

## ReadiUse™ Rapid Luminometric ATP Assay Kit

Catalog number: 21601, 21602, 21603  
Unit size: 100 Tests, 1000 Tests, 10000 Tests

Component	Storage	Amount		
		Cat No. 21601	Cat No. 21602	Cat No. 21603
Component A: ReadiUse™ ATP Assay Reagent	Freeze (<-15 °C), Minimize light exposure	10 ml	100 mL	10 x 100 mL

### OVERVIEW

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 cytotoxicity, 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 catalyzes 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. AAT Bioquest's ReadiUse™ Rapid Luminometric ATP Assay Kit (DTT free) comes with all the essential components in a ready-to-use format. It provides a fast, simple and homogeneous luminescence assay for monitoring 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 on a chemiluminescent microplate reader. The assay is extremely sensitive and can detect 50 cells/well. It has stable luminescent signal with half-life more than 2 hours. This ReadiUse™ Rapid Luminometric ATP Assay Kit does not use DTT, which eliminating the unpleasant odor.

### 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 ready-to-use ReadiUse™ Rapid Luminometric ATP Assay Reagent (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 kits components at room temperature before starting the experiment.

### KEY PARAMETERS

Instrument: Luminescence microplate reader  
Recommended plate: Solid white

### PREPARATION OF STANDARD SOLUTION

#### ATP standard

For convenience, use the Serial Dilution Planner:  
<https://www.aatbio.com/tools/serial-dilution/21601>

Optional, ATP is not provided.

Make 1 mM ATP stock solution with ddH<sub>2</sub>O or appropriate buffer, then perform serial dilution to achieve ATP concentrations ranging from 10 pM to 10 µM.

### 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

**Table 1.** Layout of ATP Standards and test samples in a white 96-well microplate. SD=ATP Standard, BL=Blank Control, TS=Test Sample

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

**Table 2.** Reagent composition for each well

Well	Volume	Reagent
SD1-SD7	100 µL	Serial Dilution of ATP (e.g. 10 pM to 10 µM)
BL	100 µL	ATP Assay Buffer (Component A)
TS	100 µL	Sample

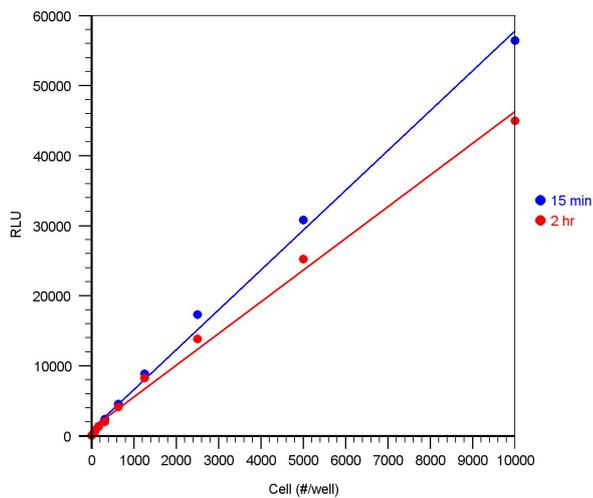
1. Treat cells (or samples) with test compounds by adding 10 µL of 10X compounds for a 96-well plate or 5 µL of 5X compounds for a 384-well plate in desired compound buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.
  2. Incubate the cell plate in a 37 °C, 5% CO<sub>2</sub> incubator for the desired period of time, such as 24, 48 or 96 hours.
  3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of ReadiUse™ Rapid Luminometric ATP Assay Reagent.
  4. Incubate at room temperature for 10 - 20 minutes.
- Note** Aliquot and store the unused ReadiUse™ Rapid Luminometric ATP Assay Reagent at -20 °C, and avoid repeated freeze/thaw cycles.
4. Monitor the luminescence intensity with a standard luminometer.

### 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 baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Cell 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.** Cell number correlates with the luminescent signal. Different Jurkat cell number (2-fold dilution) was measured using the RadiUse™ Rapid Luminometric ATP Assay Kit in a 96-well white plate using a ClarioStar plate reader (BMG Labtech). The kit can detect as low as 50 cells. There is a linear relationship ( $r^2 > 0.99$ ) between the luminescent signal and cell number after 15 minutes or 2 hours incubation. The half-life of luminescent signal is more than 2 hours.

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