ADP / ATP Ratio Assay Kit

ADP / ATP Ratio Assay Kit is a detection kit for the quantification of ADP / ATP Ratio in cells.

Catalog number: ARG82132

Package: 100 tests

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INTRODUCTION

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 are much more pronounced in necrosis versus apoptosis.

PRINCIPLE OF THE ASSAY

This 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.

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

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.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on dry ice. Store all components at -20°C. Shelf life of 12 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Substrate	120 µL	-20°C
Cosubstrate	120 µL	-20°C
ATP Enzyme	120 µL	-20°C
ADP Enzyme	120 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Luminometer
- Centrifuge
- White opaque 96 well microplate.
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 of ATP Reagent (see REAGENT PREPARATION).

REAGENT PREPARATION

- **ATP Reagent:** For each well, mixing 95 µL of Assay Buffer with 1 µL of Substrate, 1 µL of Cosubstrate and 1 µL of ATP Enzyme.
- **ADP Reagent:** For each well, mixing 5 µL of distilled water with 1 µL of ADP Enzyme.

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Keep thawed Enzyme Mix in a refrigerator or on ice during assays. Store unused reagents including the enzyme at-20°C.

ATP Assay

1. Add **90 µL** of **ATP Reagent** to each well and mix by tapping the **sample** at **white opaque 96 well plate**.
2. After **1 minute**, read luminescence (**RLU A**) on a luminometer.

ADP Assay

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

CALCULATION OF RESULTS

1. Calculation of ADP/ATP Ratio.

   ADP/ATP Ratio = (RLU C – RLU B) / RLU A

2. 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:

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