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. 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 0.02 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: 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 white microplate for luminescent assays. For consistency, it is recommended that the time between the two luminescence measurements be the same for all samples.

1. *Standard Curve*. Prepare 500 µL 30 µM ADP Premix by mixing 5 µL 3 mM Standard and 495 µL distilled water (for cell culture samples dilute ADP in culture media). Dilute standard as follows. Transfer 10 µL standards into wells of a white opaque 96-well plate.

No	Premix + H_2O/media	Vol (µL)	ADP (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

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 ADP calculation.

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 ATP Reagent (see below).

2. *ATP Reaction*. 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 10 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. Immediately following reading RLU A, add 5 µL *ADP Reagent* to each well and mix by tapping the plate or pipetting up and down. Incubate for 2 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:

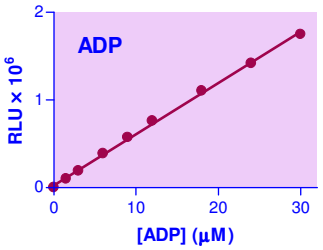
$$[\text{ADP}]_{\text{sample}}\ (\mu\text{M}) = \frac{(\text{RLU B})_{\text{sample}} - (\text{RLU A})_{\text{sample}}}{\text{Slope}}$$

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, white flat-bottom 96-well plates, luminescence plate reader.

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.



ADP 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.