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Product Information

ADP/ATP Ratio Assay Kit

Catalog Number **MAK135**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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.

The ADP/ATP Ratio Assay kit provides a simple and direct procedure for measuring ADP and ATP levels in cells for the screening of apoptosis, necrosis, and cell proliferation. 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 the intracellular ATP concentration.

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

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.

Components

The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer Catalog Number MAK135A	10 mL
Substrate Catalog Number MAK135B	120 µL
Cosubstrate Catalog Number MAK135C	120 µL
ATP Enzyme Catalog Number MAK135D	120 µL
ADP Enzyme Catalog Number MAK135E	120 µL

Reagents and Equipment Required but Not Provided.

- Luminometer
- 96 well flat-bottom plate – It is recommended to use white plates with clear bottoms for luminescence assays

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

This kit is shipped on dry ice. Storage at –20 °C is recommended.

Procedure

Notes: Assays can be carried out in tubes or in a 96 well plate. The signal of the reaction decreases by ~1% each minute. For best results, it is recommended the times between the three luminescence measurements be the same for all samples.

Sample Preparation

The assay reagents are compatible with all culture media.

For suspension cells, transfer 10 µL of the cultured cells (10^3–10^4) into the 96 well plate.

Adherent cells (10^3–10^4) can be directly cultured in the assay microplate.

Assay Reaction
Bring Assay Buffer, Substrate, and Cosubstrate to room temperature before beginning assay. Thaw ADP enzyme on ice. Aliquot and store unused reagents at –20 °C.

ATP Assay
1. Prepare ATP reagent according to the scheme in Table 1. Add 90 µL of ATP reagent to each well in plate and tap plate briefly to mix. If working with adherent cells, first remove culture medium and then add 90 µL of ATP reagent to each well in plate and tap plate briefly to mix.

Table 1.
ATP Reagent

Reagent	Volume (1 well)
Assay Buffer	95 µL
Substrate	1 µL
Cosubstrate	1 µL
ATP Enzyme	1 µL

2. Incubate plate for 1 minute at room temperature. Read luminescence (relative light units) on a luminometer for the ATP assay (RLU_A).

ADP Assay
1. Incubate the plate for an additional 10 minutes.

2. During the 10 minute incubation, prepare ADP Reagent according to the scheme in Table 2.

Table 2.
ADP Reagent

Reagent	Volume (1 well)
Water	5 µL
ADP Enzyme	1 µL

3. After 10 minute incubation, read luminescence for ATP (RLU_B). This measurement provides the background prior to measuring ADP (i.e., the residual ATP signal).

4. Immediately following the reading of RLU_B, add 5 µL of ADP Reagent to each well and mix by tapping the plate or pipetting. After 1 minute, read luminescence (RLU_C).

Calculations
Calculate the ADP/ATP ratio using the formula below.

$$\text{ADP/ATP ratio} = \frac{RLU_C - RLU_B}{RLU_A}$$

Notes: 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.
1. Test with markedly elevated ATP levels with no significant increase in ADP levels in comparison to control cells is often seen in proliferation.
2. Test with lower ATP levels with an increase in ADP levels in comparison to control cells is often seen in apoptosis.
3. Test with markedly lower ATP levels with greatly increased ADP levels in control cells is often seen in necrosis.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For luminescence assays, use white (opaque) plates with clear bottoms.
	Pipetting errors in the Reaction Mix	Prepare a master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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