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 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.

KIT CONTENTS

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

Storage conditions: The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 12 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

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

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_2O/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.

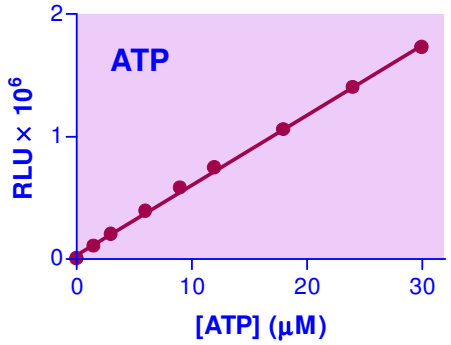
   For each 96-well, mix 95 µL Assay Buffer with 1 µL Substrate and 1 µL ATP Enzyme. Add 90 µL Reconstituted Reagent to each well.

   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 ~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.



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.