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 avoid 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. 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 using a luminometer can be used the quantitation of ATP.

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

The PhosphoWorks™ ATP Assay Kit 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. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format, and is extremely sensitive (detects 10-100 cells/well). The high sensitivity of this assay permits the detection of ATP in many biological systems, environmental samples and foods.

Kit Key Features

Sensitive:	Can detect as low as 10cells/well.
Continuous:	Stable luminescence, suitable for manual or automated operations with no mixing or separations required.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	**#21609 (1 plate)**	**#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)

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 min → Read 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 96-well) or 250-2,000cells/20 µL/well (for 384-well) plates.

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 96-well) or 500-5,000 cells/20 µL/well (for 384-well) poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with break 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 for achieving the best results.

2. Prepare ATP assay solution:

2.1 Thaw all the components to room temperature before use.

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

2.3 Add 20 µL of Component A (ATP Monitoring Enzyme) into the solution prepared from step 2.2.
Note: Aliquot and store the unused Components A and C at -20°C, avoiding 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 (for 96-well plates) 10X or 5 µL (for 384-plates) 5X compounds in desired compound buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.

3.2 Incubate the cell plates in 37°C, 5% CO_2 incubator for the 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) per well of ATP assay solution (from step 2.3) for 10-20 min at room temperature.

3.4 Read luminescence intensity using a standard luminometer.

4. Establish standard ATP calibration curve:

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 10 pM to 10 nM are appropriate.

4.2 Add the same amount of the diluted ATP solution into an empty plate (100 µL for 96-well plate, 25 µL for 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 from 10 to 20 min at room temperature.

4.5 Record the luminescence intensity using 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 can be varied depending upon the sources of the growth media or the microtiter plates. Calculate the amount of ATP in test cell (or samples) from the standard curve. The typical data are included in Figures 1 (ATP titration curve) and 2 (Cell number response curve).

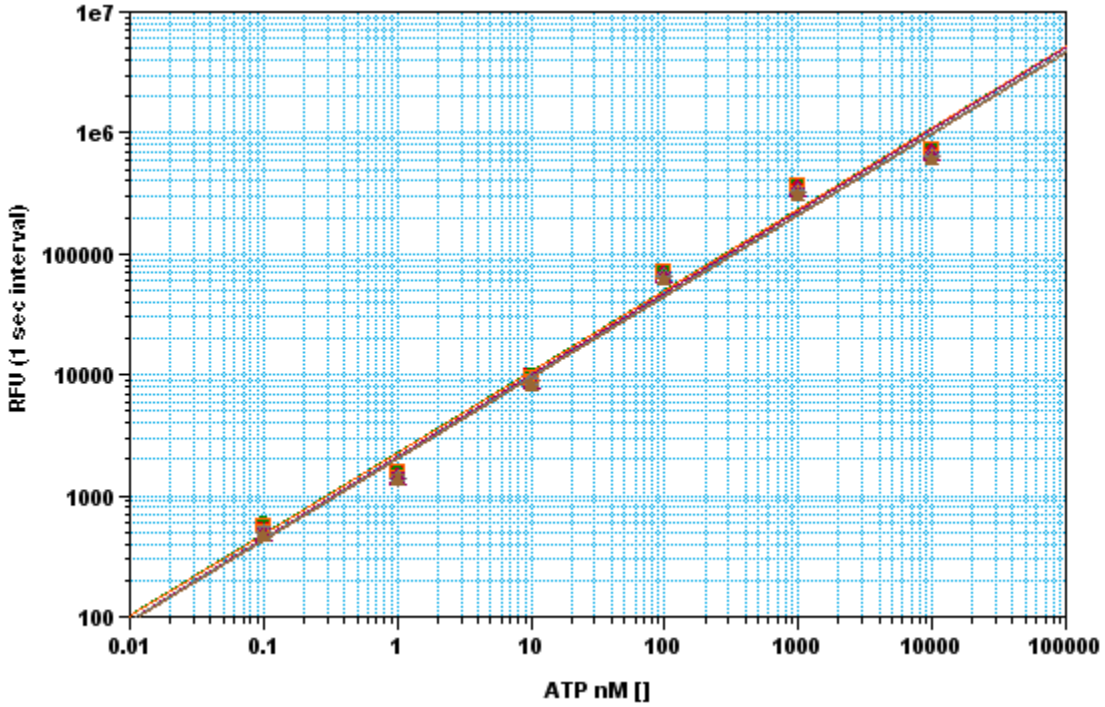

Figure 1. ATP dose response was measured with the PhosphoWorks™ Luminescence ATP Assay Kit on 96-well white plate using a NOVOstar plate reader (BMG Labtech). The linear luminescence signal for ATP concentrations from 10 µM to 0.1 nM was detected up to 5 hr (Z' factor = 0.7) without signal decayed (above figure shows 20 min, 1, 2, 3, 4, and 5 hr signal). The integrated time was 1 sec.

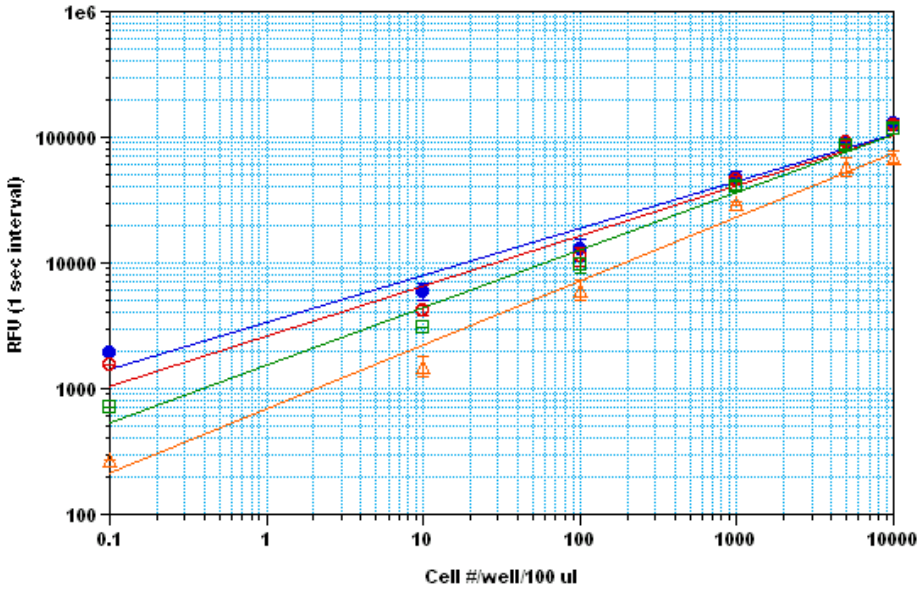

Figure 2. CHO-K1 cell number was measured with the PhosphoWorks™ Luminescence ATP Assay Kit on 96-well white plate using a NOVOstar plate reader (BMG Labtech). The luminescence signal for CHO-K1 cells down to 10 cells per well was detected up to 2 hr (Z' factor = 0.6). The integrated time was 1 sec.

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.