamster Ovary (CHO) cells cultured in DMEM/F12 supplemented with 5% FCS and 1% Pen/Strep (100 µL cell suspension/well). The luminescence was measured on the PerkinElmer TopCount® Microplate Scintillation and Luminescence Counter at 22 °C.

3. Advantages of ATPlite

- *Long-lived luminescent signal*
  - half-life ($t_{1/2}$) greater than 5 hours, depending on cell type and medium

- *Rapid*
  - results generated in 15 - 25 minutes

- *Simple and reproducible*
  - no separation steps
  - only two reagent additions

- *Homogeneous assay*
  - no cell harvesting or centrifugation required

- *Sensitive*
  - down to 5 cells in 100 µL medium (derived from CHO and HL-60 cells in 100 µL medium)

- *Wide linear dynamic range*
  - ≥ 10^5 (as derived from CHO and HL-60 cells)

4. Contents of kit

6016943 - ATPlite 300 assay kit
Each assay kit contains the following components:

1. 1 x 20 mL of mammalian cell lysis solution
2. 1 x 20 mL of substrate buffer solution
3. 3 vials of substrate solution (lyophilized)
4. 1 vial of ATP standard (lyophilized)
5. Instruction booklet

6016941 - ATPLite 1,000 assay kit
Each assay kit contains the following components:

1. 1 x 60 mL of mammalian cell lysis solution
2. 1 x 60 mL of substrate buffer solution
3. 10 vials of substrate solution (lyophilized)
4. 2 vials of ATP standard (lyophilized)
5. Instruction booklet

6016947 - ATPLite 5,000 assay kit
Each assay kit contains the following components:

1. 1 x 270 mL of mammalian cell lysis solution
2. 1 x 270 mL of substrate buffer solution
3. 10 vials of substrate solution (lyophilized)
4. 2 vials of ATP standard (lyophilized)
5. Instruction booklet

6016949 - ATPlite 10,000 assay kit
Each assay kit contains the following components:

1. 1 x 520 mL of mammalian cell lysis solution
2. 1 x 520 mL of substrate buffer solution
3. 4 bottles of substrate solution (lyophilized)
4. 4 vials of ATP standard (lyophilized)
5. Instruction booklet

5. Storage

Upon arrival, store kit at 2 - 8 °C.
DO NOT FREEZE !

Reconstituted substrate solution can be stored at 2 - 8 °C, however the activity declines during storage (approximately 30% lower activity after 1 week).

Reconstituted substrate solution can also be stored frozen at - 20 °C for longer periods of time. After thawing crystals may appear. These crystals can be dissolved by swirling the vial when it has reached room temperature.

6. Safety precautions

- FOR IN VITRO RESEARCH USE ONLY
- Mammalian cell lysis solution contains 0.1 M of alkaline solution. In case of accidental spillage, wash affected areas thoroughly with water.
- Good laboratory procedures should be applied for the handling and use of the kit.

7. Instrumentation and materials required

1. Detection instrument such as the PerkinElmer TopCount, MicroBeta®, LumiCount®, VICTOR3™ Multi Label Reader, VICTOR Light, EnVision™ or EnSpire®.
2. Sterile, tissue culture treated, white or black 96- or 384-well microplates such as the PerkinElmer CulturPlate and ViewPlate®.
3. ATP-free dispensing materials.

8. ATPlite assay procedure (for 96-well microplate)

1. Allow the reagents to equilibrate to room temperature.

2. For the 300 and 1,000 assay kit reconstitute one lyophilized substrate solution vial by adding 5 mL of substrate buffer solution. Agitate gently until the solution is homogeneous.

   For the 5,000 assay kit reconstitute one lyophilized substrate solution vial by adding 25 mL of substrate buffer solution. Agitate gently until the solution is homogeneous.

   For the 10,000 assay kit reconstitute one lyophilized substrate solution bottle by adding 125 mL of substrate buffer solution. Agitate gently until the solution is homogeneous.

3. Add 50 µL of mammalian cell lysis solution to 100 µL of cell suspension per well of a microplate and shake the plate for five minutes in an orbital shaker at 700 rpm. This lyses the cells and stabilizes the ATP.

4. Add 50 µL substrate solution to the wells and shake the microplate for five minutes in an orbital shaker at 700 rpm.

5. Dark adapt the plate for ten minutes and measure the luminescence.

ATPlite general assay procedure for 96-well microplates is outlined in Figure 3.

