PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*

Catalog number: 21612, 21613
Unit size: 1 Plate, 10 Plates

Component	Storage	Amount	
		Cat No. 21612	Cat No. 21613
Component A: ATP Monitoring Enzyme	Freeze (<-15 °C), Avoid Light	1 vial	1 vial
Component B: ATP Sensor (Light-sensitive)	Freeze (<-15 °C), Dessicated, Avoid Light	1 vial	10 vials
Component C: Reaction Buffer	Freeze (<-15 °C), Avoid Light	1 vial (10 mL)	2 vials (50 mL/vial)

OVERVIEW

Adenosine triphosphate (ATP) plays a fundamental role in cellular energenics, metabolic regulation and cellular signaling. The PhosphoWorks™ ATP Assay Kit provides a fast, simple and homogeneous luminescence assay for the determination of cell proliferation and cytotoxicity in mammalian cells. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format. The high sensitivity of this assay permits the detection of ATP in many biological systems, environmental samples and foods. This PhosphoWorks ATP Assay Kit does not use DTT, and has the stable luminescence signal as long as 4 hours. It has stable luminescence with no mixing or separations required, and formulated to have minimal hands-on time.

AT A GLANCE

Protocol summary

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

Important To achieve the best results, it's strongly recommended to use the white plates. Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Luminescence microplate reader
Recommended plate:	Solid white

PREPARATION OF WORKING SOLUTION

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

2. Add 20 µL of ATP Monitoring Enzyme (Component A) into the bottle of Component B+C and mix well to make ATP working solution.

Note Avoid potential ATP contamination from exogenous biological sources.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Run ATP assay:

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.

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. Add 100 µL (96-well plate) or 25 µL (384-well plate) of ATP working solution into each well.

4. Incubate at room temperature for 10 - 20 minutes.

5. Monitor luminescence intensity with a standard luminometer.

Generate a standard ATP calibration curve:

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

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.

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).

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of ATP working solution.

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

5. Record the luminescence intensity with a standard luminometer.

6. Generate the ATP standard curve.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RLU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate ATP samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

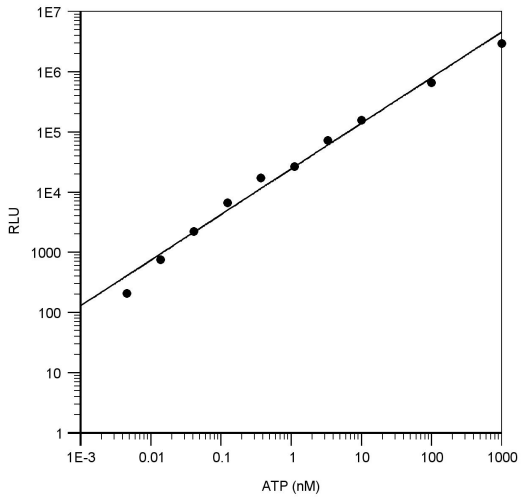

Figure 1. ATP dose response was measured with the PhosphoWorks™ Luminescence ATP Assay Kit *DTT-Free* on a 96-well white plate using a NOVOstar plate reader (BMG Labtech). The kit can detect (3 pmole/well) 0.03 nM ATP within 20 min. The integration time was 1 sec. The half life is more than 2 hours.

DISCLAIMER