



## 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.

Luciferase

$\text{ATP} + \text{D-luciferin} + \text{O}_2 = \text{oxyluciferin} + \text{AMP} + \text{PPi} + \text{CO}_2 + \text{light}$

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. Due to a special formulation of the reagent system which greatly stabilizes the light signal generated by the luciferase reaction, the luminescence from the initial ATP measurement remains stable throughout this assay. Therefore, the second light intensity measured represents the total ADP and ATP concentration in the sample.

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

Assay Buffer: 10 mL

Substrate: 120 µL

Cosubstrate 120 µL

ATP Enzyme: 120 µL

ADP Enzyme: 120 µL

Storage conditions: store all reagents at -20°C. This product 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. Sample Preparation. For 96-well assays, plate 20,000 to 50,000 cells at 100 µL/well. For 384-well assays, plate 5,000 to 15,000 cells at 25 µL/well. Use 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. 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. For each 384-well, mix 30 µL Assay Buffer with 0.3 µL Substrate, 0.3 µL Cosubstrate and 0.3 µL ATP Enzyme.

Add ATP Reagent to each well (90 µL/96well, 25 µL/384well) and mix by tapping the plate. Incubate for 10 minutes at room temperature.

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

3. ADP Assay. Prepare ADP Reagent: for each 96-well, mix 12 µL dH<sub>2</sub>O with 1 µL ADP Enzyme. For each 384-well, mix 3 µL dH<sub>2</sub>O with 0.25 µL ADP Enzyme.

Add ADP Reagent to each well (10 µL/96well, 2.5 µL/384well) and mix by tapping the plate or pipetting up and down. Incubate for 10 minutes at room temperature. Read luminescence (RLU B) on a luminometer.

4. Calculation of ADP/ATP Ratio. Subtract RLU A from RLU B, then divide by Data A:  $ADP/ATP \text{ Ratio} = RLU B - RLU A$

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. (Ratio <0.1)
2. Test gives lower ATP levels with an increase in ADP levels in comparison to control cells = apoptosis. (Ratio ~0.1-1.0)

3. Test gives markedly lower ATP levels with greatly increased ADP levels in comparison to control cells = necrosis. (Ratio > 1.0)

#### PUBLICATIONS

1. Schafer ZT, et al. (2009). Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment. *Nature*. 461(7260):109-113.

2. Bekeredjian R, et al. (2010). Conditional HIF-1alpha expression produces a reversible cardiomyopathy. *PLoS One*. 5(7): e11693.

3. Chandak PG, et al. (2010). Efficient phagocytosis requires triacylglycerol hydrolysis by adipose triglyceride lipase. *J Biol Chem*. 285(26):20192-201.

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