revvity

USER MANUAL

Technology: Luminescence Assay Systems

ATPlite 1glow-L

Single Addition ATP Detection Assay System
Lyophilized Format

Part number:	6096746	6096741	6096749
Volume:	10 mL	100 mL	1,000 mL

Storage: 2 - 8°C upon receipt

Version: 2 **Date:** March 2026

For research only. Not for use in diagnostic procedures

INTRODUCTION

ATPlite™ 1glow-L is a single enhanced signal stability Adenosine TriPhosphate (ATP) monitoring reagent based on firefly *(Photuris pennsylvanica)* 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 compounds1,2,3,4,5,6.

ATPlite 1glow-L is a true homogeneous high sensitivity ATP monitoring assay reagent for the quantification of viable cells. Because of the increased signal stability and on deck longevity, the reagent can be used in high throughput environments. These high throughput systems can have up to an hour or longer after the addition of the reagent and reading the resulting luminescence. The ATPlite 1glow-L signal stability is > 3 hr at room temperature making this ideal for processing high numbers of 96- or 384-well microplates. The maximum cell number that can be applied has been determined to be 50,000 cells per well for 96-well and 32,000 cells per well for 384-well microplates.

The simplicity of the ATPlite 1glow-L assay in a 96-well microplate (with reference to a 384-well microplate) is illustrated in Figure 1.

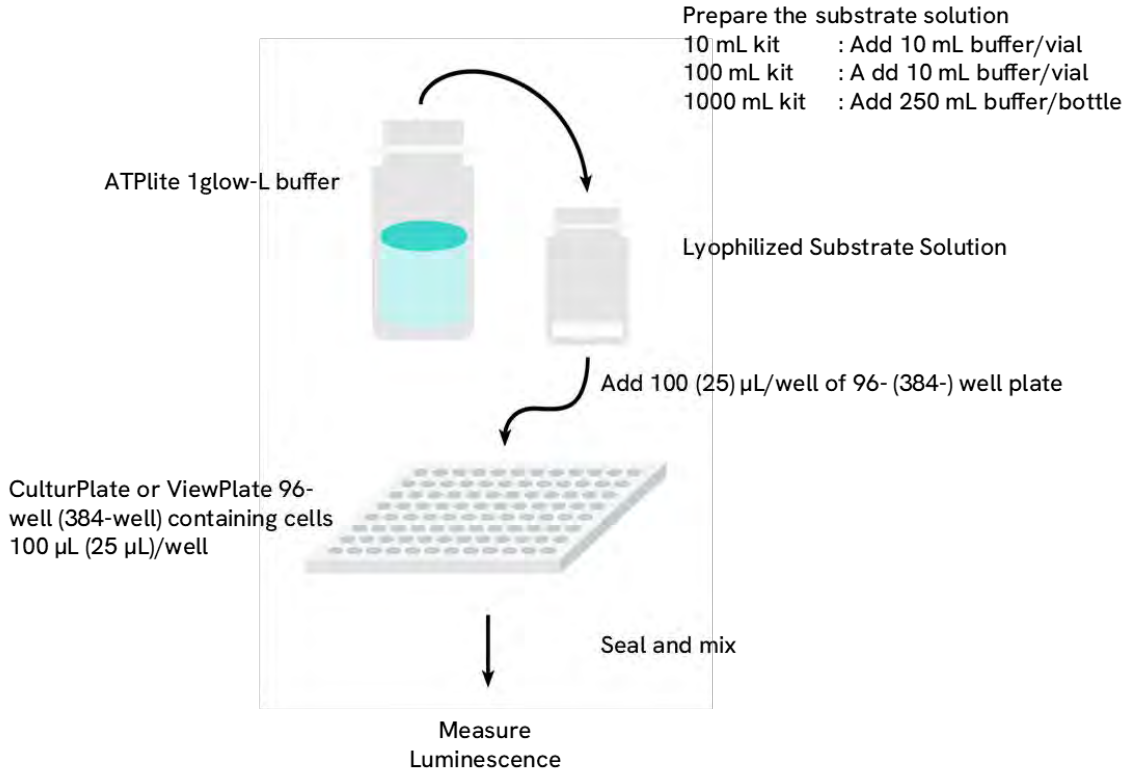

Figure 1. ATPlite 1glow-L assay set up.

