EnzyLight™ ATP Assay Kit (EATP-100)
Rapid bioluminescent determination of ATP

DESCRIPTION

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.

BioAssay Systems' EnzyLight™ 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.

$$\text{ATP} + \textit{D}\text{-luciferin} + O_2 \xrightarrow{\textit{Luciferase}} \text{oxyluciferin} + \text{AMP} + PP_i + CO_2 + \text{light}$$

This non-radioactive, homogeneous cell-based assay is performed in microplates. The reagent is compatible with all culture media and 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 0.1 µM ATP or 40 cells 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 for processing thousands of samples per day.

APPLICATIONS

ATP determination in cells and other biological samples.

KIT CONTENTS

Assay Buffer:	**10 mL**
Substrate:	**120 µL**
ATP Enzyme:	**120 µL**
Standard:	**100 µL 3 mM ATP**

Storage conditions: store all reagents at -20°C. This kit is shipped on dry ice. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE

1. *Standard Curve*. Prepare 1000 µL 30 µM ATP Premix by mixing 10 µL 3 mM Standard and 990 µL distilled water (for cell culture samples dilute ATP in culture media). Dilute standard as follows. Transfer 100 µL standards into wells of a white opaque 96-well plate.

No	Premix + H_2O/media	Vol (µL)	ATP (µM)
1	150 µL + 0 µL	150	30
2	120 µL + 30 µL	150	24
3	90 µL + 60 µL	150	18
4	60 µL + 90 µL	150	12
5	45 µL + 105 µL	150	9
6	30 µL + 120 µL	150	6
7	15 µL + 135 µL	150	3
8	0 µL + 150 µL	150	0

Samples. Add 100 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 1-100 µL supernatant to each well and bring the volume to 100 µL with PBS. Test several doses of the sample and choose the readings that are within the standard curve range for ATP calculation.

For cell cultures, plate cells (100 µL/96well plate, 25 µL/384well plate) in white opaque tissue culture plates. If desired, add 5 µL test compounds and controls dissolved in PBS or culture medium per well. Rock plate lightly to mix and incubate for desired period of time (e.g. overnight).

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.

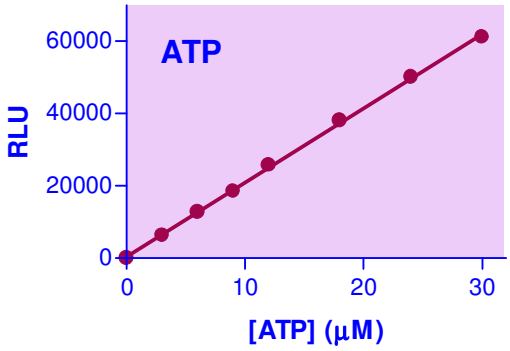
   For each 384-well, mix 30 µL Assay Buffer with 0.3 µL Substrate and 0.3 µL ATP Enzyme. Add 25 µL Reconstituted Reagent to each well.

   Mix by tapping the plate. Incubate for 10 minutes at room temperature.

3. Read luminescence on a luminometer. For most luminometers (Berthold Luminometer, LJL Analyst, Top Count, MicroBeta Counters, CLIPR and LeadSeeker), integration time of 0.1 to 5 sec is appropriate.

GENERAL CONSIDERATIONS

Signal stability. After adding the Reconstituted Reagent, the luminescence signal is stable for about 20 min and decreases slowly thereafter.



ATP Standard Curve in Water

PUBLICATIONS

1. Schwarzer C., et al. (2008). Oxidative stress caused by pyocyanin impairs CFTR Cl(-) transport in human bronchial epithelial cells. Free Radic. Biol. Med. 45(12):1653-62.
2. Chandak P.G., et al. (2010). Efficient phagocytosis requires triacylglycerol hydrolysis by adipose triglyceride lipase. J Biol. Chem. 285(26):20192-201.
3. Belleannée C., et al. (2010). Role of purinergic signaling pathways in V-ATPase recruitment to apical membrane of acidifying epididymal clear cells. Am. J. Physiol. Cell Physiol. 298(4): C817-C830.