PhosphoWorks™ Fluorimetric ATP Assay Kit

Catalog number: 21620
Unit size: 100 Tests

Component	Storage	Amount
Component A: Amplite™ Red Substrate (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Enzyme Mix	Freeze (<-15 °C), Minimize light exposure	1 bottle (lyophilized powder)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (5 mL)
Component D: ATP Standard	Freeze (<-15 °C), Minimize light exposure	2.8 mg/vial
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Adenosine triphosphate (ATP) plays a fundamental role in cellular energetics, metabolic regulation and cellular signaling. It is referred as the \"molecular unit of currency\" of intracellular energy transfer to drive many processes and chemical synthesis in living cells. ATP also serves as a signaling molecule for cell communication and plays an important role in DNA and RNA synthesis. AAT Bioquest offers a variety of bioluminescence assay kits to determine nanomolar (nM) range of ATP with recombinant firefly luciferase (Cat# 21610 & 21609). These kits require luminescence plate readers, are frequently used for cell viability or cytotoxicity assays. PhosphoWorks™ Fluorimetric ATP Assay Kit is based on a serial ATP-induced enzyme coupled reactions to produce hydrogen peroxide, which is spectrophotometrically quantified with our Amplite™ Red Substrate. The assay can detect ~0.4 µM of ATP in a 100 µL reaction volume with minimal interference from ADP and AMP. It provides a robust, simple and convenient assay for measuring ATP levels in biological samples. The PhosphoWorks™ Fluorimetric ATP Assay is complementary to our luciferase-based ATP assay kits.

AT A GLANCE

Protocol summary

1. Prepare ATP working solution (50 µL)
2. Add ATP standards or test samples (50 µL)
3. Incubate at room temperature for 10 - 30 minutes
4. Monitor fluorescence increase at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important Thaw the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 mn
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Amplite™ Red Substrate stock solution (200X):
Add 30 µL of DMSO (Component E) into the vial of Amplite™ Red Substrate (Component A) to make 200X Amplite™ Red Substrate stock solution.

2. ATP standard solution (10 mM):
Add 0.5 mL of ddH2O into the vial of ATP Standard (Component D) to make 10 mM ATP standard solution.

PREPARATION OF STANDARD SOLUTION

ATP standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/21620

Add 10 µL of 10 mM ATP standard solution into 990 µL 1X PBS buffer to generate 100 µM ATP standard solution (AS7). Take 100 µM ATP standard solution (AS7) and perform 1:3 serial dilutions to get serially diluted ATP standards (AS6-AS1) with 1X PBS buffer.

PREPARATION OF WORKING SOLUTION

1. Add 5 mL of Assay Buffer (Component C) into Enzyme Mix bottle (Component B), and mix well.

2. Add 25 µL of 200X Amplite™ Red Substrate stock solution to the Enzyme Mix bottle, mix well to make APT working solution.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of ATP standards and test samples in a solid black 96-well microplate. AS= ATP Standards (AS1 - AS7, 0.14 to 100 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
AS1	AS1	...	...
AS2	AS2	...	...
AS3	AS3		
AS4	AS4		
AS5	AS5		
AS6	AS6		
AS7	AS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
AS1 - AS7	50 µL	Serial Dilutions (0.14 to 100 µM)
BL	50 µL	1 X PBS Buffer
TS	50 µL	test sample

1. Prepare ATP standards (AS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of ATP working solution to each well of ATP standard, blank control,

and test samples to make the total ATP assay volume of 100 µL/well. For a 384-well plate, add 25 µL of ATP working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 10 - 30 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em=540/590 nm (Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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

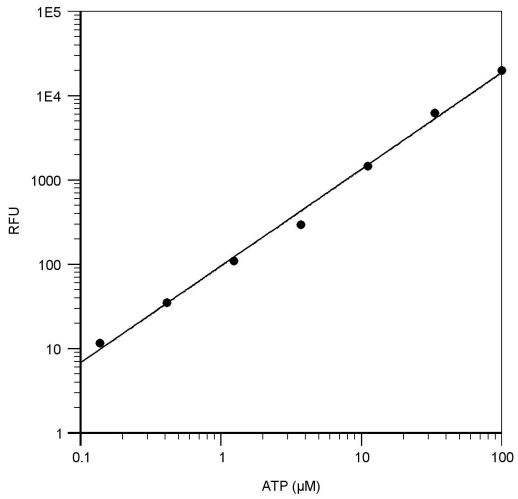

Figure 1. ATP dose response was measured with PhosphoWorks™ Fluorimetric ATP Assay Kit in a 96-well solid black plate using a Germini microplate reader (Molecular Devices).

DISCLAIMER