



ADP ATP Ratio Assay Kit

(catalog #107003; 100 assays; store at -20°C)

Description:

The changes in ADP/ATP ratio have been used to differentiate the different modes of cell death and viability. ADP/ATP Ratio Assay kit utilizes bioluminescent detection of the ADP and ATP levels for a rapid screening of apoptosis, necrosis, growth arrest, and cell proliferation simultaneously in mammalian cells. The assay utilizes the enzyme luciferase to catalyze the formation of light from ATP and luciferin, and the light can be measured using a luminometer or Beta Counter. ADP level is measured by its conversion to ATP that is subsequently detected using the same reaction. The assay can be fully automatic for high throughput and is highly sensitive than other methods used for cell viability assays (can detect 100 mammalian cells/well).

APPLICATIONS

1) Cell Proliferation; 2) Cytotoxicity and Apoptosis; 3) HTS

KEY FEATURES

Sensitive and accurate: As low as 100 cells can be accurately quantified.

Simple and high-throughput: Simple procedure; Can be readily automated on HTS liquid handling systems.

Kit Contents for 100 Assays:

1. Assay buffer 12 mL
2. Substrate 120 μ L
3. ATP Enzyme 120 μ L
4. ADP Enzyme 600 μ L

Storage:

Store kit at -20°C. Shelf life of three months. Warm up all of the component (except enzyme) to room temperature before use. Briefly centrifuge all small vials prior to opening.

Protocol:

1. Treatment cells by desired method include without induction control.
2. For each reaction, prepare enough ATP reaction mixture; mix 90 μ L assay buffer, 1 μ L substrate and 1 μ L ATP Enzyme.
3. For suspension cells, transfer 10 μ L of the cultured cells (103– 104) into luminometer plate. Add 90 μ L ATP reaction mixture.

4. For adherent cells, culture 10^3 - 10^4 cells in luminometer plate. Remove the culture medium immediately before adding 90 μ L ATP reaction mixture.
5. Gentle shaking plate for 1 minute, and then read the sample in a luminometer (Data A).
6. After incubate 10 minutes, read the plate one more time (Data B).
7. Add 5 μ L ADP Enzyme into each sample well, gentle shaking plate for 1 minute, then read the sample in a luminometer (Data C).

Calculation:

ADP/ATP Ratio is calculated as: $\frac{\text{Data C} - \text{Data B}}{\text{Data A}}$

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

- a. If test gives markedly elevated ATP values with no significant increase in ADP levels in comparison to control cells, if ADP/ATP ratio is very low, the cells are proliferation condition.
- b. If test gives similar or slightly higher levels of ATP and with little or no change in ADP compared to control, if ADP/ATP ratio is low, the cells are growth arrest condition.
- c. If test gives lower levels of ATP to control but shows an increase in ADP, if ADP/ATP ratio is high, the cells are apoptosis condition.
- d. If test gives considerable lower ATP levels than control but greatly increased ADP, if ADP/ATP ratio is much higher, the cells are necrosis condition.

LITERATURE

1. Arne Lundin (2000) Use of firefly luciferase in ATP related assays of biomass, enzymes, and metabolites *Methods in Enzymology* 305:346-370.
2. Matthew G. Vander Heiden, Navdeep S Chandel, Paul T Schumacker, Craig B Thompson (1999) Bcl-xL Prevents Cell Death following Growth Factor Withdrawal by Facilitating Mitochondrial ATP/ADP Exchange *Molecular Cell* 3(2): 159-167.
3. Russell G. Jones, David R. Plas, Sara Kubek, Monica Buzzai, James Mu, Yang Xu, Morris J. Birnbaum, Craig B. Thompson (2005) AMP Activated Protein Kinase Induces a p53-Dependent Metabolic Checkpoint *Molecular Cell* 18(3): 283-293.



Note: This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals. Avoid contact with eye, skin and clothing. Do not ingest. Wear gloves.

RELATED PRODUCTS:

ADP Colorimetric/Fluorometric Assay Kit (catalog# 107004)

ATP Colorimetric/Fluorometric Assay Kit (catalog# 107002)

Cell Viability Assay Kits (catalog# 110001)