# EnzyLight™ ADP/ATP Ratio Assay Kit (ELDT-100)
## Bioluminescent Assay for ADP/ATP Ratio

## DESCRIPTION

Changes in the ADP/ATP ratio have been used to differentiate modes of cell death and viability. Increased levels of ATP and decreased levels of ADP signify proliferating cells. Conversely, decreased levels of ATP and increased levels of ADP represent apoptotic or necrotic cells where the decrease in ATP and increase in ADP is much more pronounced in necrosis versus apoptosis.

BioAssay Systems' EnzyLight™ ADP/ATP Ratio Assay Kit provides a rapid method to measure ADP and ATP levels for the screening of apoptosis, necrosis and cell proliferation in mammalian cells. The assay involves two steps. In the first step, the working reagent lyses cells to release ATP and ADP. In the presence of *luciferase*, ATP immediately reacts with the Substrate *D*-luciferin to produce light. The light intensity is a direct measure of intracellular ATP concentration.

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

In the second step, the ADP is converted to ATP through an enzyme reaction. This newly formed ATP then reacts with the D-luciferin as in the first step.

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.
**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 and above 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

**Apoptosis and Necrosis determination** in cells.
**Cell proliferation:** effects of cytokines, growth factor, nutrients.
**Drug discovery:** high-throughput screening for anticancer drugs.

## KIT CONTENTS

| | |
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| **Assay Buffer:** | **10 mL** |
| **Substrate:** | **120 µL** |
| **Cosubstrate** | **120 µL** |
| **ATP Enzyme:** | **120 µL** |
| **ADP Enzyme:** | **120 µL** |

**Storage conditions:** The kit is shipped on ice. Store all reagents at -20°C. This product is shipped on dry ice. 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 96-well plate. For consistency, it is recommended that the time between the three luminescence measurements be the same for all samples.

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

   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 *ATP Reagent* (see below).

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

   *ATP Reagent*. For each 96-well, mix 95 µL Assay Buffer with 1 µL Substrate, 1 µL Cosubstrate and 1 µL ATP Enzyme.

   Add 90 µL *ATP Reagent* to each well and mix by tapping the plate. After 1 min, read luminescence (RLU A) on a luminometer.

3. ***ADP Assay***. Prepare *ADP Reagent*: for each 96-well, mix 5 µL $dH_2O$ with 1 µL ADP Enzyme.

   Ten minutes after reading the luminescence for ATP (RLU A), read the luminescence of the samples again (RLU B). This measurement provides the background prior to measuring ADP (i.e. the residual ATP signal).

   Immediately following reading RLU B, add 5 µL *ADP Reagent* to each well and mix by tapping the plate or pipetting up and down. After 1 min. read luminescence (RLU C) on a luminometer.

4. *Calculation of ADP/ATP Ratio*. Subtract RLU B from RLU C, then divide by RLU A:

$$\text{ADP/ATP Ratio} = \frac{\text{RLU C} - \text{RLU B}}{\text{RLU A}}$$

## RESULTS INTERPRETATION

The interpretation of different ratios obtained may vary significantly according to the cell types and conditions used. However, the following may be used as guidelines:

1. Test gives markedly elevated ATP levels with no significant increase in ADP levels in comparison to control cells = proliferation.
2. Test gives lower ATP levels with an increase in ADP levels in comparison to control cells = apoptosis.
3. Test gives markedly lower ATP levels with greatly increased ADP levels in comparison to control cells = necrosis.

## PUBLICATIONS

1. Rajamani, U., Gross, A. R., Ocampo, C., Andres, A. M., Gottlieb, R. A., & Sareen, D. (2017). Endocrine disruptors induce perturbations in endoplasmic reticulum and mitochondria of human pluripotent stem cell derivatives. Nature communications, 8(1), 219.
2. Villa-Bellosta, R., Hamczyk, M. R., & Andres, V. (2017). Novel phosphate-activated macrophages prevent ectopic calcification by increasing extracellular ATP and pyrophosphate. PloS one, 12(3), e0174998
3. Taneike, M., Nishida, K., Omiya, S., Zarrinpashneh, E., Misaka, T., Kitazume-Taneike, R. & Shah, A. M. (2016). mTOR hyperactivation by ablation of tuberous sclerosis complex 2 in the mouse heart induces cardiac dysfunction with the increased number of small mitochondria mediated through the down-regulation of autophagy. PLoS One, 11(3), e0152628.