Tribioscience

ADP/ATP Ratio Assay Kit (Bioluminescent)

(Catalog #TBS2015; 100 assays; store at -20°C)

INTRODUCTION

The changes in ADP/ATP ratio have been used to differentiate the different modes of cell death and viability. Tribioscience's ADP/ATP Ratio Assay kit utilizes bioluminescent detection of the ADP and ATP levels for 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 which is subsequently detected using the same reaction. The assay can be fully automatic for high throughput and is more 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

Assay buffer 12 mL; Substrate 120 µL;
ATP Enzyme 120 µL; ADP Enzyme 600 µL.

STORAGE AND HANDLING

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

ASSAY PROTOCOL

1. Treatment cells by desired method include without induction control.
2. For each reaction, prepare enough ATP reaction mixture; mix 90 µL assay buffer, 1µL substrate and 1µL ATP Enzyme.
3. For suspension cells, transfer 10µL of the cultured cells (10^3– 10^4) into luminometer plate. Add 90 µL ATP reaction mixture.
4. For adherent cells, culture 10^3-10^4 cells in luminometer plate. Remove the culture medium immediately before adding 90 µL ATP reaction mixture.
5. Gentling shaking plate for 1 minute, and then read the sample in a luminometer (Data A).
6. After incubating 10 minutes, read the plate one more time (Data B).
7. Add 5 µL 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:

$$\frac{\text{(Data C – Data B)}}{\text{Data A}}$$

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 in **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 ration is low, the cells are in **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 in **apoptosis condition**.
d. If test gives considerably lower ATP levels than control but greatly increased ADP, if ADP/ATP ratio is much higher, the cells are in **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-x_L 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

RELATED PRODUCTS:

Cell Viability Assay Kits (#TBS2001)
ATP Colorimetric/Fluorometric Assay Kit (#TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (#TBS2020)

For research use only.