

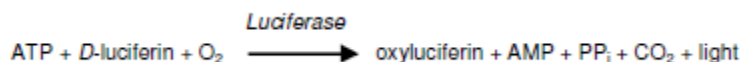
Adenosine Triphosphate (ATP) Assay Kit (Chemiluminescent)

LS-K254-100 (100 Tests) • Store at -20°C



Introduction

Adenosine 5'-triphosphate (ATP) is the chemical energy for cellular metabolism and is often referred to as energy currency of the cell. ATP is produced only in living cells during photosynthesis and cellular respiration and consumed in cellular processes including biosynthetic reactions, motility and cell division. It is a key indicator of cellular activity and has been utilized as a measure of cell viability and cytotoxicity in research and drug discovery. This ATP Assay Kit provides a rapid Method to measure intracellular ATP. The single working reagent lyses cells to release ATP, which, in the presence of luciferase, immediately reacts with the Substrate *D*-luciferin to produce light. The light intensity is a direct measure of intracellular ATP concentration.



This non-radioactive, homogeneous cell-based assay is performed in microplates. The reagent is compatible with all liquid handling systems for high-throughput screening applications in 96-well and 384-well plates.

Key Features

- Safe. Non-radioactive assay.
- Sensitive and accurate. As low as 0.02 μM ATP or a single cell can be quantified.
- Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.
- Robust and amenable to HTS: Z' factors of > 0.5 are routinely observed in 96-well and 384-well plates. Can be readily automated on HTS liquid handling systems.

Applications

- ATP determination in cells and other biological samples.

Components

Component	K254-100
	100 Tests
Assay Buffer	10 mL
Substrate	120 μL
ATP Enzyme	120 μL
Standard (3 mM ATP)	100 μL

Storage

The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 12 months after receipt.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121

www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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Assay Procedure

Assays can be carried out in a tube or in a microplate.

Procedure

1. Standard Curve. Prepare 500 μL 30 μM ATP Premix by mixing 5 μL 3 mM Standard and 495 μL distilled water (for cell culture samples dilute ATP in culture media). Dilute standard as shown in the Table. Transfer 10 μL standards into wells of a white opaque 96-well plate.

Samples. Use 10 μL sample per well in separate wells.

For tissue samples, homogenize 20 mg sample in 200 μL of cold phosphate-buffered saline, spin at 12,000 g for 5 min to pellet any debris. Transfer 1-10 μL supernatant to each well and bring the volume to 10 μL with PBS. Test several doses of the sample and choose the readings that are within the standard curve range for ATP calculation.

No	Premix + H ₂ O/media	Vol (μL)	ATP (μM)
1	50 μL + 0 μL	50	30
2	40 μL + 10 μL	50	24
3	30 μL + 20 μL	50	18
4	20 μL + 30 μL	50	12
5	15 μL + 35 μL	50	9
6	10 μL + 40 μL	50	6
7	5 μL + 45 μL	50	3
8	0 μL + 50 μL	50	0

For suspension cells, transfer 10 μL of the cultured cells (10^3 - 10^4) into a white opaque 96 well plate.

For adherent cells, culture 10^3 - 10^4 cells in white opaque microplate. At the time of assay, remove the culture medium immediately before adding 90 μL Reconstituted Reagent (see below).

2. Assay. Bring Assay Buffer and Substrate to room temperature. Thaw enzyme on ice or at 4°C. Fresh Reconstitution is recommended. Store unused reagents including the enzyme at -20°C.

For each 96-well, mix 95 μL Assay Buffer with 1 μL Substrate and 1 μL ATP Enzyme. Add 90 μL Reconstituted Reagent to each well.

Mix by tapping the plate.

3. Read luminescence on a luminometer within 1 min after adding Reconstituted Reagent.

General Considerations

Signal stability. Since the signal of the reaction decreases by $\sim 1\%$ each minute, for most accurate results, care should be taken that the time between adding the Reconstituted Reagent and luminescence reading is the same for all samples and standards.

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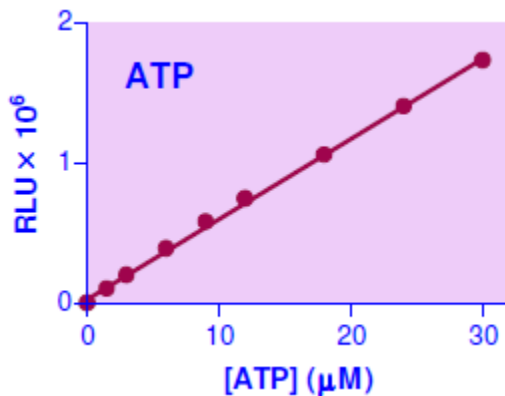
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Sample Data



ATP Standard Curve in Water

Version: V.08.09.2018

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