PhosphoWorks™ Luminometric ATP Assay Kit

Steady Glow

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 21609(1 plate), 21609-Bulk (10 plates)	Keep in freezer and protect from light	Luminescence microplate readers

Introduction

Adenosine triphosphate (ATP) plays a fundamental role in cellular energenics, 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.

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

As Kit 21610, this assay is also based on the detection of ATP using firefly luciferase to catalyze the release of light with ATP and luciferin. Complementary to Kit 21610, this kit provides a more stable luminescence signal that lasts for a few hours, making it convenient to be used with the luminometers that are not equipped with liquid handling capacity. Although Kit 21609 is less sensitive than Kit 21610, its more stable signal provides advantages for some particular applications, such as rapid diagnostic applications. This kit has been used successfully for rapid food safety inspection with a hand-held luminometer.

Kit Key Features

Sensitive:	Detect as low as 10 cells per well.
Continuous:	Stable luminescence, and suitable for manual or automated operations without mixing or a separation step.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	**Amount**	
	Cat. # 21609 (1 plate)	**Cat. # 21609-Bulk (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)

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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 250-2,000 cells/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 recommend to use 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 the whole vial of 10 mL Reaction Buffer (Component C) into ATP Sensor (Component B), and mix well.

2.3 Add 20 µL of ATP Monitoring Enzyme (Component A) into the solution prepared at Step 2.2.
Note: Aliquot and store the unused Components A and C at -20 °C. 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 a desired period of time, such as 24, 48 or 96 hours.

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

3.4 Monitor 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 of dilutions of ATP in PBS buffer with 0.1% BSA by including a sample without ATP (as a control) for measuring background luminescence.
Note: Typically ATP concentrations from 1 nM to 10 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, and 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 Monitor 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 cell or samples. An ATP titration curve is shown in Figure 1 and a cell number response curve is shown in Figure 2.

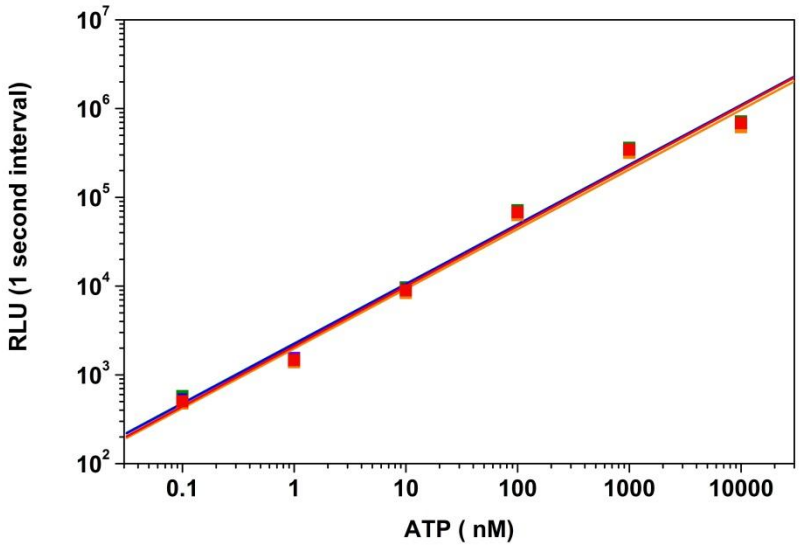

Figure 1 ATP dose response was measured with the PhosphoWorks™ Luminescence ATP Assay Kit on a 96-well white plate using a NOVOstar plate reader (BMG Labtech). The linear luminescence signal for ATP concentrations from 100 µM to 0.1 nM was monitored for up to 5 hours (Z' factor = 0.7) without signal decay (above figure shows 20 minutes, 1, 2, 3, 4, and 5 hour signal). The integrated time was 1 second.

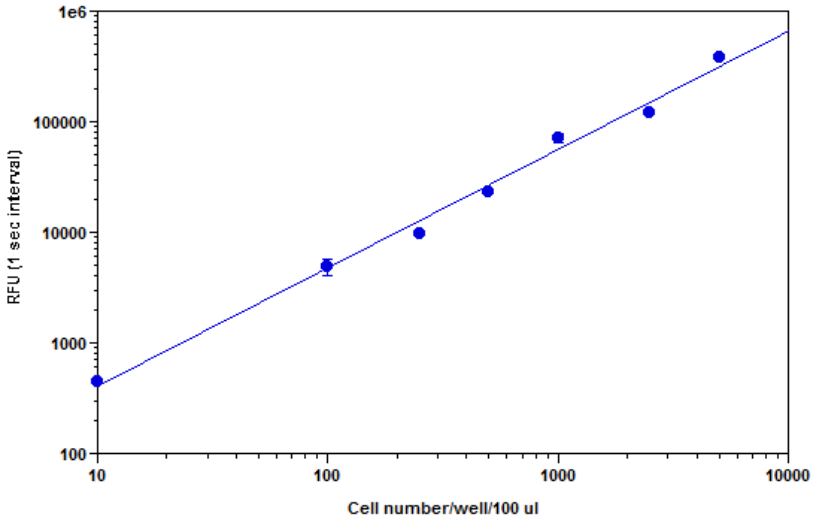

Figure 2 CHO-K1 cell number was measured with the PhosphoWorks™ Luminescence ATP Assay Kit on a 96-well white plate using a NOVOstar plate reader (BMG Labtech). The luminescence signal for CHO-K1 cells down to 100 cells per well was monitored for up to 2 hours (Z' factor = 0.6). The integrated time was 1 second.

References

1. McElroy, W.D. (1947) The Energy Source for Bioluminescence in an isolated System. Proc. Natl. Acad. Sci. USA **33**,342.
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.
6. Sun, Y. and Chai, T. C. (2006) Augmented extracellular ATP signaling in bladder urothelial cells from patients with interstitial cystitis *Am J Physiol Cell Physiol* 290, C27-34.