EnzyLight™ ADP Assay Kit (EADP-100)
Rapid bioluminescent determination of ADP

DESCRIPTION

BioAssay Systems' EnzyLight™ ADP Assay Kit provides a rapid method to measure ADP levels. 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. 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.
Sensitive and accurate. As low as 0.1 µM ADP 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 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

ADP determination in cells and other biological samples.

KIT CONTENTS

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

Storage conditions: store all reagents at -20°C. This product is shipped on dry ice. Shelf life of at least 6 months.

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. *1. Standard Curve*. Prepare 1000 µL 30 µM ADP Premix by mixing 10 µL 3 mM Standard and 990 µL distilled water (for cell culture samples dilute ADP 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)	ADP (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 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).

For other biological samples, transfer 100 µL (25 µL for 384 well plates) of the sample to wells in a white opaque titer plate.

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 Substrate 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, LJL Analyst, Top Count, MicroBeta Counters, CLIPR and LeadSeeker), integration time of 0.1 to 5 sec is appropriate.

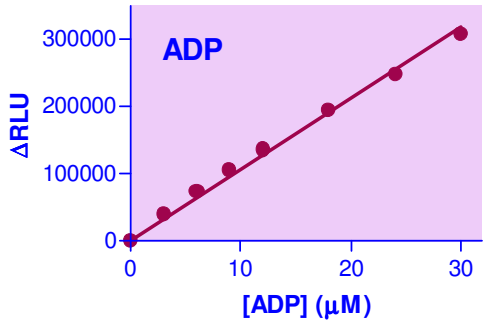
3. ***ADP Assay***. Prepare *ADP Reagent*: for each 96-well, mix 12 µL dH_2O with 1 µL ADP Enzyme. For each 384-well, mix 3 µL dH_2O with 0.3 µL ADP Enzyme.

   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 Concentration*. Subtract RLU A from RLU B for standards and samples. Plot the ΔRLU versus ADP concentration for the standards. From the slope of this plot, the *Sample* ADP concentration can be computed with the following equation:

$$[\text{ADP}]_{\text{sample}}\ (\mu M) = \frac{(\text{RLU B})_{\text{sample}} - (\text{RLU A})_{\text{sample}}}{\text{Slope}}$$



ADP Standard Curve in Water

LITERATURE

[1]. Bradbury DA, et al (2000). Measurement of the ADP:ATP ratio in human leukaemic cell lines can be used as an indicator of cell viability, necrosis and apoptosis. *J Immunol Methods*. 240:79-92.

[2]. Chen-Scarabelli C, et al (2004). Turning necrosis into apoptosis: the exacting task that can enhance survival. *Am Heart J*. 148(2):196-9.

[3]. Crouch S, et al (1993). The use of ATP Bioluminescence as a measure of cell proliferation and cytotoxicity. *J Immunol Methods*, 160(1): 81-8.