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 1glow-L reagent is based on the production of light caused by the reaction of ATP with added luciferase and D-luciferin. This is illustrated in Figure 2.

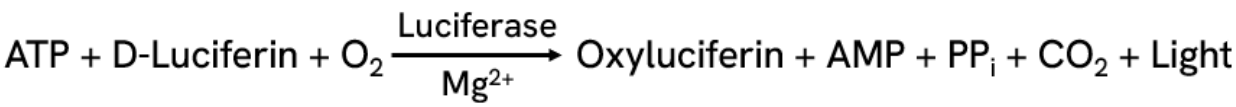

Figure 2. Assay principle for ATP-based luminescence assays for measuring cell proliferation and cytotoxicity.

Cellular ATP is quantified following direct lysis of the cells with suitable detergent or lysis buffer. ATP released from the lysed cells reacts with added luciferin in the presence of luciferase, oxygen and magnesium to produce light. The emitted light is then quantified with the use of a luminescence reader, whereby the measured luminescence signal is directly proportional to the amount of ATP present in the sample; and hence light intensity is representative of the number and health of cells present in the sample.

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

The ATPlite 1glow-L reagent has been formulated in such a way that after a 2 minute room temperature incubation, a bright, sustained signal will be measured, when using up to the maximum cell number per well.

CELL DENSITY SIGNAL LINEARITY

To show that there is a linear correlation between the luminescence signal and the cell number the following experiment was performed with ATPlite 1glow-L reagent.

Serial 2-fold dilutions of U 937 cells in RPMI-1640 supplemented with 10% FBS with cell numbers from 3 to 50,000 cells per well were made in a 96-well black CulturPlate™ at a final volume of 100 µL cell suspension per well. The plate was incubated for 1 hour at 37°C / 5% CO_2. The plate was taken from the incubator and equilibrated at room temperature for 30 minutes. Next, 100µL of ATPlite 1glow-L reconstituted reagent was added to the wells and the plate was shaken for 2 minutes at 700 rpm on an orbital shaker. The resulting luminescence was measured in an EnVision Nexus™.

The results presented in Figure 3 show that there is a linear correlation between cells per well and luminescence. The inset shows linearity from 3 – 200 cells per well.

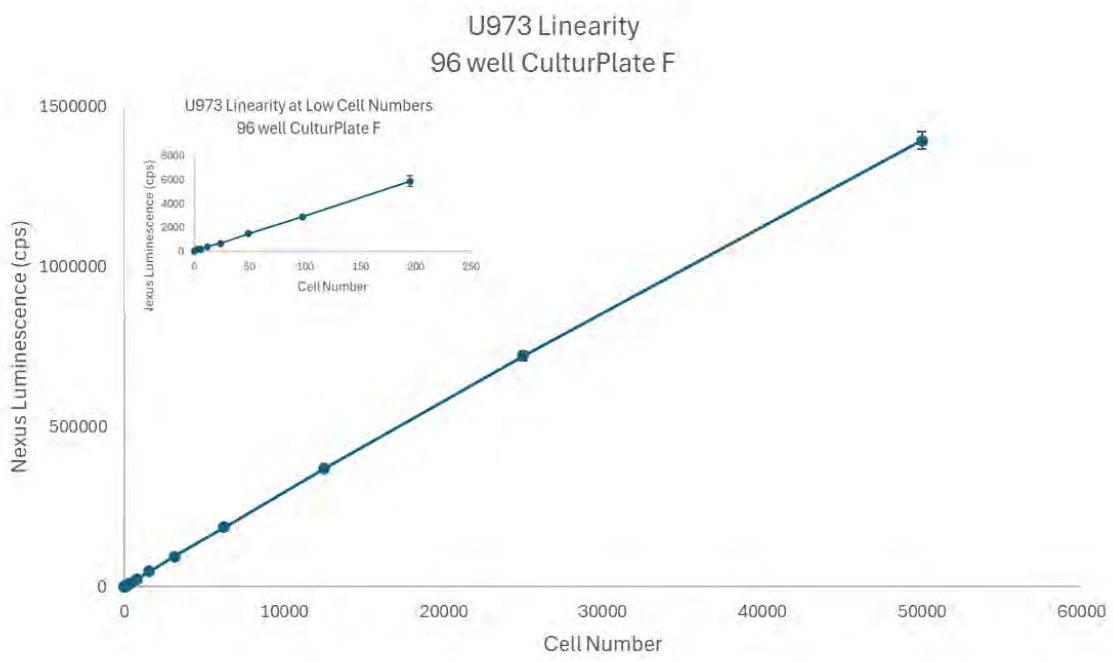

Figure 3. Serial dilution (2-fold) U 937 cells in RPMI-1640 10% FBS. The inset shows linearity at low cell numbers per well.

