PhosphoWorks™ Luminometric ATP Assay Kit (DTT Free)

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 21612 (1 plate), 21613 (10 plates)	Keep in freezer and protect from light	Luminescence microplate readers

Introduction

Adenosine triphosphate (ATP) plays a fundamental role in cellular energetics, metabolic regulation and cellular signaling. The quantitation of ATP can be used for a variety of biological applications. Because ATP is the energy source for almost all living organisms that rapidly degrades in the absence of viable organisms, its existence can be used to identify the presence of viable organisms. The measurement of ATP has been used for cell cytoxicity, detection of bacteria on surfaces, quantification of bacteria in water, somatic cells in culture and food quality.

The use of firefly bioluminescence to measure ATP was first proposed by McElroy when he discovered that ATP was essential for light production. Firefly luciferase is a monomeric 61 kD enzyme that catalyses a two-step oxidation of luciferin, which yields light at 560 nm. The first step involves the activation of the protein by ATP to produce a reactive mixed anhydride intermediate. In the second step, the active intermediate reacts with oxygen to create a transient dioxetane, which quickly breaks down to the oxidized product oxyluciferin and carbon dioxide along with a burst of light. When ATP is the limiting component, the intensity of light is proportional to the concentration of ATP. Thus the measurement of the light intensity can be used for quantifying ATP using a luminometer.

$$\text{Luciferin} + \text{ATP} + O_2 \xrightarrow{Mg^{2+}} \text{Oxyluciferin} + \text{AMP} + \text{Pyrophosphate} + CO_2 + \text{light}\ (\sim 560\ \text{nm})$$

AAT Bioquest's PhosphoWorks™ Luminometric ATP Assay Kit (DTT free) comes with all the essential components and provides a fast, simple and homogeneous luminescence assay for the determination of cell proliferation and cytotoxicity in mammalian cells. This assay is based on the detection of ATP using firefly luciferase to catalyze the release of light by ATP and luciferin. It can be performed in a convenient 96-well or 384-well microtiter-plate format and run with many luminescence instruments. The assay is extremely sensitive and can detect 100 cells/well. Its high sensitivity permits the detection of ATP in many biological systems, environmental samples and foods. This Phospho Works ATP Assay Kit does not use DTT. It has stable luminescence with no mixing or separations required, and formulated to have minimal hands-on time.

Kit Key Features

Sensitive:	Detect as low as 100 cells/well.
Continuous:	Stable luminescence, suitable for manual or automated operations without mixing or a separation step.
Convenient:	Formulated to have minimal hands-on time.
Odorless:	No DTT, odor free.

Kit Components

Components	**Amount**	
	Cat. # 21612 (1 plate)	**Cat. # 21613 (10 plates)**
Component A: ATP Monitoring Enzyme	1 vial	1 vial
Component B: ATP Sensor (Light-sensitive)	1 vial	10 vials
Component C: Reaction Buffer	1 vial (10 mL)	2 vials (50 mL/vial)

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells (samples) with test compounds (100 µL/96-well plate or 25 µL/384-well plate) → Add equal volume of ATP assay solution → Incubate at room temperature for 10 - 20 minutes → Monitor the luminescence intensity

1. Prepare cells (or samples):

1.1 For adherent cells: Plate cells overnight in growth medium at 1,000 - 10,000 cells/90 µL/well (for a 96-well plate) or 250-2,000cells/20 µL/well (for a 384-well plate).

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 2,000 - 20,000 cells/90 µL/well for a 96-well poly-D lysine plate or 500 - 5,000 cells/20 µL/well for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.
Note1: Each cell line should be evaluated on an individual basis to determine the optimal cell density for proliferation or cytotoxicity induction. For toxicity assays, start with more cells.

Note2: For all luminescent experiments, it is recommended using white plates to achieve the best results.

2. Prepare ATP assay solution:

2.1 Thaw all the components to room temperature before use.

2.2 Transfer 10 mL of Component C (Reaction Buffer) into Component B (ATP Sensor), and mix well.

2.3 Add 20 µL of Component A (ATP Monitoring Enzyme) into the solution prepared at Step 2.2.
Note: Aliquot and store the unused components A and C at -20 °C, and avoid repeated freeze/ thaw cycles and potential ATP contamination from exogenous biological sources.

