

ApoSENSOR™ ADP/ATP Ratio Assay Kit

(Catalog #K255-200; Store kit at -70°C)

I. Introduction:

The changes in ADP/ATP ratio have been used to differentiate the different modes of cell death and viability. Increased levels of ATP and decreased levels of ADP have been recognized in proliferating cells. In contrast, decreased levels of ATP and increased levels of ADP are recognized in apoptotic cells. The decrease in ATP and increase in ADP are much more pronounced in necrosis than apoptosis. The ApoSENSOR™ 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 (detects 100 mammalian cells/well).

II. Kit Contents:

Component	K255-200
	200 assays
Nucleotide Releasing Buffer	50 ml
ATP Monitoring Enzyme (green cap)	1 vial
ADP Converting Enzyme (blue cap)	1 vial
Enzyme Reconstitution Buffer (red cap)	450 µl

III. ApoSENSOR ADP/ATP Ratio Assay Protocol:

A. Reagent Reconstitution and General Consideration:

- Reconstitute ATP Monitoring Enzyme and ADP Converting Enzyme each with 220 µl of the Enzyme Reconstitution Buffer. Mix gently by inversion (**Note:** The reconstituted Enzymes will be milky, cloudy solution, not clear solution). Aliquot enough enzymes (1 µl per assay) for the number of assays to be performed in each experiment and freeze immediately at -70°C for future use. The reconstituted enzymes are stable for up to 3 months at -70°C after reconstitution. For more accurate handling, the enzyme can be dilute 10 fold with Nucleotide Releasing Buffer just before use, then use 10 µl of the enzymes each for each assay.
- The ApoSENSOR™ kit is significantly more sensitive than other methods used for cell viability assays. The method can detect as few as 10 cells, but as a general guide, we recommend using 1 x 10⁴ cells per assay. Avoid contamination with ATP from exogenous biological sources, such as bacteria or fingerprints.
- Ensure that the Nucleotide Releasing Buffer is at room temperature before use. The optimal temperature is 22°C. Keep other enzymes on ice during the assay and protect from light as much as possible.
- The assay can be performed using either a single tube or a white walled 96-well luminometer plate (100 µl/well culture volume is recommended).

Assay Protocol:

- Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
- For suspension cells, transfer 10 µl of the cultured cells (10³ – 10⁴) into luminometer plate. Add 100 µl of the Nucleotide Releasing Buffer.

For adherent cells, remove culture medium and treat cells (10³ – 10⁴) with 100 µl of Nucleotide Releasing Buffer for 5 minutes at room temperature with gentle shaking.
- To measure the ATP levels in the cells, add 1 µl of the ATP Monitoring Enzyme into the cell lysate. Read the sample in 1 minute in a luminometer (Data A).
- To measure ADP levels in the cells, read the samples (from step 3) in 10 minutes (Data B), then add 1 µl of ADP Converting Enzyme. Read the samples again in 1 minute in a luminometer (Data C).

Note: The results can be analyzed using cuvet-based luminometers or Beta Counters. When Beta Counter is used it should be programmed in the "out of coincidence" (or Luminescence mode) for measurement. The entire assay can directly be done in a 96-well plate. It can also be programmed automatically using instrumentation with injectors (When using injector the ATP Monitoring Enzyme and the ADP Converting Enzyme can be diluted with the Nuclear Releasing Buffer at 1:50 for injector).

IV. Calculation of ADP/ATP Ratio:

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

V. Interpretation of Results:

Cell Fate	ADP Level	ATP Level	ADP/ATP
Proliferation	Very Low	High	Very Low
Growth Arrest	Low	Slightly Increased	Low
Apoptosis	High	Low	High
Necrosis	Much Higher	Very Low	Much Higher

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:

- Test gives markedly elevated ATP values with no significant increase in ADP levels in comparison to control cells = proliferation.
- Test gives similar or slightly higher levels of ATP and with little or no change in ADP compared to control = growth arrest.
- Test gives lower levels of ATP to control but shows an increase in ADP = apoptosis.
- Test gives considerable lower ATP levels than control but greatly increased ADP = necrosis.

V. Related Products:

- ApoSENSOR™ ATP Determination Kit (Cell Viability Assay Kit)