Monitoring luminescence over time (Figure 4) shows that the decrease in luminescence is independent of the cell numbers per well.

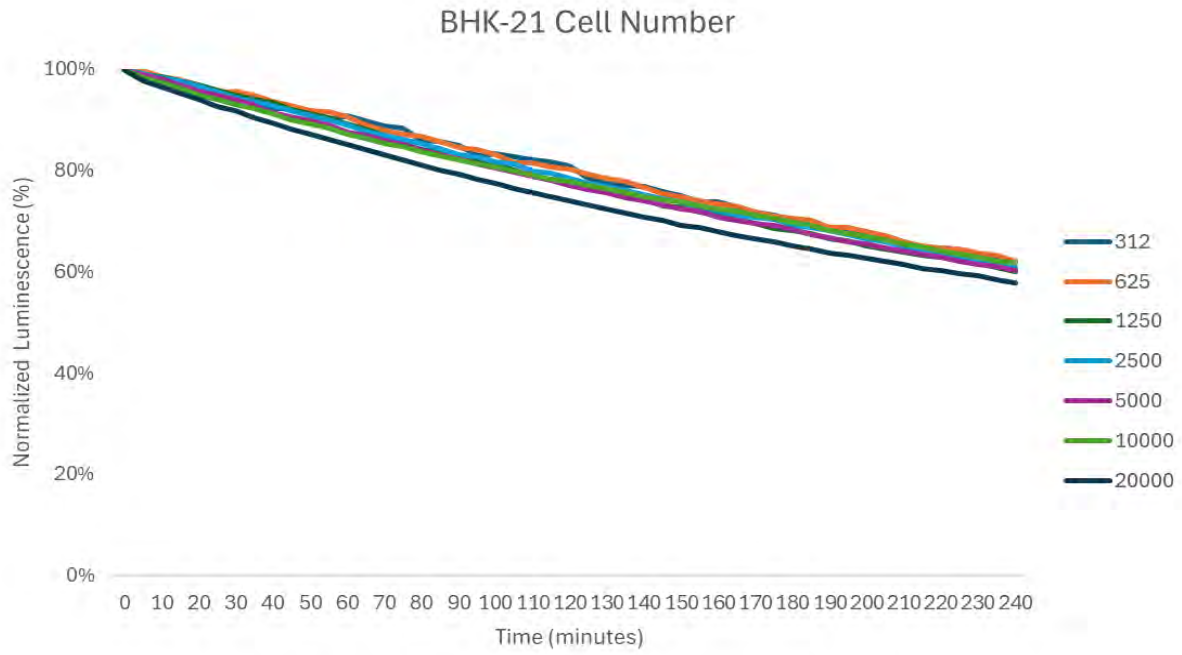

Figure 4. Relative luminescence versus time for different cell numbers per well. A two-fold serial dilution of BHK-21 adherent cells in RPMI-1640, 10% FBS and cells were seeded in 100 µL of medial in a black 96-well CulturPlate from 312 to 20,000 cells/well. Cells were allowed to attach for 4 hours at 37°C, followed by room temperature equilibration for 30 min. ATP was measured by adding 100 µL ATPlite 1glow-L reagent, followed by 2 minutes shaking at 700 rpm. Detection was performed in an EnVision Nexus with at the ultra-sensitive luminescence setting.

EFFECT OF ORGANIC SOLVENTS

Organic solvents are often used to introduce screening compounds, resulting in the presence of a small percentage of organic solvents in the culture medium. The effect of organic solvents on the light signal of ATPlite 1glow-L reagent was investigated in the following experiment.