3. Run ATP assay:

3.1 Treat cells (or samples) with test compounds by adding 10 µL of 10X compounds for a 96-well plate or 5 µL of 5X compounds for a 384-well plate in desired compound buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.

3.2 Incubate the cell plate in a 37 °C, 5% CO_2 incubator for the desired period of time, such as 24, 48 or 96 hours.

3.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of ATP assay solution (from Step 2.3), and incubate at room temperature for 10-20 minutes.

3.4 Monitor the luminescence intensity with a standard luminometer.

4. Generate a standard ATP calibration curve:

Note: An ATP standard curve should be generated together with the above assay if the absolute amount of ATP in samples needs to be calculated.

4.1 Make a series dilutions of ATP in PBS buffer with 0.1% BSA by including a sample without ATP (as a control) to measure background luminescence.
Note: Typically ATP concentrations ranging from 0.1 nM to 1 µM are appropriate.

4.2 Add the same amount of the diluted ATP solution into an empty plate (100 µL for a 96-well plate or 25 µL for a 384-well plate).

4.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of ATP assay solution (from step 2.3).

4.4 Incubate the reaction mixture at room temperature for 10 to 20 minutes.

4.5 Record the luminescence intensity with a standard luminometer.

4.6 Generate the ATP standard curve.

Data Analysis

The luminescence in blank wells with the growth medium is used as a control, and is subtracted from the values for the cell (or sample) wells. The background luminescence of the blank wells varies depending upon the sources of the growth media or the microtiter plates. Use the standard curve to calculate the amount of ATP in test cells or samples. An ATP titration curve is shown in Figure 1 and a cell number response curve is shown in Figure 2.

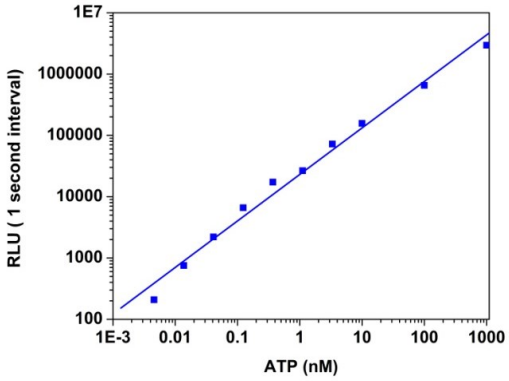

Figure 1. ATP dose response was measured with the PhosphoWorks™ Luminescence ATP Assay Kit *DTT-Free* on a 96-well white plate using a NOVOstar plate reader (BMG Labtech). The kit can detect (3pmole/well) 0.03 nM ATP within 20 min. The integration time was 1 sec. The half life is more than 2 hours.

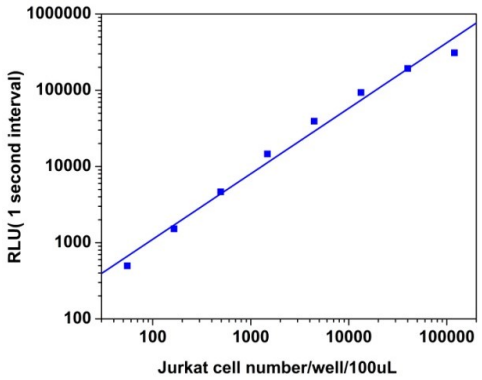

Figure 2. Jurkat cell number was measured with the PhosphoWorks™ Luminescence ATP Assay Kit *DTT-Free * on a 96-well white plate using a NOVOstar plate reader (BMG Labtech). The kit can detect as low as 100 cells. The integration time was 1 sec.

References

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2. de Wet JR, Wood KV, Helinski DR, DeLuca M, (1985) Cloning of firefly luciferase cDNA and the expression of active luciferase in Escherichia coli, Proc. Natl. Acad. Sci USA 82,7870-7873.
3. Khan, H.A. (2003) Bioluminometric assay of ATP in mouse brain: Determinant factors for enhanced test sensitivity, J. Bioscience 28, 379-382.
4. Drew, B and C. Leeuwenburgh (2003) Method for measuring ATP production in isolated mitochondria: ATP production in brain and liver mitochondria fo Fischer-344 rats with age and caloric restriction, Am J. Physiol. Regul. Integr. Comp. Physiol., 285, R1260-R1268.
5. Hara, K. Y. and Mori, H. (2006) An efficient method for quantitative determination of cellular ATP synthetic activity, *J Biomol Screen* 11**,** 310-7.