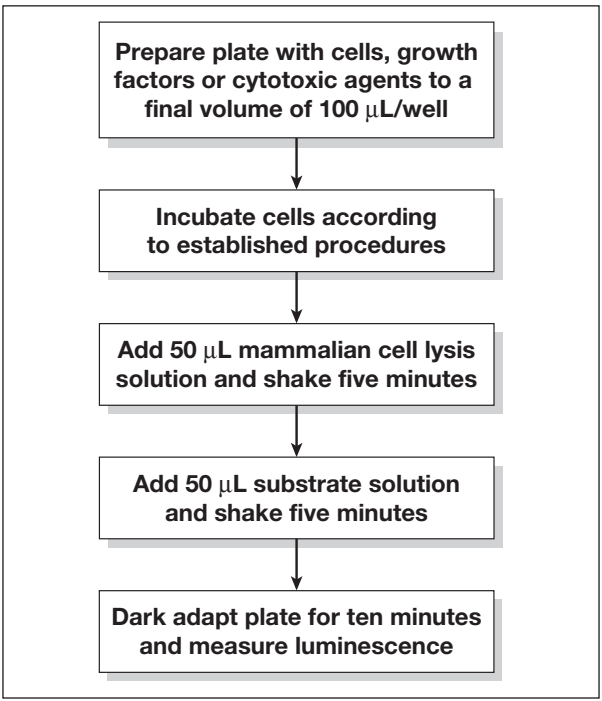

Figure 3: The ATPlite assay flow chart.

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 and hands or fingertips is avoided. Open vials carefully and do not touch the mouth of the bottle. Be careful removing the rubber stoppers from the vials.*
Use ATP-free pipette tips. Handle microplates carefully and use lids to avoid dust or other contamination.

9. ATP Standard

In cases where it is necessary to quantify the ATP released from the cells, perform the following procedure in a 96-well microplate:

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 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 dilution series in water from a concentration of 1 x 10^{-5} M down to blank.
    b. Pipette a series of 100 µL of complete culture medium without cells into the wells of the plate.

    c. Add to these wells 50 µL of the mammalian cell lysis solution and shake the plate for five minutes in an orbital shaker at 700 rpm.
    d. Add 10 µL of the ATP dilution series to the wells and shake the plate for five minutes in an orbital shaker at 700 rpm.

    e. Add 50 µL of the substrate solution and shake for five minutes in an orbital shaker at 700 rpm.

    f. Dark adapt the plate for ten minutes and measure the luminescence.

    g. Calculate the standard curve.

Note: *Reconstituted ATP standard is stable for weeks when stored at - 20 °C. Diluted ATP solutions are stable for eight hours when stored on ice.*

10. Recommendations for use

1. Care should be taken not to contaminate the components of the kit with ATP. This 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 ATP-free dispensing materials.

2. When handling the plates prior to measurement, work in SUBDUED lighting out of direct sunlight or direct bright fluorescent lighting. Bright light may cause plate phosphorescence resulting in higher background levels. Phosphorescence has a half-life of several minutes.

3. If more than one vial of lyophilized substrate solution is reconstituted for the assay, they should be combined before adding them to the microplates.

11. Ordering information

ATPLite	Reorder No.
300 Assay kit	**6016943**
1,000 Assay kit	**6016941**
5,000 Assay kit	**6016947**
10,000 Assay kit	**6016949**

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**

12. References

1. Kangas L., Grönroos M. and Nieminen A.L. 1984. Bioluminescence of cellular ATP: a new method for evaluating agents in vitro. *Medical Biology*, **62**, 338 - 343

2. Lundin A., Hasenson M., Persson J. and Pousette A. 1986. Estimation of biomass in growing cell lines by ATP assay. *Methods Enzymol.* **133**, 27 - 42

3. Crouch S.P.M., Kozlowski R., Slater K.J. and Fletcher J. 1993 The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. *J. Immunol. Methods*, **160**, 81 - 88

4. Petty R.D., Sutherland L.A., Hunter E.M. and Cree I.A. 1995 Comparison of MTT and ATP - based assays for the measurement of viable cell number. *J. Biolumin. Chemilumin.* **10**, 29 - 34

5. Storer R.D., McKelvey T.W., Kraynak A.R., Elia M.C., Barnum J.E., Harmon L.S., Nichols W.W. and Deluca J.G. 1996 Revalidation of the in vitro alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and the results for 81 compounds. *Mutation Research*, **368**, 59 - 101

6. Cree I.A. and Andreotti P.E. 1997 Measurement of cytotoxicity by ATP - based luminescence assay in primary cell cultures and cell lines. *Toxicology in Vitro*, **11**, 553 - 556

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U.S. Pat. 6503723; European Pat. 1117825 and foreign equivalent patents.

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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs.

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