A volume of 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 incubated for 4 hours 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. ATPlite 1glow-L reagent, 100 µL, was added to the wells and the plate shaken for 2 minutes at 700 rpm using an orbital plate shaker. The resulting luminescence was measured on an EnVsion Nexus. The results presented in Figure 5 show that with Isopropanol, the luminescence decreases gradually with increasing concentration.

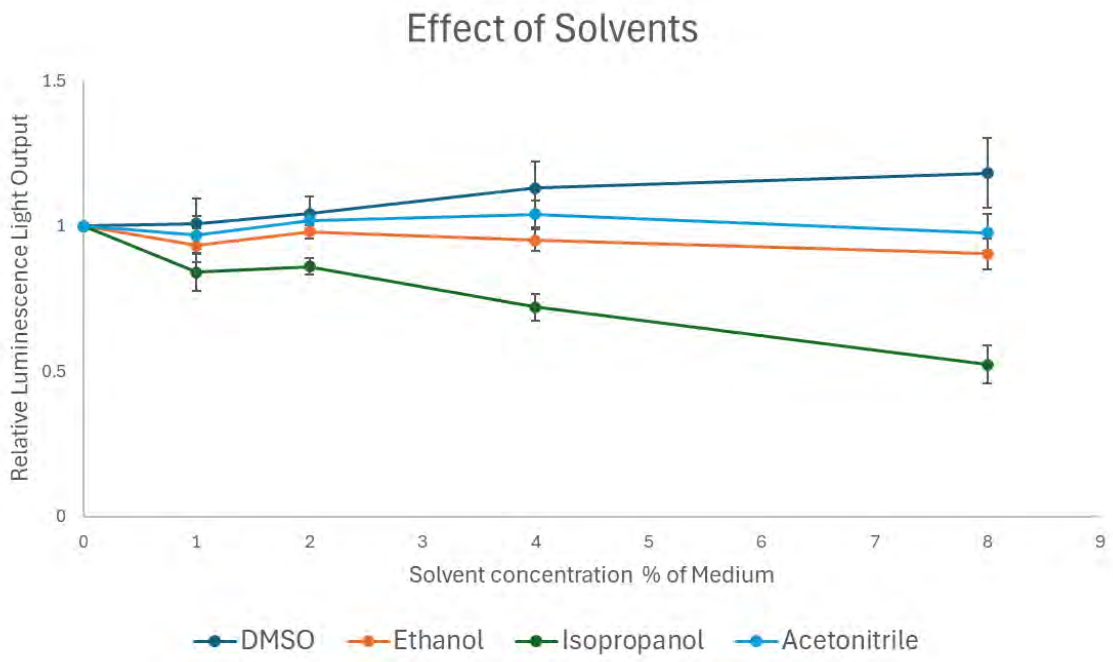

Figure 5. Effect of organic solvents on ATPlite 1glow-L signal intensity.

ADVANTAGES OF ATPlite 1glow-L

Simple and reproducible	Homogeneous single reagent addition to cells cultured in a microplate
High throughput	Scalable for 96- and 384-well microplates
Enhanced signal stability	Signal is stable at room temperature for > 3 hr
Signal to cell density correlation	Linear signal for up to 50,000 cells/well 96-well microplates and12,500 cells/well 384-well microplates
Automation friendly	On deck reagent stability
Sensitive, bright signal	Can be used on less sensitive detectors, e.g., multi-label
Rapid signal detection	No luminescence signal stabilization time required

REAGENT HANDLING AND KIT CONTENTS

Kit Components*	6096746 10 mL 100 pts 96-well** 400 pts 384-well	6096741 100 mL 1000 pts 96-well 4000 pts 384-well	6096749 1,000 mL 10,000 pts 96-well 40,000 pts 384-well
Substrate Buffer Solution	1 x 10 mL	1 x 100 mL	2 x 500 mL
Substrate Solution (lyophilized)***	1 vial	10 vials	4 bottles
ATP Standard (lyophilized)	1 vial	2 vials	4 vials
CulturPlate 96	1	-	-

*Upon arrival and for long-term storage, the reagent should be stored at 2 - 8°C.

**The number of assay points is based on an assay of 100 µL per well in a 96-well microplate or 25 µL per well in a 384-well microplate.

***Reconstituted ATPlite 1glow-L reagent is stable (~25% loss of activity) for 7 days at ambient temperatures. At 2 - 8°C storage the loss in activity is less than 10% after 2 weeks. Freshly prepared reagents can be aliquoted and stored at -80°C.

HANDLING

Care should be taken during handling the ATPlite 1glow-L reagent bottle to ensure that the contents is not contaminated with ATP. Such contamination will cause high background levels. Bare hands and fingers are a very potent source of ATP-contamination, therefore the use of clean gloves is strongly recommended. The use of ATP-free containers and pipette tips is recommended.

PREPARING THE REAGENT

Equilibrate the Lyophilized Substrate and Reconstitution Buffer to room temperature (20 – 22°C). Add the Reconstitution Buffer according to the table below to a Lyophilized Substrate vial. Mix the contents of the vial gently by inverting the bottle several times then leave for 5 minutes before use.

ATPlite 1glow-L Kit Size and Product Number	Number of Substrate Vials	Volume of Reconstitution Buffer per Vial
10 mL 6096746	1	10 mL
100 mL 6096741	10	10 mL
1000mL 6096749	4	250 mL

Do not vortex or shake vigorously to avoid excessive foaming.

Open the vial and bottle carefully to avoid ATP contamination of the reagent.

STABILITY

For storage of the kit, we recommend storage at 2 - 8°C. The reconstituted ATPlite 1glow-L Substrate is stable for an 8 hr workday on the bench top with no loss of data quality and maintains > 80% of activity after 5 days at ambient (22°C) temperature. At 2 – 4°C storage, the reconstituted Substrate reagent maintains > 90% activity after > 1 week.

MIXING

Adequate mixing after the addition of ATPlite 1glow-L reagent is highly recommended for maximum signal and decreased signal development time. Using laboratory orbital microplate shaker (diameter of the orbit: 3 mm) set at 700 rpm for 2 minutes gave consistent results using 96-well plates. Alternate settings such as increasing the rpm to 1,100 and mixing for 2 minutes allows for consistent mixing in 384-well microplates.

ADDITIONAL REQUIRMENTS

1. 22°C water bath
2. Detection instrument such as the Revvity MicroBeta^{2} ™, VICTOR Nivo™, VICTOR Kira ™, or EnVision Nexus.

3. Sterile, tissue culture treated, white or black 96- or 384-well microplates such as the Revvity CulturPlate and ViewPlate.
4. Pipette (multichannel) or automated pipetting device.
5. A microplate shaker suitable for efficient mixing of the plate used.
6. ATP-free area for dispensing and performing the assay.

ATPlite 1glow-L ASSAY PROCEDURE

1. Plate preparation
   Seed mammalian cells into a microplate and treat with growth factors or cytotoxic agents as required. Optimize cell density, reagent volumes, and treatment concentrations for your specific experimental conditions.

2. Cell incubation
   Incubate the cells under appropriate conditions according to your experimental design.

3. Equilibration to room temperature
   Remove the microplate from the incubator and allow it to equilibrate to room temperature for at least 30 minutes.

4. Reagent addition
   Add an equal volume of ATPlite 1glow-L reagent to each well. For 96 (384)-well microplates add 100 µL (25 µL) of the reagent to each well containing 100µL (*25*µL) cells/media/additives.

5. Mixing
   Cover the plate with a lid and place it on an orbital shaker. Shake for 2 minutes at a speed that avoids cross-contamination between wells. For a 96-well plate, shake with a 3 mm orbit set to 700 rpm. For a 384-well plate, shake for 2 minutes at 1,100 rpm. Optimize shaking conditions to improve assay performance.

6. Luminescence measurement
   Seal the plate with TopSeal-A PLUS and measure luminescence.

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 1glow-L general assay procedure for 96 (384)-well format is outlined in Figure 6.

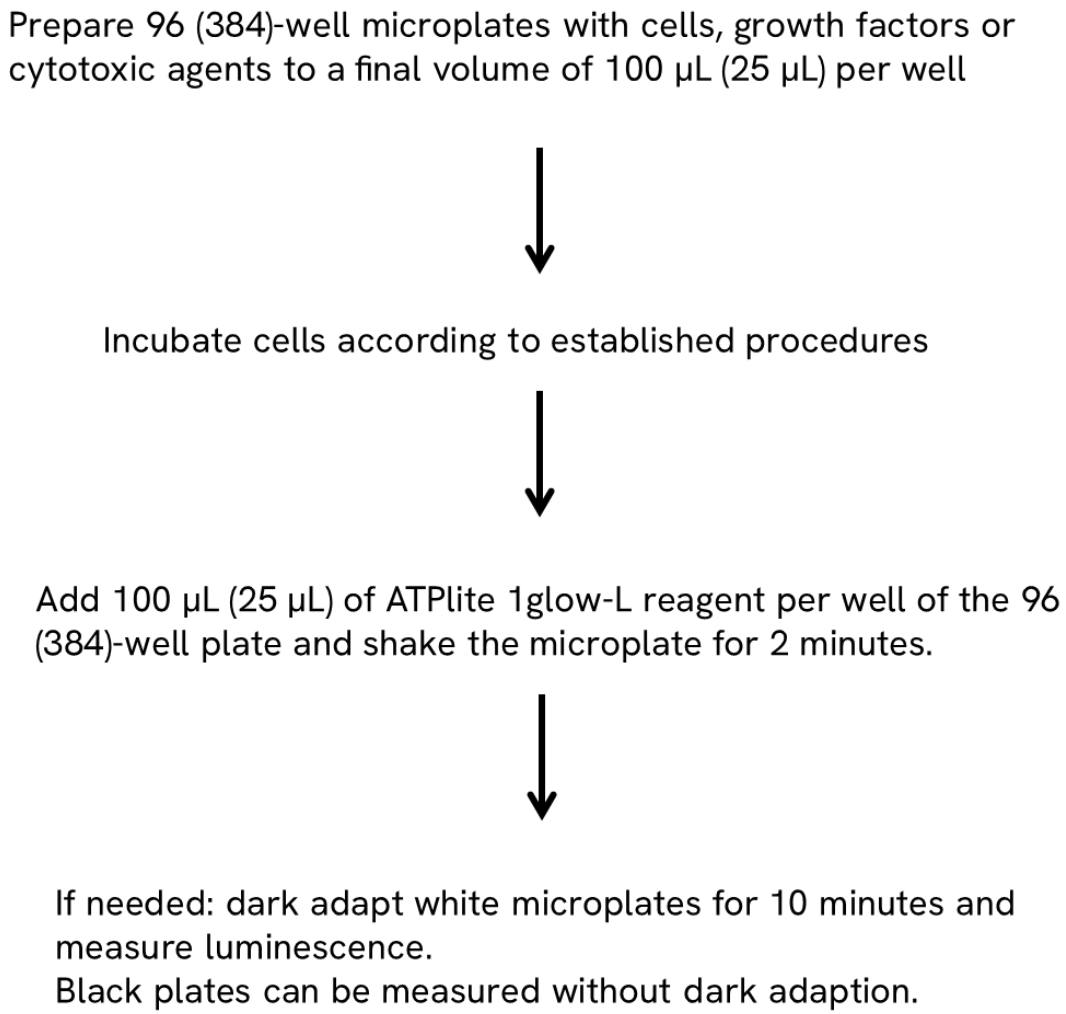

Figure 6. ATPlite 1glow-L assay flow chart

ATP STANDARD

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

1. Prepare a 10mM stock solution of ATP by reconstituting a vial of ATP lyophilized standard supplied with the kit in water.
    a. If the ATP amount printed on the label is 11.7 µmole, add 1,170 µL of water.

b. If the ATP amount printed on the label is 9.6 µmole, add 960 µL of water.

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 an equal volume of ATPlite 1glow-L 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 ATPases 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 1glow-L reagent*

NOTES ON 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 1glow-L 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 1glow-L 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.

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.

ORDERING INFORMATION

Revvity Product	Reorder No.
ATPlite 1glow-L 10 mL	6096746
ATPlite 1glow-L 100 mL	6096741
ATPlite 1glow-L 1000 mL	6096749
CulturPlate 96 (white)/CulturPlate 384 (white)	6055680/6007680
CulturPlate 96 F (black)/CulturPlate 384 (black)	6055660/6007660
ViewPlate 96 (white)/ViewPlate 384 (white)	6005181/6007480
ViewPlate 96 F (black)/ViewPlate 384 (black)	6005225/6007460
BackSeal-96/384 White	6005199
BackSeal-96/384 Black	6005189
TopSeal-A PLUS	6050185

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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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